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Keywords:	skin physiological function, transepidermal water loss, skin hydration, skin pH, ceramide, cleaning agent, water washing, wiping

Wash or wipe? A comparative study of skin physiological changes between water washing and wiping after skin cleaning

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Abstract

Background/purpose: Presently, skin-cleaning agents that claim to be removed by water or wiping alone are commercially available and have been used for the purpose of bed baths. However, there is a lack of knowledge on how water washing and wiping differently affect skin physiological functions or ceramide content. The aim of this study was to compare the effects of water washing and wiping on skin physiological functions and ceramide content.

Methods: Three kinds of the cleaning agents with different removal techniques (i.e., water washing and wiping) were used in this study. Skin physiological functions (i.e., transepidermal water loss, skin hydration, and skin pH) and skin ceramide content were measured before and after seven consecutive days of the application of each cleaning agent.

Results: No significant differences in skin physiological functions or ceramide content were observed between water washing and wiping.

Conclusion: Cleaning agents that claim to be removed by water washing or wiping do not affect skin physiological functions or ceramide content by either removal method.

Running Head

How does washing or wiping affect skin after cleaning?

Key words

skin physiological function; transepidermal water loss; skin hydration; skin pH; ceramide; cleaning agent; water washing; wiping

1. Introduction

Bathing is one of the most fundamental activities to keep our body clean. It usually involves washing away dirt and sweat from the skin with lukewarm water, cleansing oily dirt with soap or a cleaning agent, rinsing the soap/cleaning agent off, and drying. These processes are not only a cleaning procedure but also have beneficial effects on the prevention of several kinds of diseases (1, 2) and the eradication of bacterial skin colonization (3, 4).

Bed baths are a type of bathing technique in which the caregiver wipes the patient's skin by means of a soft, wet towel with or without soap. This technique is particularly effective for patients who are bedridden and/or receiving home-care services. When soap is used, the caregiver is required to purge the remaining soap from the skin by water washing. Because soap remnants may irritate the skin (5) and cause deterioration in skin physiology (6), plenty of water and a basin are required to remove remnant soap on the skin. It is, however, a burdensome task for caregivers, particularly at the bedside and at home (7). In addition, such water washing is not available when water usage is restricted, for example, after natural disasters.

As an alternative to the traditional bed bath with soap and water, cleaning agents that can be removed just by wiping and without water have been introduced. Such "removable-by-wiping" cleansing is considered to be comparable to the traditional soap-and-water bed bath (8), less invasive (9), and more cost effective (10). Because wiping can cleanse the skin as well as water washing can, there is a growing acceptance of "removable-by-wiping" cleaning agents in both clinical and home-care settings (8, 10).

Although such "removable-by-wiping" agents claim that both water washing and wiping can be interchangeably used for removal, one question remains: how do the different removal techniques (i.e., water washing and wiping) affect skin physiology? Considering the fact that wiping cannot completely remove soap on the skin (11) and that residual soap irritates and causes skin deterioration (5, 6), we need to know whether wiping alone can maintain skin integrity comparable to water washing.

Furthermore, because the cleaning agents are amphiphilic (i.e., have an affinity for both water and lipids), skin lipids, particularly ceramides, may be ablated during wiping. Ceramides are the main components of lipids in the stratum corneum (SC) and are responsible for skin barrier function. Depletion of ceramides leads to skin dryness (12). Therefore, we also need to know how wiping without water affects skin ceramide retention.

To address these questions, the aim of this study was to reveal the effect of different removal techniques (i.e., water washing and wiping) on skin physiological functions [e.g., skin pH, hydration, and transepidermal water loss (TEWL)] and ceramide content in the SC.

2. Materials and methods

2.1. Ethical consideration

This study was approved by the Medical Ethics Committee of Kanazawa University (approval number: HS27-9-1) and was performed in accordance with the Declaration of Helsinki.

2.2. Cleaning agents

Three kinds of cleaning agents, A, B, and C, were chosen based on their market share and availability in Japan. Since the aim of this study was not product evaluation, the name and manufacturer of each agent are withheld. According to each manufacturer's instructions, all of the cleaning agents could be removed by both water washing and wiping. Each of the cleaning agents had different properties; agent A was a creamy foam (Fig. 1A), agent B was an airy foam (Fig. 1B), and agent C was a cream (Fig. 1C). The contents of each cleaning agent are shown in Table 1.

2.3. Participants and allocation

In this study, 15 healthy participants (age: 21–22 years) were recruited and provided written informed consent. The participants were randomly divided into three groups corresponding to the three cleaning agents (see Section 2.2) with five participants to each agent.

2.4. Experimental setup

The experimental setup of cleaning/removal was as follows (Fig. 2). First, the washing area was defined as a circle with a radius of 3.5 cm, centered 10 cm distal to the cubital fossa (red circle, Fig. 2A). The trained researcher applied the "protective cleaning method" [Fig. 2B, (13)] on both forearms of each participant for 30 s. The amount of each cleaning agent was as follows: 2 mL for agents A and B, and 0.6 g for agent C.

After the washing procedure, the cleaning agent on the right arm was removed by applying one liter of lukewarm water (40 ± 1°C) to the washed region in 20 s, followed by gentle tapping (not wiping) five times with a gauze (Cueb CARE Gauze; Koshiya Medical Care Corp., Ishikawa, Japan). The cleaning agent on the left arm was wiped off five times in a clockwise direction by means of a gauze. In both methods, the gauze was changed with a new one for every tap or wipe.

After removal of the cleaning agent, the washed regions were covered with gauze and a film dressing (AIRWALL Fuwari; Kyowa Ltd., Osaka, Japan) to avoid external disturbance. In addition, the participants were requested not to dip the washed regions into water (e.g., bathing, washing dishes, etc.). The overall cleaning/removal procedure was performed once a day for seven consecutive days by the trained researcher (Fig. 2C).

2.5. Measurement of TEWL, skin hydration, and skin pH

To test the differences of skin physiological changes between water washing and wiping, skin physiological functions and skin ceramide content were measured before and after the cleaning/removal experiment (Fig. 2C). The TEWL, skin hydration, and skin pH were measured using Vapometer (Delfin Technologies Ltd, Kuopio, Finland) (14), Corneometer® CM 825 (Courage+Khazaka electronic GmbH, Cologne, Germany) (15), and skin-pH-meter PH 900

(Courage+Khazaka electronic GmbH) (16), respectively, at a specific area on the skin (Fig. 2A). Skin physiological functions were measured by one trained researcher in an air-conditioned room (25 ± 2 °C, 50 ± 10% relative humidity). Each measurement was performed in triplicate and the average of three data was used for the analysis.

2.6. Semi-quantification of skin ceramide content

The skin ceramide content was measured as described previously (17) with some modifications. In brief, the cells of the SC (2nd to 5th layers) were collected by applying an adhesive tape (1.4 cm × 4.2 cm; #08380; A-ONE G. K., Tokyo, Japan) to the skin (Fig. 2A). The collected tape was divided into two parts, one for analysis of ceramide content and one for determination of total protein content as a surrogate index of total SC (18).

For the analysis of ceramide content, lipids, including ceramides, were extracted from the SC cells using 200 µL of methanol with sonication for 30 min (ASU-2; AS ONE Corp., Osaka, Japan). The extracted lipid solution was spotted on a polyvinylidene difluoride membrane (Immobilon-P®; Merck Millipore Corp., Hesse, Germany), followed by a dot-blot analysis using mouse IgM anti-ceramide antibody (ALX-804-196; 1:10 dilution; Enzo Life Sciences, Inc., NY, USA) and horseradish peroxidase (HRP)-conjugated anti-mouse IgM antibody (ab97230; 1:1000 dilution; Abcam plc., Cambridge, UK). The ceramide signals were developed by the Clarity Western ECL Substrate (Bio-Rad Laboratories, Inc., CA, USA) and captured by the C-DiGit blot scanner (LI-COR, Inc., NE, USA). The intensity of each ceramide spot was calculated with Image Studio™ Lite software (version 4.0; LI-COR, Inc).

The total SC amount for the normalization of the ceramide content was estimated by the total protein amount collected by the tape stripping (18). The collected tapes were individually soaked in 100 µL of 1 M NaOH with vigorous shaking for 30 min, followed by neutralization with 100 µL of 1M HCl. The total protein amount was then determined by a spectrophotometer (680 XR; Bio-Rad Laboratories, Inc.) at 595 nm with the Quick Start™

Bradford Protein Assay (#5000205JA; Bio-Rad Laboratories, Inc.). The normalized relative amount of skin ceramide was then calculated by simply dividing the raw value of the ceramide signal by the protein amount of the corresponding sample.

2.7. Statistics

Data are expressed as means and standard deviations (SD). Comparison of the baseline data between the right (i.e., water washing) and left (i.e., wiping) forearms was performed using Welch's *t*-test. Two-way analysis of variance of mixed design [time (pre- versus post-experiment) × removal technique (water washing versus wiping)] was used for interaction analysis. A *P*-value of <0.05 was considered statistically significant. All statistical analyses were performed with the SPSS Statistics software (version 23; International Business Machines Corp., NY, USA).

3. Results

The baseline (i.e., pre-experiment) data of the right (water washing) and left (wipe) forearms were not significantly different in all participant groups (Table 2), which enabled direct comparison between the right and left arms. Tables 3, 4, and 5 show the pre-/post-results of the skin physiological functions (TEWL, skin hydration, and skin pH) and normalized skin ceramide content with agents A, B, and C, respectively. There were no interactions between the removal technique and change in skin physiological functions for the cleaning agents used in this study. Figure 3 summarizes skin physiological functions and ceramide content. In the agent B group, a statistically significant increase in skin hydration ($P = 0.0059$) and decrease in skin pH ($P = 0.0015$) were observed between pre- and post-experiment, regardless of water washing or wiping. The ladder plots of the individual data are shown in Figure S1.

4. Discussion

In this study, we tested the effect of different removal techniques (i.e., water washing and wiping) of several cleaning agents on skin physiological function. In addition, we evaluated ceramide content in the SC, which affects skin barrier function. Because it is known that cleaning agent residues (containing several kinds of surfactant) can deteriorate skin physiological function, it is important to know how removal methods affect the skin.

Even if a single application of the irritant (i.e., cleaning agent ingredients) may cause skin reactions, such as erythema, barrier function disruption, or reduction of skin hydration, it is relatively easy for users to discontinue use because such irritations are usually severe enough to cause apparent drawbacks (5, 6, 19, 20). The problem is that mild but repetitive application of such agents may cause low but sustained irritation of the skin (5, 19, 21, 22). In this study, therefore, we evaluated changes in skin physiological function after repetitive use of cleaning agents. In addition, we focused on the "removable-by-wiping" cleaning agents because of their potential usefulness and cost-effectiveness for home-care services.

Because the residue of cleaning agents on the skin is reportedly greater in wiping compared to water washing (23), it is speculated that just wiping may not be a sufficient removal technique and that cleaning agent remnants may deteriorate skin physiological function in the wiping group compared to water washing. However, in this seven-day experiment with three kinds of cleaning agents, none produced adverse effects on skin physiological function or ceramide content, regardless of water washing or wiping (Tables 3–5). In the agent C group, one participant was excluded because of significant erythema. However, this was caused by the film dressing, which was used as a cover of the region of interest, and not due to the cleaning agent or the removal technique, as the covered areas of both forearms (i.e., wiping and water washing) were affected. In sum, removal of cleaning agents by just wiping has comparable effects on skin physiological functions and skin ceramide content to water washing.

Significant changes in skin hydration and pH were observed with cleaning agent B (Fig. 3). Although we do not have any solid reason for this, specific component(s) of agent B may have affected skin physiological functions, particularly skin hydration and skin pH. For example, triethanolamine (TEA)-cocoyl hydrolyzed collagen, which was only found in agent B, has been patented worldwide for its mildness (24); however, this cannot fully explain the observed effects on the skin.

There are two major limitations in this study. One is the small number of participants. As we wanted to test three kinds of cleaning agents, we had no choice but to limit the number of participants assigned to each agent. However, the small sample size makes it difficult to generalize the results of this study. The other limitation is the age of the participants. The study participants were all young (21 or 22 years); therefore, care must be taken with the elderly who are more susceptible to external insult.

5. Conclusion

In this study, we compared the effects of removal techniques (i.e., water washing and wiping) of cleaning agents on skin physiological functions (e.g., TEWL, skin hydration, and skin pH) and skin ceramide content. As a result, we did not find any differences between water washing and wiping on skin physiological functions and skin ceramide content.

Cleaning agents that claim to be removed by both water and just wiping produce no differences in skin physiological function or ceramide content depending on which removal method is used.

Acknowledgments

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Conflict of interest

The authors have declared that no competing interests exist.

References

1. Taylor CR, Lillis C, LeMone P, Lynn P. Fundamentals of Nursing: The Art and Science of Nursing Care. 7th ed. Philadelphia, USA: Lippincott Williams & Wilkins, 2010.

2. Bulechek GM, Butcher HK, Dochterman JM, M WC. Nursing Intervention Classification (NIC). 6th ed. Missouri, USA: Elsevier Mosby, 2013.

3. Sloot N, Siebert J, Hoffler U. Eradication of MRSA from carriers by means of whole-body washing with an antiseptic in combination with mupirocin nasal ointment. Zentralbl Hyg Umweltmed 1999;202:513-523.

4. Jury LA, Guerrero DM, Burant CJ, Cadnum JL, Donskey CJ. Effectiveness of routine patient bathing to decrease the burden of spores on the skin of patients with Clostridium difficile infection. Infect Control Hosp Epidemiol 2011;32:181-184.

5. Branco N, Lee I, Zhai H, Maibach HI. Long-term repetitive sodium lauryl sulfate-induced irritation of the skin: an in vivo study. Contact Dermatitis 2005;53:278-284.

6. Korting HC, Braun-Falco O. The effect of detergents on skin pH and its consequences. Clin Dermatol 1996;14:23-27.

7. Sloane PD, Hoeffer B, Mitchell CM, McKenzie DA, Barrick AL, Rader J, et al. Effect of person-centered showering and the towel bath on bathing-associated aggression, agitation, and discomfort in nursing home residents with dementia: a randomized, controlled trial. J Am Geriatr Soc 2004;52:1795-1804.

8. Gillis K, Tency I, Roelant E, Laureys S, Devriendt H, Lips D. Skin hydration in nursing home residents using disposable bed baths. Geriatr Nurs 2016;37:175-179.

9. Birch S, Coggins T. No-rinse, one-step bed bath: the effects on the occurrence of skin

tears in a long-term care setting. Ostomy Wound Manage 2003;49:64-67.

10. Schoonhoven L, van Gaal BG, Teerenstra S, Adang E, van der Vleuten C, van Achterberg T. Cost-consequence analysis of "washing without water" for nursing home residents: a cluster randomized trial. Int J Nurs Stud 2015;52:112-120.

11. Abe T, Higasi K, Kudo S. Frequency of washing and skin surface pH at the partial bath. Bull Fac Educ Hirosaki Univ 1999;81:75-83.

12. Coderch L, Lopez O, de la Maza A, Parra JL. Ceramides and skin function. Am J Clin Dermatol 2003;4:107-129.

13. Matsumoto M, Matsuo J, Dai M, Nishizawa T, Matsui K, Ichikawa Y, et al. Influence of differences in washing methods on skin texture. Int J Cosmet Sci 2014;36:175-181.

14. De Paepe K, Houben E, Adam R, Wiesemann F, Rogiers V. Validation of the VapoMeter, a closed unventilated chamber system to assess transepidermal water loss vs. the open chamber Tewameter. Skin Res Technol 2005;11:61-69.

15. Hashimoto-Kumasaka K, Takahashi K, Tagami H. Electrical measurement of the water content of the stratum corneum in vivo and in vitro under various conditions: comparison between skin surface hygrometer and corneometer in evaluation of the skin surface hydration state. Acta Derm Venereol 1993;73:335-339.

16. Ehlers C, Ivens UI, Møller ML, Senderovitz T, Serup J. Comparison of two pH meters used for skin surface pH measurement: the pH meter 'pH900' from Courage & Khazaka versus the pH meter '1140' from Mettler Toledo. Skin Res Technol 2001;7:84-89.

17. Mugita Y, Minematsu T, Huang L, Sugama J, Ichikawa Y, Nakagami G, et al. A convenient and highly sensitive method for quantification of ceramide in tape-stripping samples by dot-blot analysis as a skin barrier assessment technique in murine macerated skin. J Jpn Soc Wound Ostomy Continence Manage 2013;17:201-212.

18. Dreher F, Arens A, Hostynek JJ, Mudumba S, Ademola J, Maibach HI. Colorimetric method for quantifying human Stratum corneum removed by adhesive-tape stripping. Acta

Derm Venereol 1998;78:186-189.

19. De Jongh CM, Verberk MM, Withagen CE, Jacobs JJ, Rustemeyer T, Kezic S. Stratum corneum cytokines and skin irritation response to sodium lauryl sulfate. Contact Dermatitis 2006;54:325-333.

20. Lee CH, Maibach HI. The sodium lauryl sulfate model: an overview. Contact Dermatitis 1995;33:1-7.

21. Lee E, An S, Im MS, Kim HK, Lee TR. An improved method for measurement of change in skin roughness caused by cleansing products under mild application conditions. Skin Res Technol 2011;17:320-325.

22. Lodén M, Buraczewska I, Edlund F. The irritation potential and reservoir effect of mild soaps. Contact Dermatitis 2003;49:91-96.

23. Zhu H, Jung EC, Phuong C, Hui X, Maibach H. Effects of soap-water wash on human epidermal penetration. J Appl Toxicol 2016;36:997-1002.

24. Nnanna IA, Cheng GY, Xia J. Potential Applications of Protein-Based Surfactants. In: Nnanna IA, Xia J, eds. Protein-Based Surfactants: Synthesis: Physicochemical Properties, and Applications. New York: Marcel Dekker, Inc., 2001: 227-260.

Figure legends

Figure 1 Cleaning agents used in this study. (A), (B), and (C) correspond to the agents A, B, and C, respectively.

Figure 2 Experimental setup. (A) Regions of washing (red circle), skin pH measurement (blue circle), and skin TEWL, hydration, and ceramide content measurement (green circle) are shown. (B) “Protective cleaning method” used in this study. (C) Time course of this study.

Figure 3 Changes in skin physiological functions and skin ceramide content before and after seven-day cleaning by water washing and wiping. Black squares denote the water washing group, whereas red circles denote the wiping group.

Figure S1. Detailed ladder plots of Figure 3.

Table 1 Ingredients of each cleaning agent used in this study

Agent A	**Agent B**	**Agent C**
Aqua	Chlorhexidine digluconate	Aqua
Butylene glycol	Polysorbate-60	Ethylhexyl palmitate
Lauramine oxide	TEA-cocoyl hydrolyzed collagen	Butylene glycol
Polyglyceryl-10 laurate	Cetyl alcohol	Dipropylene glycol
Octyldodeceth-20	Alcohol denat.	Squalene
Decyl glucoside	Aqua	Macadamia oil
Dipotassium glycyrrhizate		Jojoba oil
Cetyl-PG hydroxyethyl palmitamide		Carbomer
Alcohol		Acrylates/C10-30 alkyl acrylate crosspolymer
		Ascorbyl tetraisopalmitate

In order of appearance.

Table 2 P-values of the Welch's t-test between right and left forearms at the baseline

	Agent A group	**Agent B group**	**Agent C group**
Participants, *n*	5	5	4†
TEWL	0.36	0.71	0.53
Skin hydration	0.78	0.70	0.88
Skin pH	0.74	0.48	0.58
Skin ceramide content	0.58	0.76	0.98

TEWL: Transepidermal water loss.
Refer to Tables 3, 4, and 5 for the actual data.
†Five participants were recruited, and one participant dropped out due to the reaction against film dressing.

Table 3 Changes of skin physiological functions and ceramide content before and after using agent A (n = 5)

	Removal technique	Pre	Post	*P*-value for interaction
TEWL, $g/m^2/h$	water washing	3.72 (0.26)	3.81 (0.58)	0.35
	wiping	3.73 (0.33)	3.39 (0.70)	
Skin hydration, a.u.	water washing	31.98 (12.52)	31.35 (5.65)	0.93
	wiping	30.64 (10.54)	30.55 (4.58)	
Skin pH	water washing	4.85 (0.31)	4.71 (0.36)	0.78
	wiping	4.95 (0.52)	4.73 (0.40)	
Skin ceramide content, $\times 10^6$ a.u.	water washing	1.83 (0.51)	3.49 (3.55)	0.17
	wiping	2.73 (1.32)	1.56 (1.26)	

Mean (SD).

Table 4 Changes of skin physiological functions and ceramide content before and after using agent B (n = 5)

	Removal technique	**Pre**	**Post**	***P*-value for interaction**
TEWL, $g/m^2/h$	water washing	4.02 (0.30)	3.85 (0.46)	0.96
	wiping	4.11 (0.63)	3.91 (0.54)	
Skin hydration, a.u.	water washing	26.87 (4.85)	35.05 (6.00)	0.64
	wiping	26.95 (4.43)	37.63 (7.56)	
Skin pH	water washing	5.22 (0.22)	4.69 (0.34)	0.41
	wiping	5.19 (0.35)	4.43 (0.34)	
Skin ceramide content, $\times 10^6$ a.u.	water washing	2.25 (1.88)	1.50 (0.92)	0.44
	wiping	3.02 (3.16)	1.29 (0.96)	

Mean (SD).

Table 5 Changes of skin physiological functions and ceramide content before and after using agent C (n = 4†*)*

	Removal technique	Pre	Post	*P*-value for interaction
TEWL, g/m^2/h	water washing	4.03 (0.75)	3.43 (0.81)	0.71
	wiping	3.68 (0.58)	3.33 (0.69)	
Skin hydration, a.u.	water washing	26.79 (2.63)	30.41 (6.42)	0.72
	wiping	25.60 (5.04)	30.79 (6.05)	
Skin pH	water washing	4.92 (0.22)	4.86 (0.13)	0.19
	wiping	5.18 (0.32)	4.75 (0.17)	
Skin ceramide content, ×10^6 a.u.	water washing	1.29 (0.77)	1.15 (0.32)	0.36
	wiping	0.97 (0.93)	1.45 (0.36)	

Mean (SD).

†Five participants were recruited, and one participant dropped out due to the reaction against film dressing.

Figure 1 Cleaning agents used in this study. (A), (B), and (C) correspond to the agents A, B, and C, respectively.

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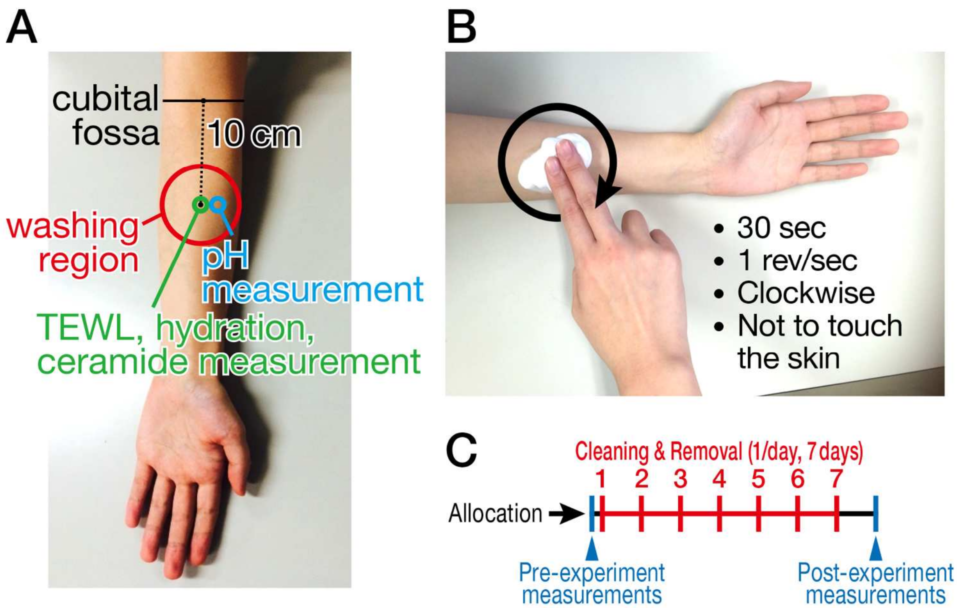

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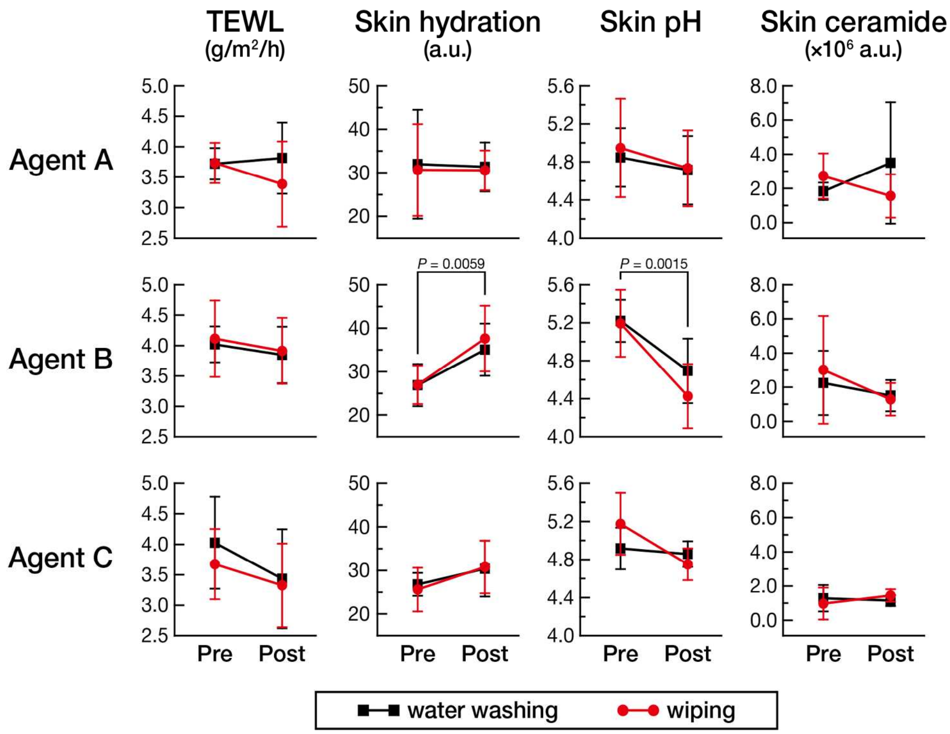

Figure 3 Changes in skin physiological functions and skin ceramide content before and after seven-day cleaning by water washing and wiping. Black squares denote the water washing group, whereas red circles denote the wiping group.

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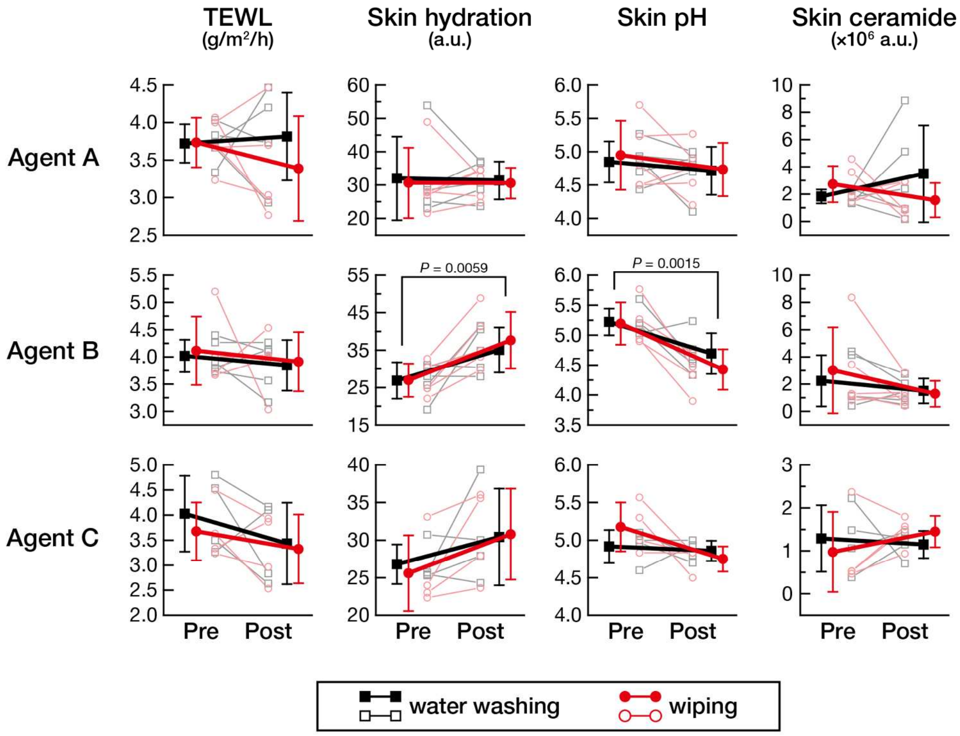

Figure S1. Detailed ladder plots of Figure 3.

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