

ORIGINAL ARTICLE

N95 respirators alter facial skin physiological functions and lipidome composition in health care personnel

Min Li^{1,2} | Feifei Wang^{3,4} | Meng Tao^{1,5}  | Yue Zhang¹ | Ruoxin Pan¹ | Duoduo Gu¹ | Hui Zhong⁴ | Yang Xu¹ 

¹Department of Dermatology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China

²Department of Dermatology, Fifth People's Hospital of Suzhou, Suzhou, China

³Yunnan Characteristic Plant Extraction Laboratory, Yunnan Yunke Characteristic Plant Extraction Laboratory Co., Ltd, Kunming, China

⁴State Key Laboratory of Natural Medicines, School of Traditional Chinese Pharmacy, China Pharmaceutical University, Nanjing, China

⁵Department of Dermatology, Zigong Fourth People's Hospital, Zigong, China

Correspondence

Yang Xu, Department of Dermatology, The First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou Road, Nanjing, 210029, China.

Email: yangxu@njmu.edu.cn

Hui Zhong, State Key Laboratory of Natural Medicines, School of Traditional Chinese Pharmacy, China Pharmaceutical University, 24 Tongjia Xiang, Nanjing, 211198, China. Email: cpuzhonghui@126.com

Abstract

Background: During the coronavirus disease 2019 pandemic, wearing medical respirators and masks was essential to prevent transmission.

Objective: To quantify the effects of N95 mask usage by measuring facial skin biophysical characteristics and changes in the lipidome.

Methods: Sixty healthy volunteers wore N95 respirators for 3 or 6 h. Facial images were acquired and physiological parameters were measured in specific facial areas, before and after mask-wearing. Lipidome analysis was also performed.

Results: After N95 respirator usage, facial erythema was observed in both the 3 and 6 h groups. Both sebum secretion and trans-epidermal water loss increased significantly in mask-covered cheeks and chins after 6 h of mask wearing compared with before mask wearing ($p < 0.05$). Principal component analysis revealed significant differences in lipid composition after mask wearing compared with before. The ceramide subclass NS exhibited a positive correlation with stratum corneum hydration, whereas the AP subclass was negatively correlated with trans-epidermal water loss in the 6 h group.

Conclusion: Prolonged wear of N95 respirators may impair facial skin function and alter lipidome composition.

KEYWORDS

face mask, healthcare personnel, lipidome composition, N95, skin function, skin physiological parameters

1 | INTRODUCTION

Healthcare personnel (HCP) are routinely exposed to respiratory infections. N95 respirators are widely used by HCP to prevent the

transmission of pathogens and filter small airborne particles. It is common for HCP to wear N95 respirators continuously for several hours a day, especially during respiratory disease epidemics and pandemics, such as that caused by coronavirus disease 2019.

Prolonged mask-wearing can cause skin discomfort, including pruritus, stinging, erythema, oily skin, skin sensitivity, and even mask-related acne.¹ Previous studies have demonstrated mask-induced skin changes in both HCP and the general population.^{2–5} However, changes in skin function and surface lipidome composition are yet to be determined.

The objective of this study was to evaluate changes in skin function by measuring physiological parameters and analyzing the composition

Abbreviations: Cer, ceramides; EI, erythema index; FA, fatty acyls; GL, glycerolipids; GP, glycerophospholipids; HCP, healthcare personnel; LC-MS/MS, liquid chromatography–mass spectrometry; MI, melanin index; OPLS-DA, orthogonal partial least squares discriminant analysis; PCA, principal component analysis; SC, stratum corneum; SCH, stratum corneum hydration; SL, saccharolipids; SP, sphingolipids; SS, sebum secretion; ST, sterol lipids; TEWL, trans-epidermal water loss.

Min Li and Feifei Wang contributed equally to this manuscript

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of the skin surface lipidome after N95 respirator usage by HCP at a university hospital.

2 | MATERIALS AND METHODS

2.1 | Study participants and environment

The study protocol was approved by the Institutional Research Committee of the First Affiliated Hospital of Nanjing Medical University. HCP of the First Affiliated Hospital of Nanjing Medical University aged 18–60 years without atopic dermatitis, a face mask allergy, or other facial skin conditions were recruited. All participants were instructed to wash their faces thoroughly before the assessment, and not to apply any cosmetic products. After an acclimatization period of 30 min, participants were divided into two groups and asked to wear a N95 respirator in an air-conditioned room under standardized conditions ($21 \pm 2^\circ\text{C}$, $50 \pm 5\%$ humidity) for either 3 or 6 h. All participants were fully informed of the details and objectives of the study and provided written informed consent.

2.2 | Biophysical evaluations

Facial images were captured using the VISIA Skin Analysis System (Canfield Scientific Inc, Parsippany, NJ, USA) before putting on the respirator and immediately after its removal. Biophysical measurements were performed at the positions shown in Figure 1: the intersection of the horizontal reference line at the nasal base and the perpendicular line passing through the outer canthus on the left cheek (A), the intersection between the horizontal line of the angle of the mouth and the vertical extension line of the center of the left pupil (B), and the highest point in the middle of the chin (C).

Skin stratum corneum (SC) hydration (SCH), trans-epidermal water loss (TEWL), sebum secretion (SS), pH, melanin index (MI), and erythema index (EI) were assessed by the Corneometer CM825, Tewameter TM300, Sebumeter SM815, Skin-pH-Meter PH905, and Mexameter MX18 (Courage+Khazaka Electronic GmbH, Cologne, Germany), respectively, in an air-conditioned room under standardized conditions ($21 \pm 2^\circ\text{C}$, $50 \pm 5\%$ humidity).

2.3 | Skin surface lipidome analysis

SC samples were collected from HCP at the intersection between the horizontal line of the angle of the mouth and the vertical extension line of the center of the right pupil (point D, Figure 1), using Corneofix adhesive tape (Courage+Khazaka Electronic GmbH) pressed onto the skin surface with slight pressure for 60 s. The tape patches were then added to 300 μL methanol and ultrasonic-assisted extraction was performed for 3 min in an ice-water bath. Following the addition of 1.2 mL methyl tert-butyl ether, the tape was removed. After vortex vibration for 30 s, the supernatant was added to 300 μL deionized water and ultrasonic-assisted extraction was performed for 5 min



FIGURE 1 Positions of biophysical measurements (A, B, and C) and skin lipid collection (D). The exact locations are as follows: (A) Intersection of the horizontal reference line at the nasal base and the perpendicular line passing through the outer canthus on the left cheek; (B) intersection between the horizontal line of the angle of mouth and the vertical extension line of the center of the left pupil; (C) the highest point in the middle of the chin; and (D) intersection between the horizontal line of the angle of mouth and the vertical extension line of the center of the right pupil.

in an ice-water bath. The samples were incubated at -40°C for 1 h and centrifuged at 3000 rpm for 15 min at 4°C (Eppendorf, Hamburg, Germany). The supernatants were collected and dried at 37°C in a vacuum concentrator. The dried samples were reconstituted in 100 μL methanol:dichloromethane (1:1 v/v). Following centrifugation at 12 000 rpm for 15 min at 4°C , the supernatant was transferred to a fresh glass vial for liquid chromatography–mass spectrometry (LC-MS/MS). The quality control sample was prepared by mixing 20 μL of the supernatant from each sample.

The samples were analyzed by LC-MS/MS using an ultra-high-performance liquid chromatography system (Agilent Technologies, Santa Clara, CA, USA) equipped with a Phenomenex Kinetex C18 column (2.1 mm $\times$ 100 mm $\times$ 1.7 μm). The mobile phase A consisted of 40% water, 60% acetonitrile, and 10 mmol/L ammonium formate. The mobile phase B consisted of 10% acetonitrile and 90% isopropanol, which was added with 50 mL 10 mmol/L ammonium formate for every 1000 mL mixed solvent. The elution gradient was as follows: 0–1.0 min, 40% B; 1.0–12.0 min, 40%–100% B; 12.0–13.5 min, 100% B; 13.5–13.7 min, 100%–40% B; 13.7–18.0 min, 40% B. The column temperature was 55°C .

2.4 | Multivariate analysis

LC-MS/MS raw data were converted into ABF format files using Analysis Base File Converter software. The data were imported into

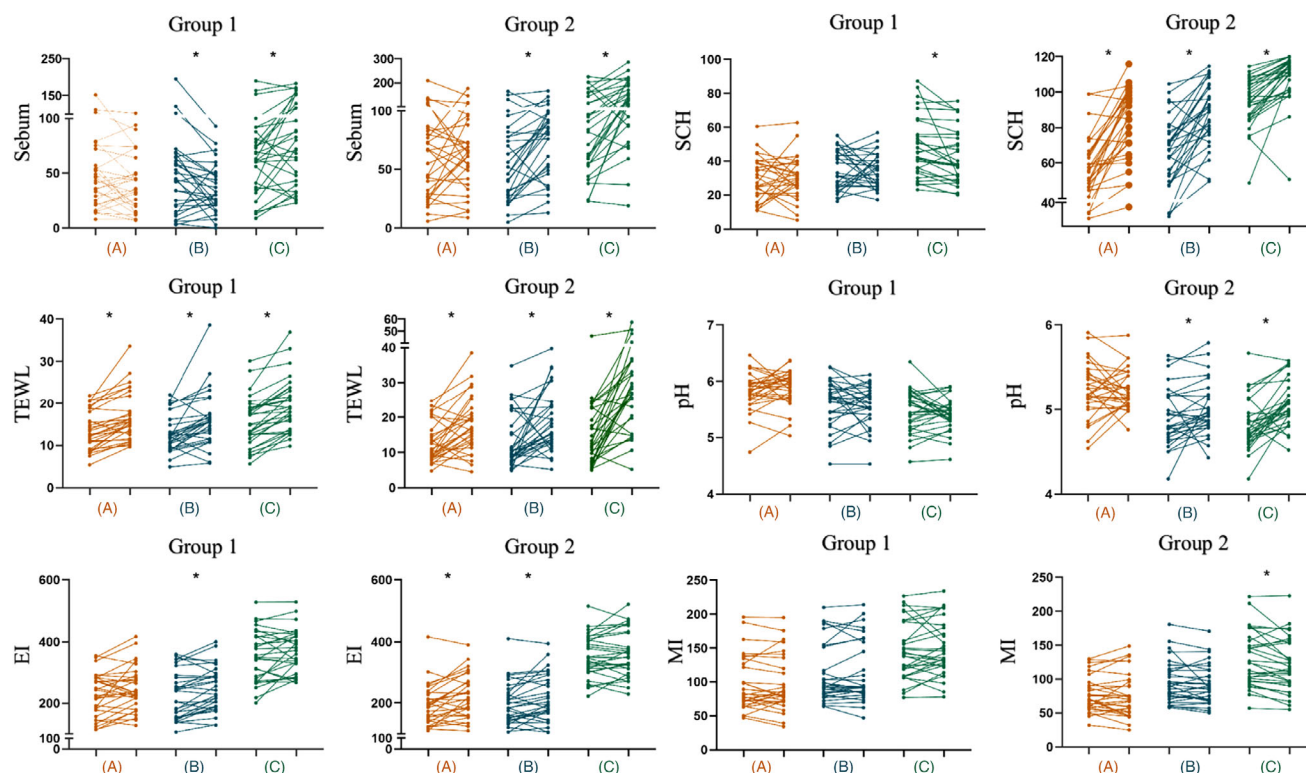


FIGURE 2 Changes in the skin physiological parameters sebum, SCH, TEWL, pH, EI, and MI in Groups 1 and 2 before and after mask wearing at facial positions A, B, and C. * $p < 0.05$. EI, erythema index; MI, melanin index; SCH, stratum corneum hydration; TEWL, trans-epidermal water loss.

Mass Spectrometry-Data Independent Analysis software for peak extraction, denoising deconvolution, and peak alignment. The obtained metabolites were annotated using the LIPID Map database. Multivariate analyses were performed using R software, including principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA).

2.5 | Statistical analysis

Statistical significance was assessed by paired Student's *t*-tests, and correlations were examined by Spearman's correlation coefficient analysis using GraphPad Prism software version 8.3.0 (GraphPad Software, La Jolla, CA, USA). Differences were considered significant when the *p*-value was < 0.05 .

3 | RESULTS

3.1 | Clinical manifestations and biophysical parameters

Sixty healthy volunteers (49 women and 11 men) participated in this study and were divided into two groups, with 30 participants in each group. Group 1 wore an N95 respirator continuously for 3 h, and Group 2 wore an N95 respirator continuously for 6 h. Group 1 had an average age of 25.43 ± 1.382 years, and comprised 25 women and five men, and

Group 2 had an average age of 25.33 ± 1.295 years, and comprised 24 women and six men ($p > 0.05$) (Table S1).

Erythema was observed on the cheeks and chin of HCP in both groups after mask-wearing but was more marked in Group 2 than in Group 1, as shown under white light and in red area images (representative images are presented in Figure S1).

Skin sample analysis revealed that SS at point C was significantly increased in both groups after mask-wearing compared with before, whereas SS at point B decreased in Group 1 but increased in Group 2. No significant changes were observed in the non-mask-covered area (point A). SCH was significantly increased in both mask-covered and non-mask-covered areas (points A, B, and C) in Group 2, whereas in Group 1, SCH was decreased only on the chin (point C). TEWL increased in both mask-covered and non-mask-covered areas in both groups. Skin pH increased in mask-covered areas (points B and C) in Group 2, while no significant changes were observed in Group 1. EI increased in the mask-covered cheek in Groups 1 and 2, whereas no changes in MI were observed (Figure 2).

3.2 | Skin surface lipidome

3.2.1 | Sebum composition

A total of 4443 skin surface lipids were detected and classified into eight categories annotated using the LIPIDMap database: glycerolipids

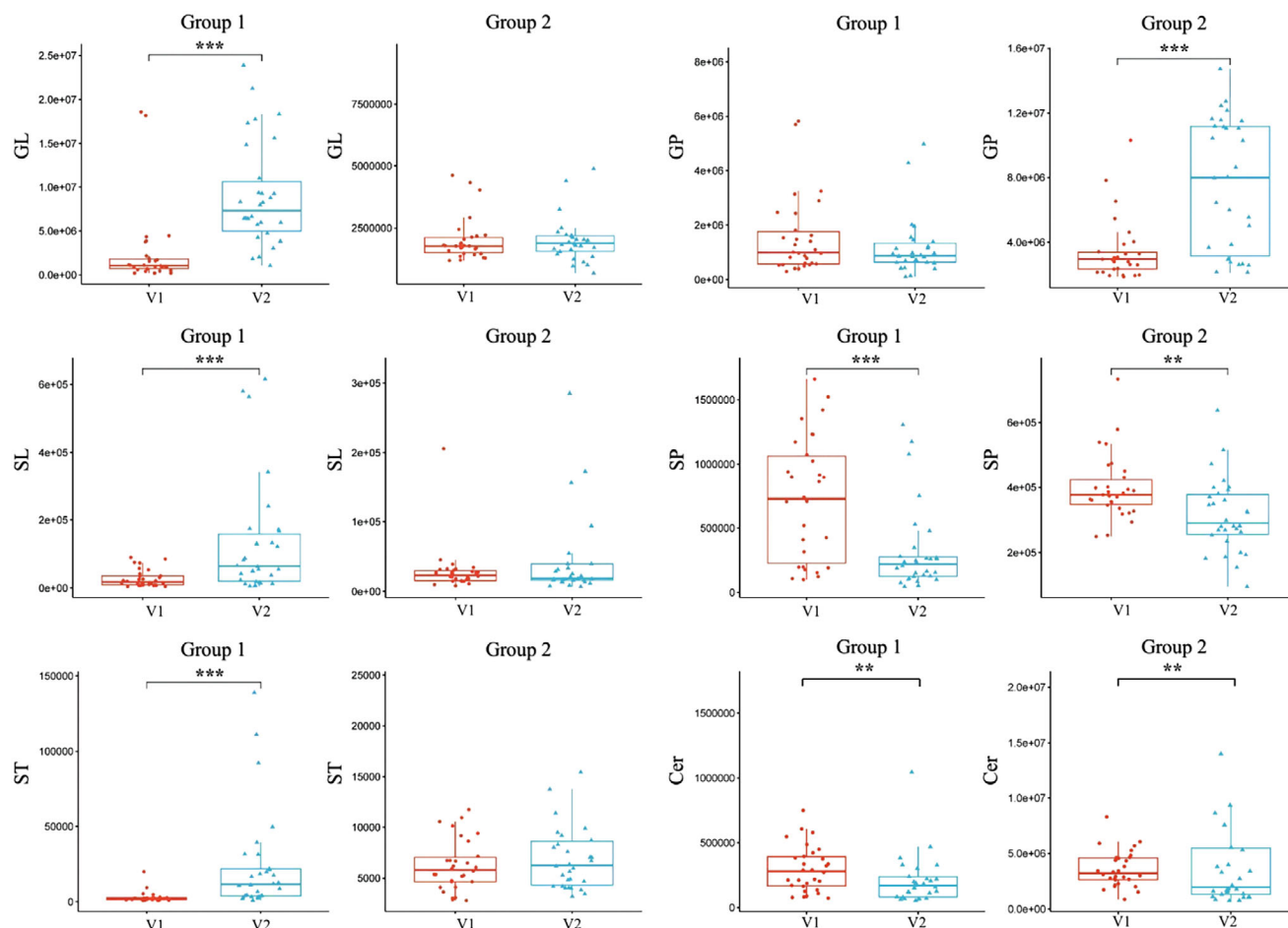


FIGURE 3 The relative abundance changes in lipid composition in Groups 1 and 2. The relative abundance changes in lipid composition in Groups 1 and 2 before (V1) and after (V2) mask wearing. ** $p < 0.01$, *** $p < 0.001$. Cer, ceramides; FA, fatty acyls; GL, glycerolipids; GP, glycerophospholipids; SL, saccharolipids; SP, sphingolipids; ST, sterol lipids.

(GL), glycerophospholipids (GP), sphingolipids (SP), sterol lipids (ST), saccharolipids (SL), fatty acyls (FA), and ceramides (Cer).

PCA revealed a significant difference in skin lipid composition after both 3 and 6 h of mask-wearing (Figure S2). Multivariate analysis of the identified lipids using OPLS-DA revealed a clear separation before and after wearing masks for 3 and 6 h (Figure S3). In Group 1, there was a significant increase in the proportion of GL and a reduction in the proportions of GP, ST, and FA after mask-wearing, compared with before. In Group 2, the proportions of GP and GL increased, whereas the proportions of FA and Cer decreased significantly after mask-wearing, compared with before (Figure S4). The abundance of lipid subclasses in Groups 1 and 2 before and after mask wearing are shown in Figure 3. It is noteworthy that the levels of SP and Cer were significantly decreased in both Groups 1 and 2 ($p < 0.01$).

3.3 | Changes in Cer and correlation with skin function

The four main Cer subclasses EOS (containing ester-linked fatty acids, ω -hydroxy fatty acids and sphingosines), AP (containing saturated

carbon chains and amide-linked α -hydroxy acids), NS (containing non-hydroxy fatty acids and sphingosines), and NP (containing non-hydroxy fatty acids and phytosphingosines), were identified and the relative abundances before and after mask wearing are shown in Figure 4. EOS, AP, and NS relative abundances showed no significant differences in the 3 h group but decreased significantly after mask wearing in the 6 h group, compared with before mask wearing. No significant differences were found in the relative abundance of NP in either group. The change in NS exhibited a positive correlation with SCH, whereas the change in AP was negatively correlated with TEWL in the 6 h group (Figure 5).

4 | DISCUSSION

The skin is the largest organ in the human body, and serves as a critical protective mechanical, chemical, immune, and biological barrier against the environment.⁶ The SC, the outermost layer of the epidermis, forms the main barrier and is composed of non-living corneocytes and a mixture of lipids organized in bilayers.⁷ Epidermal sebum is composed of sebum secreted by the sebaceous glands and epidermal lipids produced by disintegrating SC cells.⁸ The lipid components of human

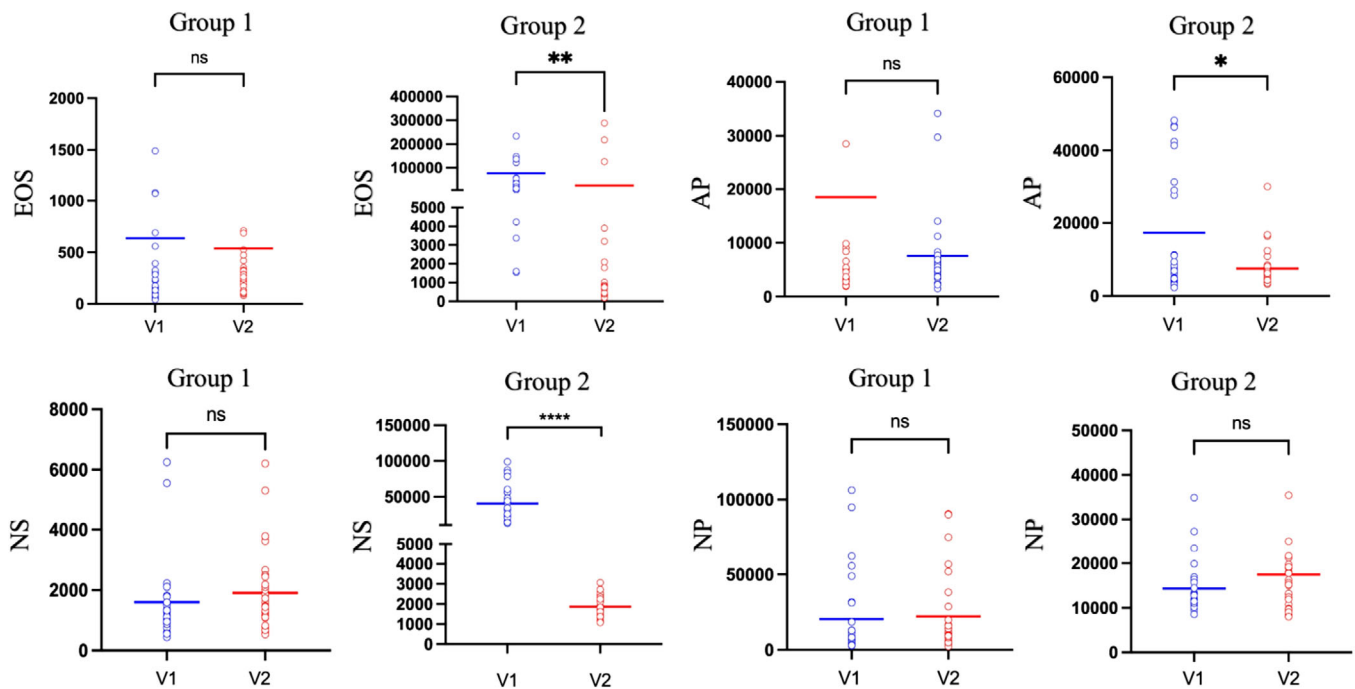


FIGURE 4 The relative abundance changes in ceramide subclasses in Groups 1 and 2. The relative abundance changes in ceramide subclasses in Groups 1 and 2 before (V1) and after (V2) mask wearing. ** $p < 0.01$.

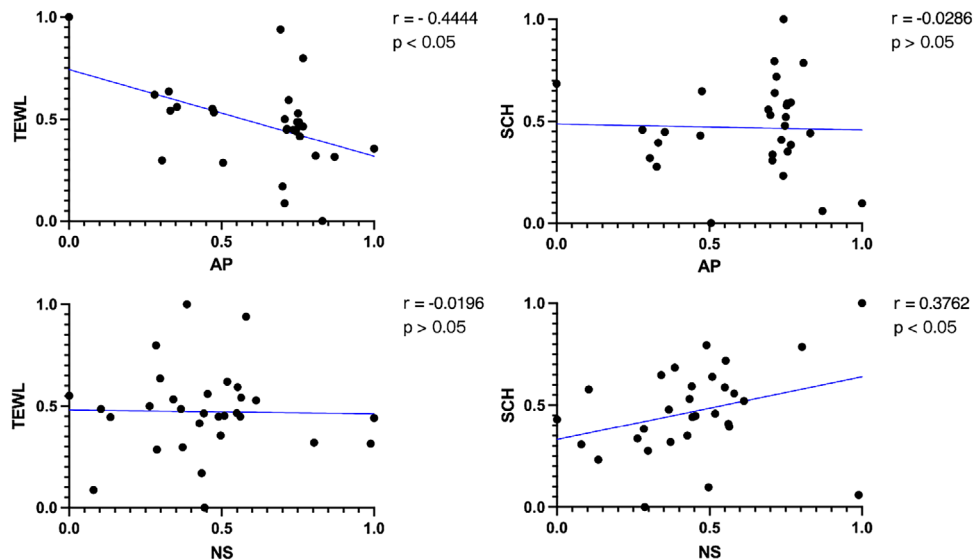


FIGURE 5 Correlation analysis of the ceramide subclasses with skin barrier function parameters. Correlation analysis of the ceramide subclasses NS and AP with skin barrier function parameters SCH and TEWL. * $p < 0.05$. SCH, stratum corneum hydration; TEWL, trans-epidermal water loss.

skin include triglycerides, fatty acids, squalene, cholesterol, wax esters, and cholesterol esters, of which squalene and wax esters are skin-specific lipids secreted by sebocytes.⁹ These lipid membranes hydrate the skin, reduce water loss, prevent the invasion of harmful substances, and maintain the stability of the internal environment. Skin barrier function can be evaluated by measuring physiological parameters, including SCH, TEWL, SS, pH, MI, and EI.¹⁰

HCP commonly wear respirators continuously for several hours, and as a result mask-related skin conditions have been reported.^{11–14} Although some studies have analyzed changes in the physiological functions of the skin, the changes in the skin lipidome and the correlation between physiological parameters and lipids remain unknown.

Effective water retention in the SC is essential for the barrier function of the skin,¹⁵ and is usually evaluated using TEWL and SCH. In this

study, TEWL increased significantly after mask wearing for 3 and 6 h compared with before, indicating impaired skin barrier function. SCH decreased in the first 3 h, indicating impaired skin barrier function, but increased after 6 h; this may have been caused by increased humidity and temperature.

Healthy skin is mildly acidic, with a pH ranging from 5.4 to 5.9.¹⁶ An acidic environment is conducive to maintaining the activity of enzymes, especially those involved in keratinization.¹⁷ Moreover, a mildly acidic environment is more able to self-regulate pH.¹⁶ Our study showed that the epidermal pH significantly increased after 6 h of mask wearing, which may impair the chemical barrier function of the skin.

In the red area images captured by VISIA, erythema was more obvious after mask wearing than before. This may be due to the increased local temperature, vascular dilation, and increased blood flow. This is consistent with the significant increase in EI values observed after both 3 and 6 h of mask wearing, compared with before mask wearing. An increased EI is generally considered a normal neurologically mediated response to heat or pressure.¹⁸ However, erythema tends to persist after prolonged exposure.¹⁹ There was no significant change in the EI values of the mandibular measurement points, possibly because of the relatively lower vascularity. No significant changes were observed in MI values, indicating no obvious changes in melanin synthesis during the mask-wearing period.

After wearing masks continuously for several hours, many people experience sweaty and oily facial skin. In a study by Dash et al., 61 out of 179 HCP (34.3%) complained of oily skin.²⁰ However, our study revealed different changes in SS in the cheek and mandible areas after mask wearing for 3 h. These differences may be related to differences in the density of the facial sebaceous glands.²¹ The decrease in SS in the cheek after 3 h, together with an increase after 6 h, indicated that there was not a simple linear change in SS as a result of mask wearing. Other factors such as epidermal dysbacteriosis may contribute to these changes.²² Further research is therefore required in this area.

Lipidomics, an important branch of metabolomics, aims to characterize the lipid composition, expression changes, and functional predictions using high-throughput analysis.²³ Previous studies have revealed changes in the composition and metabolism of skin surface sebum in many skin conditions, including acne vulgaris, atopic dermatitis, and psoriasis.²⁴ In our study, both ST and Cer showed clear decreasing trends after mask wearing.

Cer are a class of sphingolipids that form the backbone structure for all sphingolipids, such as glycosphingolipids and phosphosphingolipids, accounting for approximately 50% of total SC lipid mass.²⁵ Although they are minor constituents of cellular membranes, they are the major lipid components (along with cholesterol, free fatty acids, and other minor components) of the intercellular spaces of the SC that form the epidermal permeability barrier. In addition to functioning as a barrier, Cer are involved in signal transduction and cutaneous immunity.

Skin conditions associated with impaired permeability barrier function, such as atopic dermatitis, psoriasis, and xerosis, are characterized by alterations in Cer molecular profiles. Compared with healthy controls, the barrier-compromised skin of patients with atopic dermatitis has low levels of Cer, especially the EOS subclass.²⁶ In our study, the

Cer content showed a significant decline after mask wearing for 3 and 6 h, indicating a change in lipid composition.

At the subclass level, the relative abundance of EOS, AP, and NS declined significantly after mask wearing for 6 h. EOS molecules have an extremely long fatty acid chain, stretching from one lipid membrane layer and penetrating an adjacent layer, which holds neighboring bilayers together and reduces TEWL.²⁷ In this study, we found that AP content was negatively correlated with TEWL and NS content was positively correlated with SCH, indicating that decreased AP and NS content correlated with impaired skin barrier function. A previous study revealed that individuals with low levels of EOS and AP are more susceptible to irritant contact dermatitis, indicating a correlation between these Cer subclasses and the skin barrier.⁷ The imbalanced content of AP and NS in the skin surface may provide new insights for future research on the prevention of discomfort caused by the long-term mask usage.

5 | CONCLUSION

This study investigated the changes in skin physiological parameters and lipid composition after wearing N95 respirators, providing a theoretical basis for preventing and treating skin conditions caused by prolonged mask wearing. The decline in the Cer content is worthy of particular attention, and could potentially be addressed through the use of skincare products containing Cer.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data included in this study are available upon request by contact with the corresponding author.

ETHICS STATEMENT

All patients provided written informed consent, and the study was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (2022-SR-192).

ORCID

Meng Tao  <https://orcid.org/0000-0001-5155-1871>

Yang Xu  <https://orcid.org/0000-0001-7172-5277>

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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