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## Effects of skin washing frequency on the epidermal barrier function and inflammatory processes of the epidermis: an experimental study

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## **Abstract**

**Background:** Increased hand hygiene measures during the COVID-19 pandemic have led to an increased quantum of hand eczema (HE).

**Objectives:** To examine the effects of varying washing frequencies using current mild cleansing agents – alongside with the effect of a rehydrating cream – on the epidermal barrier function and inflammatory processes of the *stratum corneum* (SC).

**Methods:** Standardized skin washings on the volar aspects of the lower arms of skin healthy volunteers were performed using the Automated Cleansing Device either 5 or 11 times within 4 hours for 60 seconds each with a standard cleanser, a lipid-containing syndet, or a lipid-containing syndet followed by one-time application of a rehydrating cream. Skin bioengineering parameters (transepidermal water loss, SC hydration, erythema, and SC pH) and biochemical / immunological parameters (IL-1 $\alpha$ , IL-1 $\alpha$ RA, and Natural Moisturizing Factor) of SC samples collected by tape stripping were assessed.

**Results:** All applied washing procedures provided comparable, mild effects on the epidermal barrier function and skin inflammation.

**Conclusions:** Occupational skin cleansers seem to have improved regarding skin barrier damaging effects. To further corroborate this, a study design, modified on the basis of our findings, applying longer washing periods for consecutive days seems desirable.

**Keywords:** COVID-19, detergents, hand eczema, hand washing, irritant contact dermatitis, noninvasive measuring methods, occupational, risk assessment, skin barrier

## 1 Introduction

For preventing transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and development of coronavirus disease 2019 (COVID-19), hand hygiene measures must be followed strictly.<sup>1</sup> This applies to the general public and particularly specific professional groups, including health care workers (HCWs).<sup>2</sup> Hand hygiene recommendations during the COVID-19 pandemic entail longer durations and frequencies of hand washing,<sup>3</sup> which have been shown to go along with skin strain and adverse skin reactions.<sup>4-6</sup> The accumulating skin strain through water contact – which alone may be skin irritating<sup>7</sup> – and the contact with soaps or detergents lead to impairments of the skin barrier function and subsequently irritant contact dermatitis of the hands.<sup>8</sup> Due to a high amount of wet work, the prevalence of hand eczema (HE) in HCWs was already about twice as high as in the general population before the COVID-19 pandemic.<sup>9</sup> Workers subjected to a high occupational skin strain<sup>9,10</sup> are now doubly burdened by the current hygiene measures which also take place in their private surroundings. Accordingly, the elevated COVID-19 associated hygiene measures have reportedly caused an increase of HE in the general population as well as high-risk occupations.<sup>11-13</sup>

In view of individual suffering of HE patients and the health economical relevance of this disease,<sup>14,15</sup> preventative measures are needed in order to impede genesis of HE as well as to contain existing HE and to avoid chronification. Since hand hygiene measures will likely remain elevated momentarily,<sup>16</sup> demonstrably gentle hand washing regimes should be established. Against this background, this proof-of-concept study aims in an experimental set-up mimicking real-life conditions at assessing the effects of varying washing frequencies on the epidermal barrier function and inflammatory processes of the epidermis using a lipid-containing syndet compared to a standard skin cleanser. Additionally, the effect of skin cream application after washing is to be determined.

## 2 Materials & methods

### 2.1 Trial design

This study was a non-invasive, experimental, skin bioengineering, biochemical, immunological trial. Ethic approval was obtained by the sub-commission on the evaluation of medical research involving human subjects at the Medical Chamber of Lower Saxony, Hanover, Germany under

the procedure number 30/35/2020. The trial was registered at the German Clinical Trials Register (DRKS), number (DRKS-ID) DRKS00022958.

## 2.2 Participants

Only skin-healthy volunteers without atopic diathesis according to the Erlangen Atopy Score<sup>17</sup> (score value < 8) were included in the study. Detailed inclusion and exclusion criteria are listed in Table S1. Informed consent in accordance with the Helsinki Declaration of 1975, and later revisions, was obtained from all participants prior to their inclusion in the study.

## 2.3 Washing procedures

The practical study phase took place from February to April 2021. Six test areas on the volar aspects of the lower arms and one control area on the volar aspects of the upper arm with a surface of 8cm<sup>2</sup> each were marked. The test areas on the volar lower arms were randomized per participant. The test and control areas with the corresponding interventions are listed in Table 1. For each washing, a standardized amount of 3mL cleanser + 7mL distilled water (aqua dest.) was used. If cream was used, a standardized amount of 24mg cream was applied per test area (Table 1). The cream was weighed by using an electronic precision scale and a plastic top as container. Weighing took place immediately prior to application. After weighing, the cream was completely taken off of the plastic top with the aid of finger gloves and evenly distributed in the test area. Standardized washings of the test areas were performed using the Automated Cleansing Device (ACiD) with a contact pressure of the washing units of 150g and a rotation speed of 100 rotations per minute (Figure 1). The ACiD has been previously described in depth elsewhere.<sup>18-20</sup> The ingredients according to the International Nomenclature of Cosmetic Ingredients (INCI) of the commercially available study products provided by Beiersdorf AG, Hamburg, Germany are listed in Table S2.

## 2.4 Bioengineering measurements and sample collection

Before measurements were taken, participants were acclimatized to the standardized room air-conditioning (room temperature of  $21 \pm 1^\circ\text{C}$ , relative air humidity (RH) of  $50\% \pm 5\% \text{ RH}$ ) for at least 30 minutes to allow full adaptation of the skin to the environmental conditions. Skin bioengineering measurements were taken at baseline, prior to washing (T0), 15 minutes after washing (T1), 60 minutes after washing (T2), and 24 hours after the last washing (T3). The transepidermal water loss (TEWL) was assessed using the Tewameter TM 300 (Courage+Khazaka electronic GmbH, Cologne, Germany), the *stratum corneum* (SC) hydration (SCH) was assessed using the Corneometer CM 825 (Courage+Khazaka electronic

GmbH, Cologne, Germany), erythema ( $a^*$  value of chromametry) was assessed using the Chromameter CR200 (Minolta, Osaka, Japan), and the SC pH value was assessed using the Skin-pH-Meter PH 905 (Courage+Khazaka electronic GmbH, Cologne, Germany).

At T3, SC tape stripping (D-Squame sampling) was done to collect SC samples.<sup>21</sup> Sampling was conducted using round adhesive disks (22mm Standard D-Squame Skin Sampling Discs, Monaderm, Monaco) which were pressed onto the test areas for 10 seconds with the D500 D-Squame Pressure Instrument (CuDerm, Dallas, Texas, USA) in order to ensure a defined pressure. This procedure was conducted with eight discs. The fifth consecutive disk was used for Natural Moisturizing Factor (NMF) analysis and the seventh consecutive disk was used for cytokine (Interleukin 1 alpha (IL-1 $\alpha$ ) as well as Interleukin 1 alpha receptor antagonist (IL-1 $\alpha$ RA)) analysis. NMF analysis was conducted after extraction of NMF from the sampling discs using high-performance liquid chromatography with ultraviolet detection (HPLC-UV)<sup>22</sup> and the analysis of the cytokines was conducted using electrochemiluminescence immunoassays (ECLIA) (Mesoscale, Rockville, Maryland, USA).<sup>23</sup> As the amount of SC on the tape varies, the amount of cytokine in the SC on each tape was normalized by the protein content, which was determined using the Pierce Micro BCA Protein Assay Kit (Thermo Fischer Scientific, Rockford, Illinois, USA) with the bovine serum albumin supplied as standard. The results of NMF were corrected for SC protein content on the tape strips determined with the 850nm absorption infrared densitometer SquameScan 850A (Heiland Electronic, Wetzlar, Germany).

## 2.5 Statistical Analyses

Calculations were performed by using Prism 9 software (GraphPad, San Diego, California, USA). Distribution of data was tested using the Shapiro-Wilk normality test. Results for all investigated parameters at T3 were analyzed by using two-way repeated measures analysis of variance (ANOVA) and uncorrected Fisher's Least Significant Difference (LSD) followed by post-hoc correction for multiple comparisons carried out by the False Discovery Rate (FDR) method of Benjamini Hochberg (BH). Difference in data on skin bioengineering parameters TEWL, SCH, erythema, and SC pH measured at T0 to T3 were tested by two-way repeated measures ANOVA. Association between all measured parameters was performed by using Spearman correlation analysis.  $P$ -value  $< .05$  was considered significant.

### 3 Results

#### 3.1 Characteristics of the participants

25 healthy volunteers (23 women) with an age ranging from 22 to 55 years (mean  $\pm$  standard deviation (SD):  $33.8 \pm 10.7$  years) and an Erlangen Atopy Score<sup>17</sup> ranging from 0 to 7 (mean  $\pm$  SD:  $3.6 \pm 1.9$ ) were included. 19 participants (76.0%) had a Fitzpatrick<sup>24</sup> skin phototype II and 6 (24.0%) had a Fitzpatrick skin phototype III. None of the participants had used any topical immunosuppressants in the test areas in the weeks before the study.

#### 3.2 Effects of washing frequency and cream application

Two-way repeated measures ANOVA and uncorrected Fisher's LSD followed by post-hoc correction for multiple comparisons carried out by the FDR method of BH revealed that for TEWL, SCH, erythema, SC pH, NMF, IL-1 $\alpha$ , and IL-1 $\alpha$ RA there were no statistically significant differences at T3 comparing the various test areas (Table 1) with their respective control sites (Figures S1-S7). Full results of TEWL, SCH, erythema, SC pH, NMF, IL-1 $\alpha$ , and IL-1 $\alpha$ RA are provided in Tables S3-S7, respectively. Two-way repeated measures ANOVA did not reveal significant differences from T0 to T3 in any of investigated parameters.

#### 3.3 Correlation analysis of skin barrier parameters

Figure 2 shows a heatmap containing Spearman correlation coefficients and corresponding levels of significance. Skin barrier function as measured by TEWL showed an inverse correlation with NMF, and was positively correlated with SCH, SC pH, IL-1 $\alpha$ , and IL-1 $\alpha$ RA. Next to TEWL, NMF was also inversely correlated with IL-1 $\alpha$  and IL-1 $\alpha$ RA. There was no association of erythema with TEWL, SCH, SC pH, NMF, IL-1 $\alpha$ , and IL-1 $\alpha$ RA.

### 4 Discussion

To the best of our knowledge, this study is the first study to examine the effects of varying washing frequencies on the epidermal barrier function and inflammatory processes of the epidermis using a variation of cleansing agents with and without the application of a rehydrating cream under laboratory conditions using a skin cleansing device. The mimicking of almost life-like conditions and the standardization of procedures by using the ACiD can be considered as a strength of this study.

To analyze effects on the epidermal barrier function and inflammatory processes of the epidermis, measurements taken at T3 (24 hours after the last washing procedure) were examined primarily. It was shown that the applied interventions (Table 1) had a mild effect on

the examined skin bioengineering parameters TEWL, SCH, erythema, and SC pH which was not statistically significant nor of practical importance. This observation leads to the conclusion that all of the interventions did not lead to detectable impairment of the epidermal barrier function or inflammatory processes of the epidermis. Further, this finding is sustained by the reported results on biochemical / immunological parameters since there were no statistically significant changes in NMF, IL-1 $\alpha$ , and IL-1 $\alpha$ RA. The positive effect of emollient application on the epidermal barrier function after skin washing has, however, already been described in previous studies.<sup>25,26</sup> Potentially damaging effect of cleansing agents on the skin barrier function might be reduced by the application of emollients, which seems advisable after hand washing – especially when higher hand washing frequencies are applied.

Correlation analysis of skin barrier and inflammatory parameters has shown that TEWL is inversely correlated with NMF levels. Due to its hygroscopic properties, NMF contributes largely to the retention of water in the stratum corneum. Reduced NMF values would result in an increase in epidermal water loss concomitant with higher TEWL.<sup>27,28</sup> Positive association was observed between TEWL and IL-1 $\alpha$  levels, which corroborates earlier studies which have shown that skin barrier damage – depicted by higher TEWL – is associated with lower IL-1 $\alpha$  concentrations.<sup>29,30</sup>

COVID-19-associated hygiene regimes comprise elevated hand washing frequencies and longer washing durations.<sup>3</sup> The chosen duration (60 seconds) for each washing was thus considered appropriate for mimicking current hand washing durations in pandemic times. However, the applied washing frequencies of 5 times and 11 times within 4 hours might not reflect the actual skin strain HCWs are exposed to during working days, particularly considering additive effects over several days. Whilst designing this study, infection control regulations needed to be followed to protect study participants as well as the examiners. To generate results even closer to the exposure HCWs are subjected to in their working environment, it seems recommendable to conduct skin washings within a longer time period of 8 hours as well as for consecutive days when the pandemic situation allows such a study design. It should be considered to include not only skin healthy subjects in following studies but also individuals with (a history of) atopic dermatitis who have a reduced skin barrier function<sup>31</sup> and are more susceptible to developing contact dermatitis<sup>32,33</sup> providing a more representative design for a real life situation in the workplace. It can be suspected that the study design of the present study might generate different results in individuals with an atopic skin diathesis or sensitive skin rather than in individuals with normal, healthy skin.

Another limitation of this study is that the compared skin cleansers are expected to be skin-friendlier compared to cleansers used in the past. No irritant control (e.g. washing with diluted sodium lauryl sulfate (SLS)) was included due to the limited number of test areas related to the design of the ACiD. The reference cleanser contains sodium laureth sulfate (SLES) – a mild detergent by contrast with other detergents such as SLS.<sup>34,35</sup> It also contains glycerin, a humectant.<sup>36,37</sup> SLS has to a great extent been used as a model irritant in studies examining skin irritation and surfactant-induced impairment of the epidermal barrier.<sup>38-41</sup> If a cleanser containing SLS would instead have been applied, most likely, greater differences would have been observed. The current findings might allow the assumption that skin cleansers currently on the market – when SLES is used instead of harsher detergents such as SLS – have improved in comparison to products in the past in terms of skin barrier damaging effects. It thus can be denoted as a positive characteristic of this study that we depicted the current recommendations regarding usage of mild skin cleansers and have consequently followed a real-life study design as far as possible.

Within the presented experimental setting, applied skin washing frequencies did only have mild adverse effects on epidermal barrier function and on inflammatory processes in the epidermis. No differences were observed regarding the different skin cleansing products. Formulations of skin cleansers currently on the market seem to have improved in terms of skin barrier damaging effects. In the future, a study design, modified on the basis of our findings, applying washings within longer periods for consecutive days seems recommendable to generate circumstances even closer to occupational exposure of HCWs. In such a design, it is to be expected that application of an emollient after washing may have positive effects in terms of epidermal barrier homeostasis. This would especially be important for wet work professions at risk of development of occupational HE such as HCWs as well as the general population in pandemic times.

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## 6 Tables

Table 1: Test areas and control site with the corresponding conducted intervention

| No.† | Intervention                                                                                                                    |
|------|---------------------------------------------------------------------------------------------------------------------------------|
| 1    | control site, no washing, empty control                                                                                         |
| 2    | five-time washing (60s each) with a standard cleanser‡ within 4hrs                                                              |
| 3    | five-time washing (60s each) with a lipid-containing syndet‡ within 4hrs                                                        |
| 4    | five-time washing (60s each) with a lipid-containing syndet‡ within 4hrs followed by application of a rehydrating skin cream§   |
| 5    | eleven-time washing (60s each) with a standard cleanser‡ within 4hrs                                                            |
| 6    | eleven-time washing (60s each) with a lipid-containing syndet‡ within 4hrs                                                      |
| 7    | eleven-time washing (60s each) with a lipid-containing syndet‡ within 4hrs followed by application of a rehydrating skin cream§ |

†test areas were randomized per participant; ‡a standardized amount of 3ml cleanser + 7ml distilled water (aqua dest.) were applied per test area; §a standardized amount of 24mg cream was applied per test area; hrs: hours; s: seconds

## 7 Figures

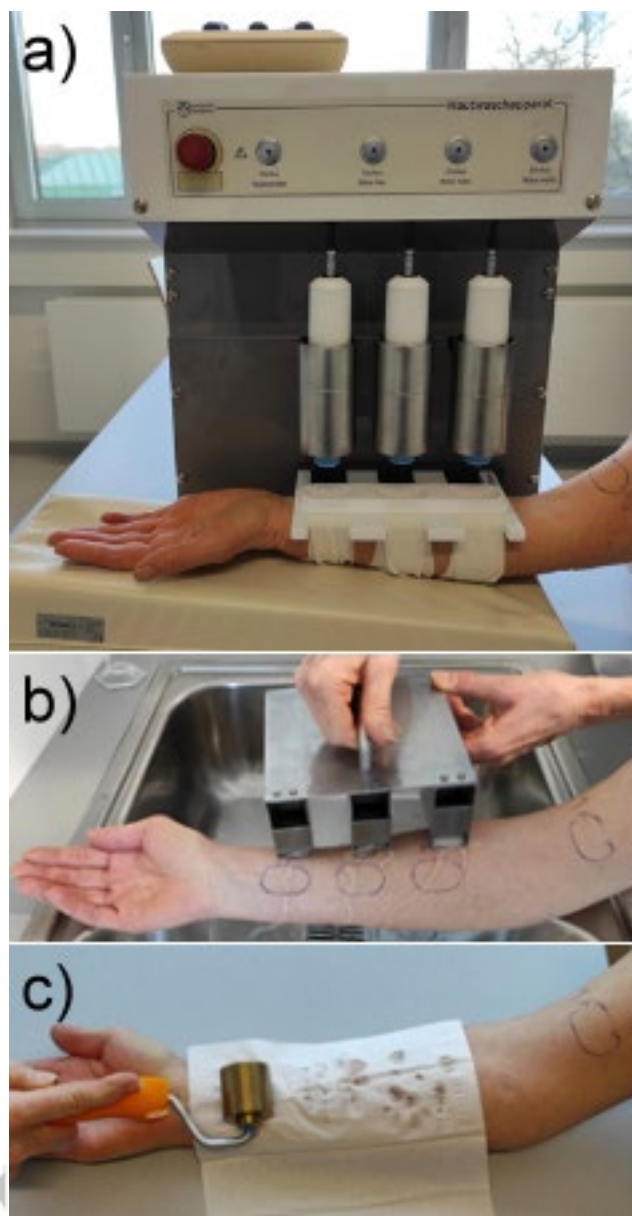


Figure 1: a) Automated Cleansing Device (ACiD) with three washing modules and the washing chamber fixed to the lower arm of the participant; b) standardized rinsing of the test areas with a defined amount of 125mL distilled water per test area using a special beaker; c) standardized drying of the test areas with a new cosmetic tissue per test area and the defined contact pressure of 298.8g

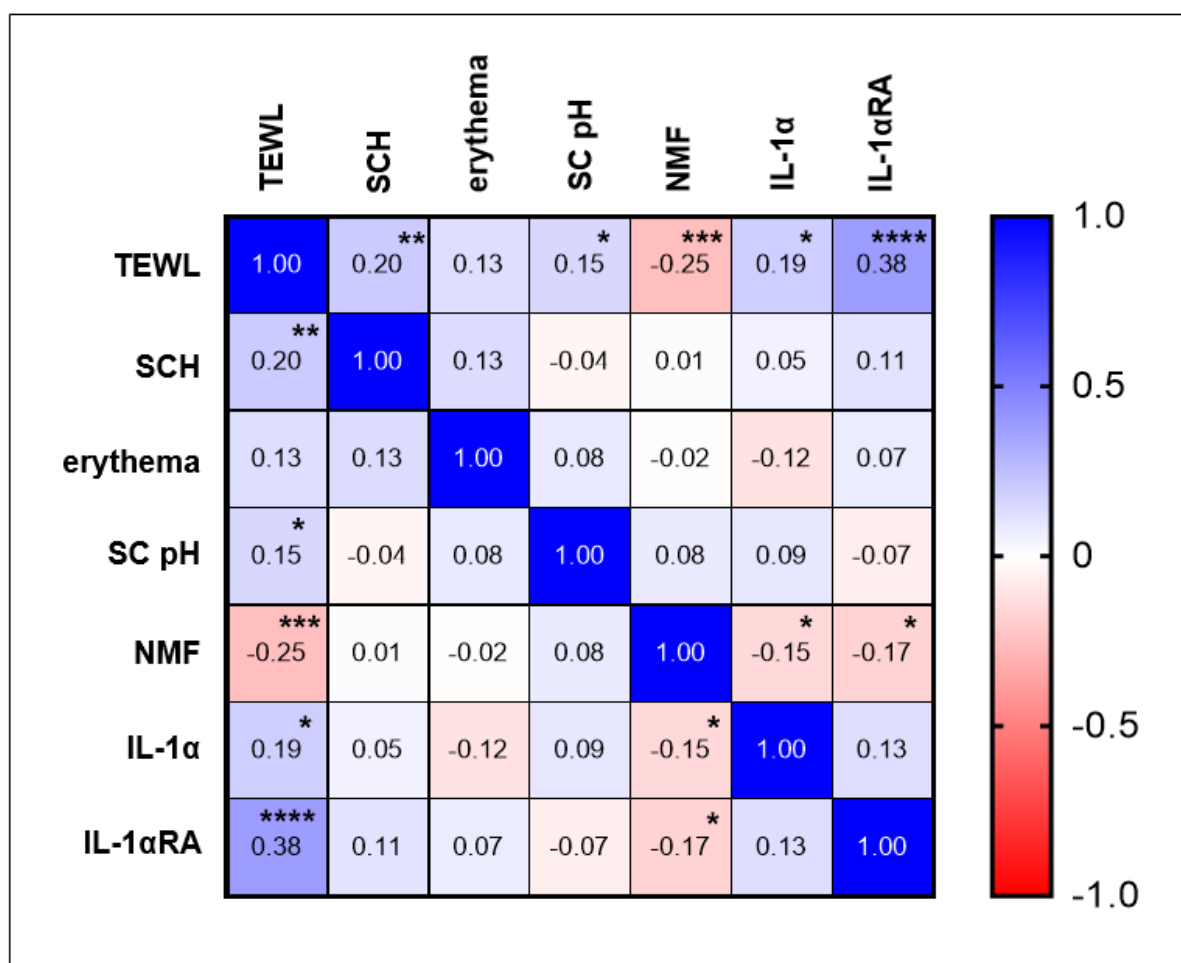
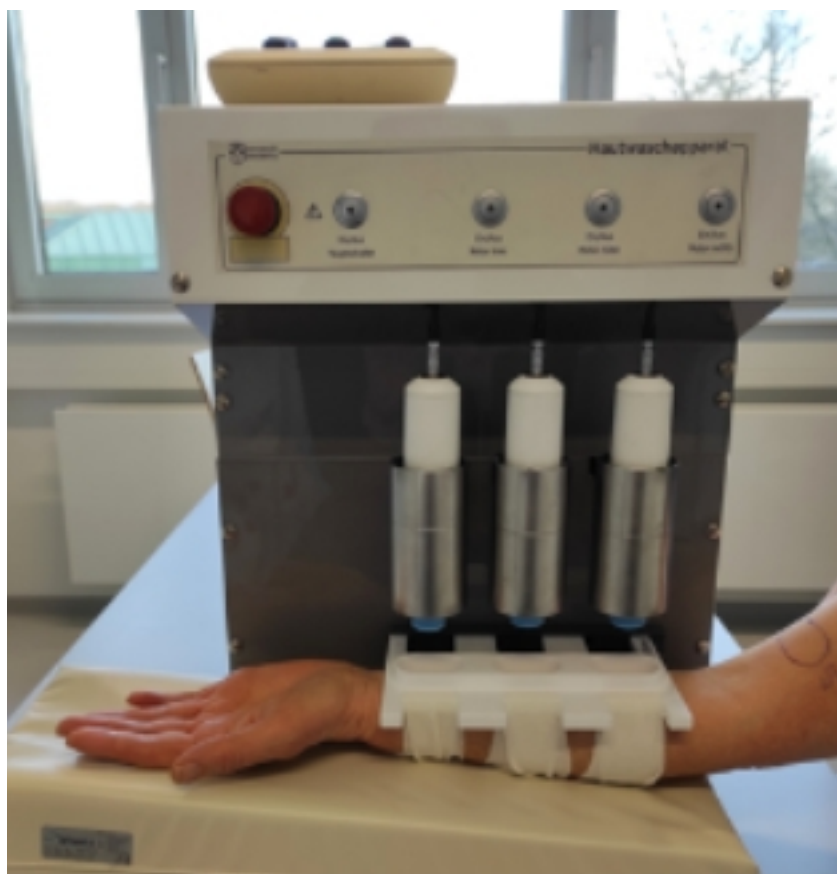


Figure 2: Correlation analysis of measured skin barrier parameters (transepidermal water loss (TEWL), stratum corneum hydration (SCH), erythema (chromametry  $a^*$  values), stratum corneum (SC) pH, Natural Moisturizing Factor (NMF), Interleukin 1 alpha (IL-1 $\alpha$ ), and Interleukin 1 alpha receptor antagonist (IL-1 $\alpha$ RA)) measured at T3 (24hrs after washing). Data are presented as Spearman correlation coefficients and corresponding level of significance. \* $P < .05$ , \*\* $P < .01$ , \*\*\* $P < .001$ , \*\*\*\* $P < .0001$



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## Supporting Information

Table S1: Inclusion and exclusion criteria

| Inclusion criteria                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Exclusion criteria                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <ul style="list-style-type: none"> <li>▪ age between 18 and 55 years</li> <li>▪ Fitzpatrick skin phototype I, II or III</li> <li>▪ clinically healthy and untreated skin on the volar lower and upper arms</li> <li>▪ willingness to not use cosmetic cleansing and caring products on the volar lower and upper arms 24 hours before participation and in the course of the study</li> <li>▪ willingness to not conduct hair removal treatments on the volar lower and upper arms one week prior participation and in the course of the study</li> <li>▪ willingness to not expose the test areas on the volar lower and upper arms to direct ultraviolet radiation (sunbathing/tanning in the salon) six weeks before participation and in the course of the study</li> </ul> | <ul style="list-style-type: none"> <li>▪ Erlangen atopy score<sup>17</sup> of <math>\geq 8</math> (atopy unlikely)</li> <li>▪ known allergies against fragrances or oat flour†</li> <li>▪ body modification (tattoos, piercings, etc.) in the test areas on the volar lower and upper arms</li> <li>▪ excessive hair growth in the test areas on the volar lower and upper arms</li> <li>▪ use of self-tanning preparations in the test areas on the volar lower and upper arms</li> <li>▪ intake of immunosuppressants (e.g., cortisone)</li> <li>▪ topical application of medical immunosuppressants (e.g., topical corticosteroids) in the test areas on the volar lower and upper arms within seven days before participation and in the course of the study</li> <li>▪ pregnancy and breast-feeding</li> </ul> |

†due to composition of study products

Table S2: Ingredients of the utilized study products according to the International Nomenclature of Cosmetic Ingredients (INCI)

| Product                                                                            | Ingredients according to the International Nomenclature of Cosmetic Ingredients (INCI) <sup>†</sup>                                                                                                                                                                                                                                                                                                                      |
|------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Standard cleanser (aqueous surfactant system)                                      | Aqua, Sodium Laureth Sulfate, Cocamidopropyl Betaine, Sodium Chloride, Citric Acid, Sodium Benzoate, Glycerin, Disodium Cocoyl Glutamate, Polyquaternium-10, PEG-200 Hydrogenated Glyceryl Palmate, Propylene Glycol, Benzophenone-4, Sodium Sulfate, Sodium Hydroxide, Panthenol, Pantolactone                                                                                                                          |
| Lipid-containing syndet (oil-in-water emulsion in contact with water) <sup>‡</sup> | Glycine Soja Oil, Laureth-4, MIPA-Laureth Sulfate, Ricinus Communis Oil, Poloxamer 101, Parfum, Aqua, Propylene Glycol, Panthenol, Tocopherol, Citric Acid, Sodium Citrate                                                                                                                                                                                                                                               |
| Rehydrating skin cream (oil-in-water emulsion)                                     | Aqua, Glycerin, Paraffinum Liquidum, Cetyl Alcohol, Glyceryl Stearate, Stearyl Alcohol, Hydrogenated Coco-Glycerides, Caprylic-Capric-Triglyceride, Octyldodecanol, Butyrospermum Parkii Butter, Cetyl Palmitate, Colloidal Oatmeal, PEG-40 Stearate, Glycyrrhiza Inflata Root Extract, Ceramide NP, Menthoxyprompanediol, Citric Acid, Sodium Citrate, Decylene Glycol, Phenoxyethanol, Caprylyl Glycol, Benzyl Alcohol |

<sup>†</sup>according to the order in which they are listed on the packaging; <sup>‡</sup>The lipid-containing syndet is a mixture of oil and surfactant which can be classified as an oil-in-water emulsion.

Table S3: Transepidermal water loss (TEWL) measured in g/m<sup>2</sup>/h taken at baseline, prior to washing (T0), 15 minutes after washing (T1), 60 minutes after washing (T2), and 24 hours after the last washing (T3). Data are presented as mean (*M*) ± standard deviation (*SD*) for all conducted interventions (test areas) as well as the control site

| No. | T0       |           | T1       |           | T2       |           | T3       |           |
|-----|----------|-----------|----------|-----------|----------|-----------|----------|-----------|
|     | <i>M</i> | <i>SD</i> | <i>M</i> | <i>SD</i> | <i>M</i> | <i>SD</i> | <i>M</i> | <i>SD</i> |
| 1   | 6.90     | 1.57      | 6.66     | 1.86      | 6.44     | 1.64      | 7.03     | 2.24      |
| 2   | 7.00     | 1.27      | 5.92     | 1.34      | 6.11     | 1.99      | 7.58     | 2.23      |
| 3   | 6.47     | 1.33      | 6.10     | 1.50      | 6.02     | 1.26      | 7.26     | 1.70      |
| 4   | 6.67     | 1.33      | 5.87     | 1.97      | 5.72     | 1.28      | 7.29     | 2.43      |
| 5   | 6.50     | 1.53      | 5.92     | 1.34      | 5.83     | 1.65      | 7.47     | 2.41      |
| 6   | 6.69     | 1.51      | 6.33     | 1.77      | 5.97     | 1.84      | 7.72     | 2.33      |
| 7   | 6.57     | 1.49      | 5.22     | 1.49      | 5.42     | 1.73      | 7.29     | 2.57      |

1, control site, no washing, empty control; 2, five-time washing (60s each) with a standard cleanser within 4h; 3, five-time washing (60s each) with a lipid-containing syndet within 4h; 4, five-time washing (60s each) with a lipid-containing syndet within 4h followed by application of a rehydrating skin cream; 5, eleven-time washing (60s each) with a standard cleanser within 4h; 6, eleven-time washing (60s each) with a lipid-containing syndet within 4h; 7, eleven-time washing (60s each) with a lipid-containing syndet within 4h followed by application of a rehydrating skin cream; *M*, mean; *SD*, standard deviation; T0, prior to washing; T1, 15 minutes after washing; T2, 60 minutes after washing; T3, 24 hours after the last washing

Table S4: Stratum corneum hydration (SCH) measured in arbitrary units (AU) at baseline, prior to washing (T0), 15 minutes after washing (T1), 60 minutes after washing (T2), and 24 hours after the last washing (T3). Data are presented as mean (*M*)  $\pm$  standard deviation (*SD*) for all conducted interventions (test areas) as well as the control site

| No. | T0       |           | T1       |           | T2       |           | T3       |           |
|-----|----------|-----------|----------|-----------|----------|-----------|----------|-----------|
|     | <i>M</i> | <i>SD</i> | <i>M</i> | <i>SD</i> | <i>M</i> | <i>SD</i> | <i>M</i> | <i>SD</i> |
| 1   | 25       | 6         | 28       | 7         | 27       | 7         | 26       | 7         |
| 2   | 25       | 7         | 29       | 7         | 26       | 6         | 25       | 7         |
| 3   | 27       | 7         | 29       | 6         | 26       | 6         | 26       | 6         |
| 4   | 26       | 7         | 39       | 8         | 33       | 7         | 29       | 6         |
| 5   | 26       | 7         | 28       | 7         | 27       | 7         | 26       | 6         |
| 6   | 26       | 6         | 27       | 6         | 26       | 7         | 27       | 5         |
| 7   | 27       | 7         | 43       | 8         | 36       | 7         | 29       | 7         |

1, control site, no washing, empty control; 2, five-time washing (60s each) with a standard cleanser within 4h; 3, five-time washing (60s each) with a lipid-containing syndet within 4h; 4, five-time washing (60s each) with a lipid-containing syndet within 4h followed by application of a rehydrating skin cream; 5, eleven-time washing (60s each) with a standard cleanser within 4h; 6, eleven-time washing (60s each) with a lipid-containing syndet within 4h; 7, eleven-time washing (60s each) with a lipid-containing syndet within 4h followed by application of a rehydrating skin cream; *M*, mean; *SD*, standard deviation; T0, prior to washing; T1, 15 minutes after washing; T2, 60 minutes after washing; T3, 24 hours after the last washing

Table S5: Erythema (chromametry  $a^*$  values) at baseline, prior to washing (T0), 15 minutes after washing (T1), 60 minutes after washing (T2), and 24 hours after the last washing (T3). Data are presented as mean ( $M$ )  $\pm$  standard deviation ( $SD$ ) for all conducted interventions (test areas) as well as the control site

| No. | T0       |           | T1       |           | T2       |           | T3       |           |
|-----|----------|-----------|----------|-----------|----------|-----------|----------|-----------|
|     | <i>M</i> | <i>SD</i> | <i>M</i> | <i>SD</i> | <i>M</i> | <i>SD</i> | <i>M</i> | <i>SD</i> |
| 1   | 7.43     | 1.58      | 8.04     | 1.88      | 8.11     | 1.64      | 7.71     | 1.36      |
| 2   | 7.01     | 1.28      | 7.69     | 1.43      | 7.64     | 1.59      | 7.65     | 1.32      |
| 3   | 6.82     | 1.46      | 7.61     | 1.44      | 7.50     | 1.55      | 7.84     | 1.81      |
| 4   | 6.81     | 1.20      | 7.67     | 1.54      | 7.60     | 1.46      | 7.71     | 1.55      |
| 5   | 7.01     | 1.43      | 7.89     | 2.00      | 7.93     | 2.04      | 7.86     | 1.62      |
| 6   | 7.11     | 1.36      | 7.95     | 1.51      | 8.07     | 1.50      | 8.06     | 1.61      |
| 7   | 6.96     | 1.13      | 7.83     | 1.66      | 7.85     | 1.73      | 7.99     | 1.39      |

1, control site, no washing, empty control; 2, five-time washing (60s each) with a standard cleanser within 4h; 3, five-time washing (60s each) with a lipid-containing syndet within 4h; 4, five-time washing (60s each) with a lipid-containing syndet within 4h followed by application of a rehydrating skin cream; 5, eleven-time washing (60s each) with a standard cleanser within 4h; 6, eleven-time washing (60s each) with a lipid-containing syndet within 4h; 7, eleven-time washing (60s each) with a lipid-containing syndet within 4h followed by application of a rehydrating skin cream; *M*, mean; *SD*, standard deviation; T0, prior to washing; T1, 15 minutes after washing; T2, 60 minutes after washing; T3, 24 hours after the last washing

Table S6: Stratum corneum (SC) pH measured in arbitrary units (AU) at baseline, prior to washing (T0), 15 minutes after washing (T1), 60 minutes after washing (T2), and 24 hours after the last washing (T3). Data are presented as mean (*M*) ± standard deviation (*SD*) for all conducted interventions (test areas) as well as the control site

| No. | T0       |           | T1       |           | T2       |           | T3       |           |
|-----|----------|-----------|----------|-----------|----------|-----------|----------|-----------|
|     | <i>M</i> | <i>SD</i> | <i>M</i> | <i>SD</i> | <i>M</i> | <i>SD</i> | <i>M</i> | <i>SD</i> |
| 1   | 5.3      | 0.8       | 5.2      | 0.4       | 6.0      | 0.4       | 5.3      | 0.5       |
| 2   | 5.4      | 0.9       | 5.5      | 0.4       | 6.1      | 0.6       | 5.4      | 0.7       |
| 3   | 5.4      | 0.9       | 5.9      | 0.5       | 6.3      | 0.6       | 5.5      | 0.6       |
| 4   | 5.4      | 0.8       | 5.1      | 0.3       | 6.1      | 0.7       | 5.4      | 0.6       |
| 5   | 5.4      | 0.9       | 5.5      | 0.4       | 6.1      | 0.6       | 5.5      | 0.6       |
| 6   | 5.4      | 0.8       | 6.0      | 0.5       | 6.5      | 0.6       | 5.6      | 0.7       |
| 7   | 5.5      | 1.0       | 5.2      | 0.5       | 6.0      | 0.8       | 5.4      | 0.5       |

1, control site, no washing, empty control; 2, five-time washing (60s each) with a standard cleanser within 4h; 3, five-time washing (60s each) with a lipid-containing syndet within 4h; 4, five-time washing (60s each) with a lipid-containing syndet within 4h followed by application of a rehydrating skin cream; 5, eleven-time washing (60s each) with a standard cleanser within 4h; 6, eleven-time washing (60s each) with a lipid-containing syndet within 4h; 7, eleven-time washing (60s each) with a lipid-containing syndet within 4h followed by application of a rehydrating skin cream; *M*, mean; *SD*, standard deviation; T0, prior to washing; T1, 15 minutes after washing; T2, 60 minutes after washing; T3, 24 hours after the last washing

Table S7: Natural Moisturizing Factor (NMF) measured in mmol/g protein, *Interleukin 1* alpha (*IL-1α*) measured in pg/μg protein, and *Interleukin 1* alpha receptor antagonist (*IL-1αRA*) measured in pg/μg protein at 24 hours after the last washing (T3). Data are presented as mean (*M*) ± standard deviation (*SD*) for all conducted interventions (test areas) as well as the control site

| No. | NMF      |           | IL-1α    |           | IL-1αRA  |           |
|-----|----------|-----------|----------|-----------|----------|-----------|
|     | <i>M</i> | <i>SD</i> | <i>M</i> | <i>SD</i> | <i>M</i> | <i>SD</i> |
| 1   | 0.5062   | 0.1319    | 230.1160 | 124.6466  | 153.2560 | 114.6361  |
| 2   | 0.5154   | 0.1806    | 175.9040 | 93.6727   | 166.4400 | 230.3568  |
| 3   | 0.4851   | 0.1500    | 201.3160 | 132.4089  | 140.1480 | 128.4151  |
| 4   | 0.5170   | 0.1694    | 190.2720 | 111.7877  | 148.6320 | 160.8185  |
| 5   | 0.4564   | 0.1418    | 223.2320 | 157.2157  | 147.8040 | 136.8243  |
| 6   | 0.4656   | 0.1557    | 221.5880 | 164.5992  | 158.9320 | 173.8916  |
| 7   | 0.4616   | 0.1385    | 166.2120 | 87.9869   | 115.8640 | 93.6099   |

1, control site, no washing, empty control; 2, five-time washing (60s each) with a standard cleanser within 4h; 3, five-time washing (60s each) with a lipid-containing syndet within 4h; 4, five-time washing (60s each) with a lipid-containing syndet within 4h followed by application of a rehydrating skin cream; 5, eleven-time washing (60s each) with a standard cleanser within 4h; 6, eleven-time washing (60s each) with a lipid-containing syndet within 4h; 7, eleven-time washing (60s each) with a lipid-containing syndet within 4h followed by application of a rehydrating skin cream; *IL-1α*, *Interleukin 1* alpha; *IL-1αRA*, *Interleukin 1* alpha receptor antagonist; *M*, mean; NMF, Natural Moisturizing Factor; *SD*, standard deviation

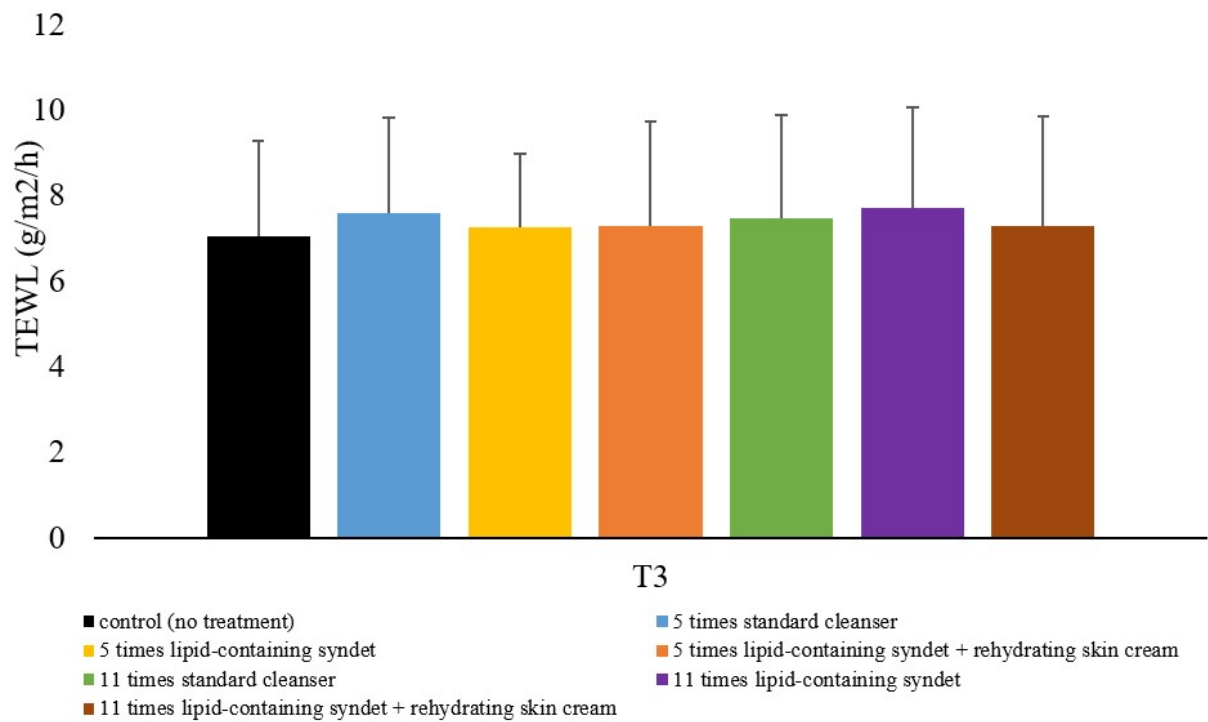


Figure S1: Comparison of TEWL at T3 (24hrs after washing) after washing the test areas in varying frequencies (5 times vs. 11 times for 60s each) whilst applying different cleansing and caring procedures. Data are presented as mean  $\pm$  standard deviation. No significant differences were observed.

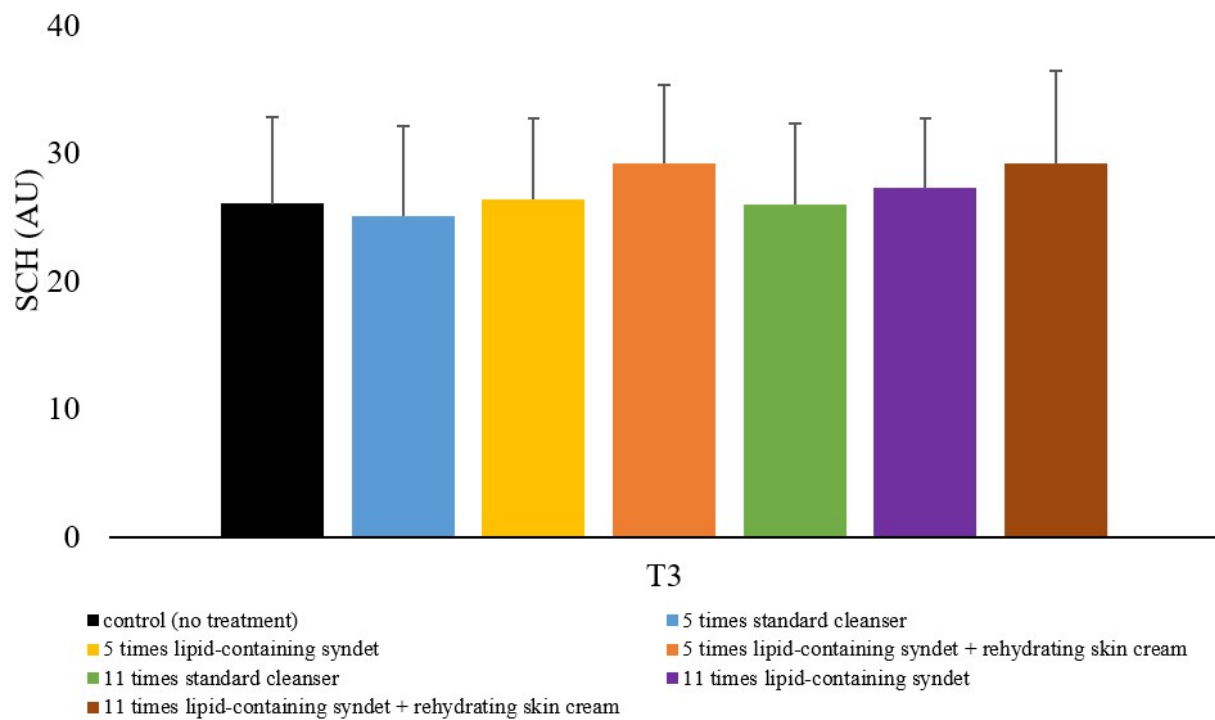


Figure S2: Comparison of stratum corneum hydration (SCH) at T3 (24hrs after washing) after washing the test areas in varying frequencies (5 times vs. 11 times for 60s each) whilst

applying different cleansing and caring procedures. Data are presented as mean  $\pm$  standard deviation. No significant differences were observed.

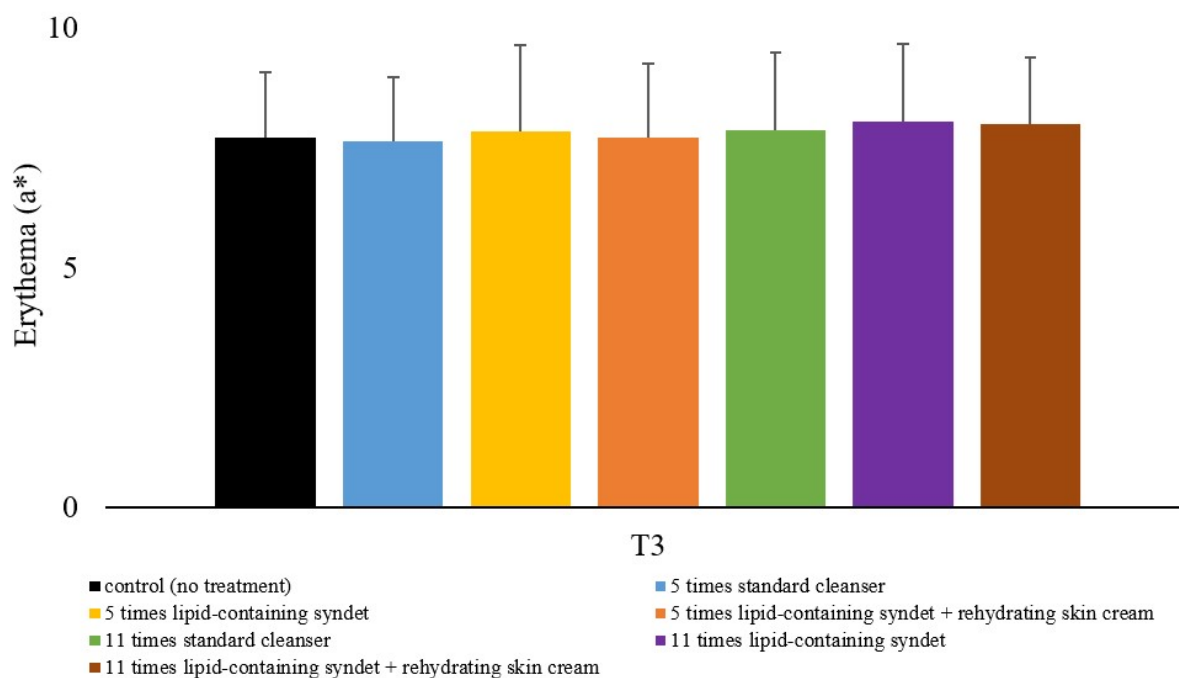


Figure S3: Comparison of erythema (chromametry a\* values) at T3 (24h after washing) after washing the test areas in varying frequencies (5 times vs. 11 times for 60s each) whilst applying different cleansing and caring procedures. Data are presented as mean  $\pm$  standard deviation. No significant differences were observed.

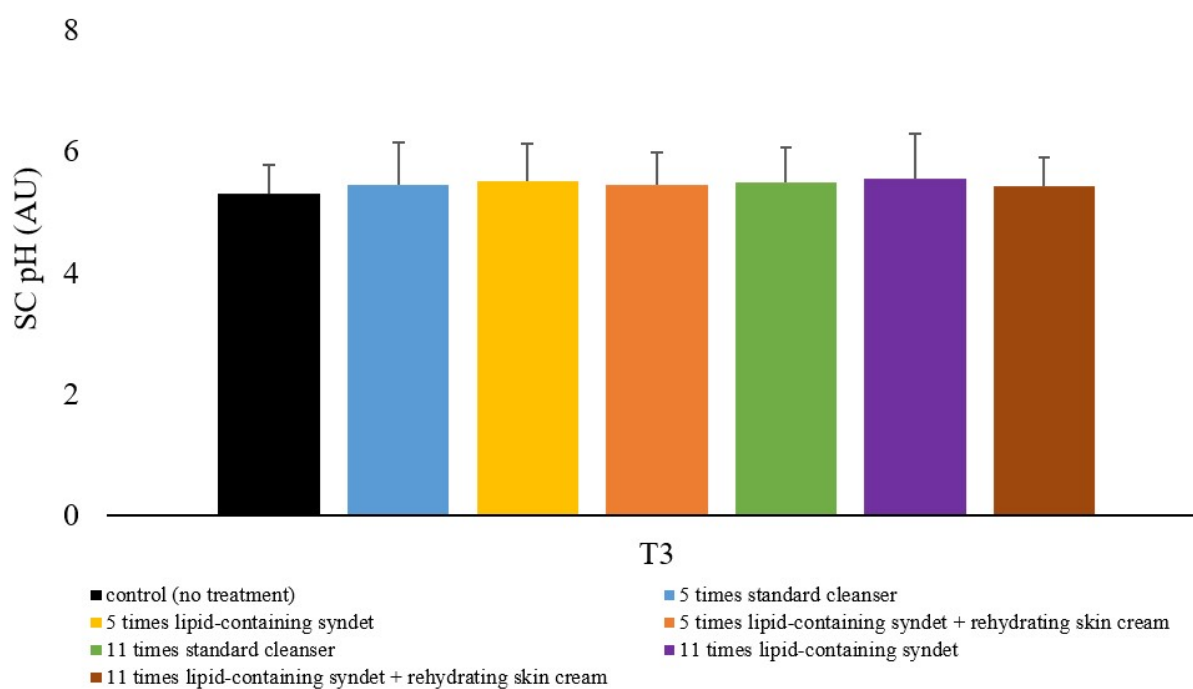


Figure S4: Comparison of stratum corneum (SC) pH at T3 (24h after washing) after washing the test areas in varying frequencies (5 times vs. 11 times for 60s each) whilst applying different cleansing and caring procedures. Data are presented as mean  $\pm$  standard deviation. No significant differences were observed.

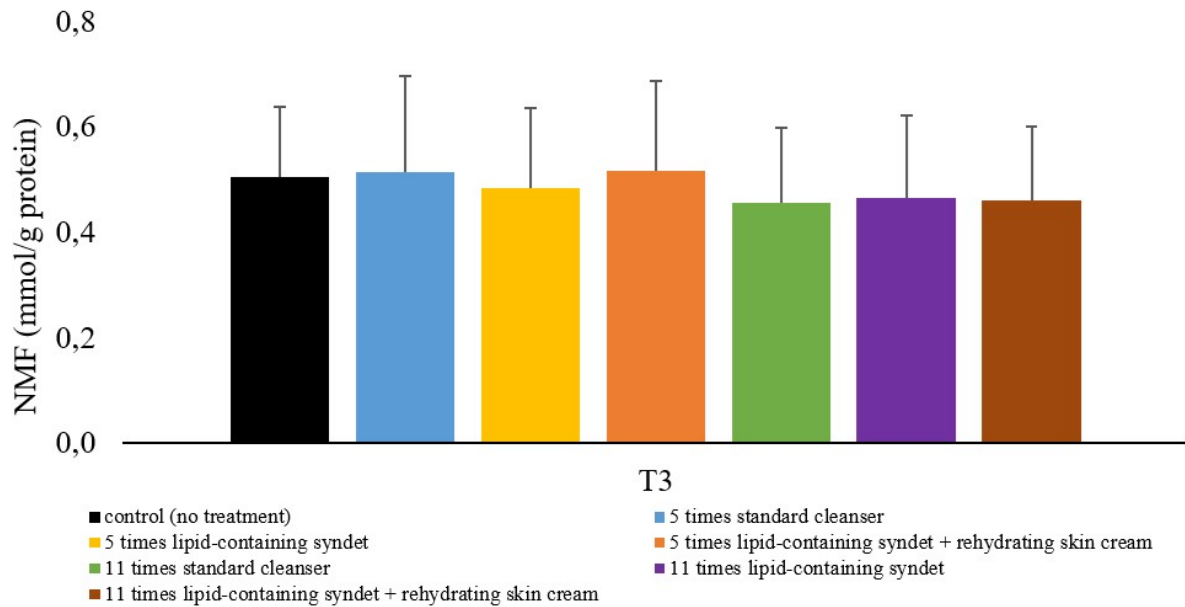


Figure S5: Comparison of Natural Moisturizing Factor (NMF) levels at T3 (24h after washing) after washing the test areas in varying frequencies (5 times vs. 11 times for 60s each) whilst applying different cleansing and caring procedures. Data are presented as mean  $\pm$  standard deviation. No significant differences were observed.

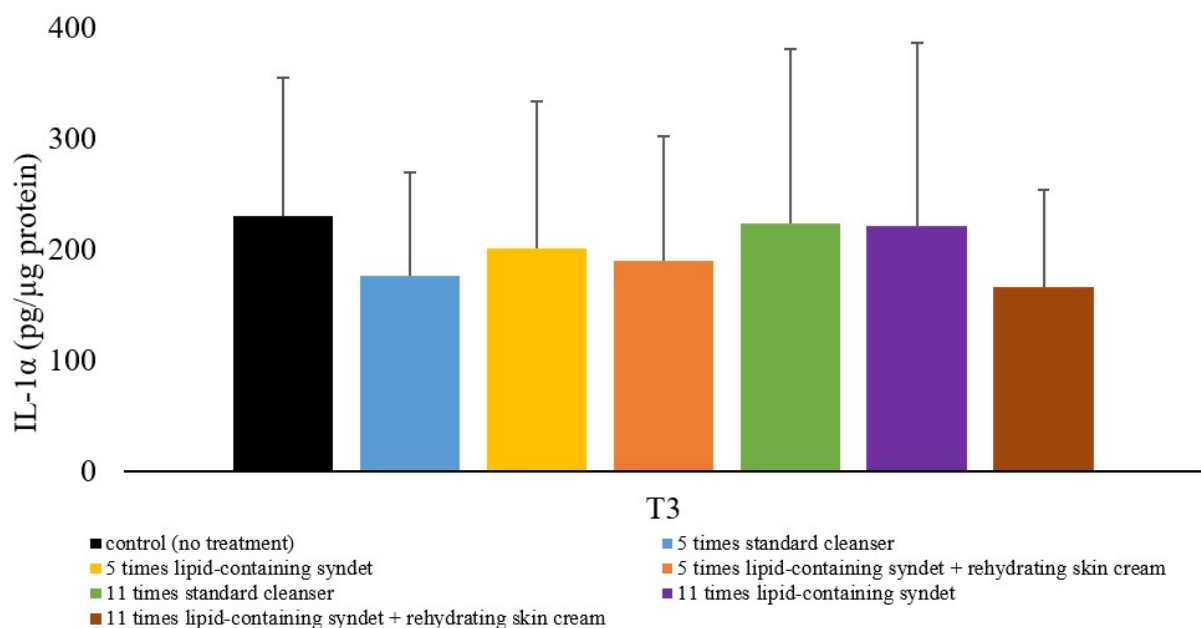


Figure S6: Comparison of *Interleukin 1* alpha (*IL-1 $\alpha$* ) levels at T3 (24h after washing) after washing the test areas in varying frequencies (5 times vs. 11 times for 60s each) whilst applying different cleansing and caring procedures. Data are presented as mean  $\pm$  standard deviation. No significant differences were observed.

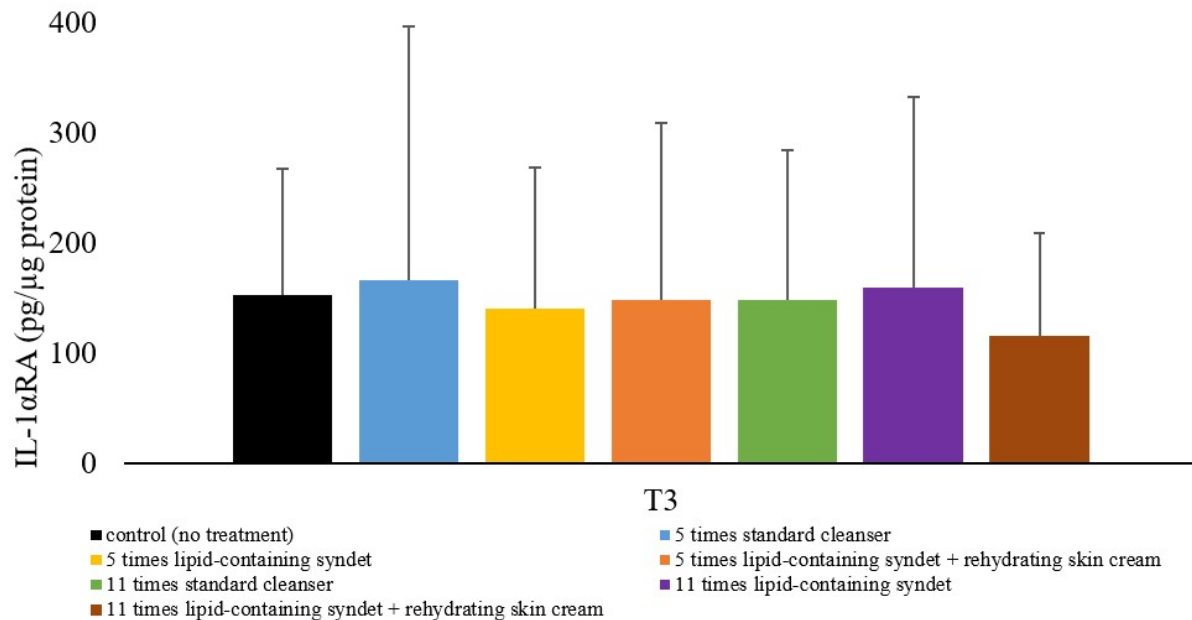


Figure S7: Comparison of *Interleukin 1* alpha receptor antagonist (IL-1 $\alpha$ RA) at T3 (24h after washing) after washing the test areas in varying frequencies (5 times vs. 11 times for 60s each) whilst applying different cleansing and caring procedures. Data are presented as mean  $\pm$  standard deviation. No significant differences were observed.