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Skin Physiology, Mucosal Functions, and Symptoms Are Modulated by Grass Pollen and Ozone Double Exposure in Allergic Patients

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Keywords
Epidermal barrier function · Mucosal functions · Grass pollen · Ozone · Skin inflammation · GA^{2}LEN pollen chamber

Abstract
Introduction: Along with climate changes, we see an increase in allergic symptoms and the number of pollen-allergic patients in many countries. Increased allergic symptoms are associated with an elevated ozone exposure which may be linked by impaired epithelial barrier function. This study aimed to quantify the clinical effect of ozone and pollen double exposure (DE). We tested whether ozone impairs barrier-related skin physiology and mucosal functions under DE with pollen in grass pollen-allergic patients versus healthy controls. ***Methods:*** This case-control study included 8 grass pollen-allergic patients and 8 non-allergic healthy subjects exposed to grass pollen and ozone in the GA^{2}LEN pollen chamber, comparing shorter and longer DE duration. Non-invasive skin physiological parameters were assessed, including stratum corneum hydration, skin redness, surface pH, and basal transepidermal water loss as a parameter for epidermal barrier function. The subjects' general well-being, bronchial, nasal, and ocular symptoms were documented. ***Results:*** Skin physiology tests revealed that DE in allergic patients deteriorates the epidermal barrier function and increases the surface pH and skin redness. DE significantly induced nasal secretion in pollen-allergic versus healthy subjects, which was more pronounced with longer DE. The general well-being was significantly impaired under DE versus pollen or ozone alone, with a negative influence of DE duration. No relevant bronchial symptoms were recorded. ***Conclusion:*** Skin physiology and nasal mucosal symptoms are negatively affected by ozone and grass pollen DE in allergic patients. The negative effects showed, in some parameters, a dose (time)-response relationship. The pH can be regarded as a possible modulatory mechanism.

Introduction

Allergies are among the most common diseases with an increasing prevalence in most industrialized parts of the world. The term "allergy" is very broad and can

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manifest in numerous organs, such as the respiratory tract, skin, and the gastrointestinal tract. Allergic reactions are seen as innate or acquired alterations in the responsiveness of the immune system to exogenous, normally innocuous substances which are recognized as allergens. Atopic diathesis is an inherited predisposition for allergic reactions manifested in epithelial tissues including allergic rhinoconjunctivitis (e.g., sensitizations to pollen), atopic dermatitis, and allergic bronchial asthma.

An increase in pollen allergies and allergic symptoms severity has been reported alongside the dramatic changes in climatic conditions and ozone (O_3) pollution, suggesting a link between allergies and a negative influence on epithelial functions [1]. The spread and increased severity of allergic rhinitis under changing environmental conditions has been reported [2].

The occurrence of allergic symptoms in sensitized patients correlates with different environmental factors such as temperature, the number of days with a temperature greater than 30°C, and sun-emitted radiation [3]. Humidity and rain can cause pollen to release high allergenic fragments into the air [4]. Since various environmental factors have a major impact on the allergenicity of pollen, the effects of climate changes are expected to increase allergic and asthmatic symptoms due to changes in pollen biology and an increased responsiveness of the affected patients. Also, a changing climate leads to variations in the timing and duration of pollen seasons [5]. Interactive effects with plants have already been demonstrated from the two widely emitted greenhouse gases, CO_2 and O_3 [6]. During an experimental study in which plants were grown under elevated concentrations of CO_2 and O_3, it could be shown that the altered air composition increased the concentration of grass pollen, estimating an increase by up to 200% of grass pollen concentration in the near future for a real-world scenario [6]. Given the widespread distribution of grass species, these results provide a perspective on significant health effects of climate change on patients with grass pollen allergies [6].

O_3 can be distinguished between stratospheric O_3, which is located 10–15 km above the ground and is referred to as the "O_3 layer" that provides protection against ultraviolet radiation, and tropospheric O_3, which is ground-level O_3 and can be harmful to humans, animals, and plants [7, 8]. There is a natural influx of stratospheric O_3 into the troposphere; however, the predominant source of tropospheric O_3 is the photochemical reaction from volatile organic compounds and nitrogen oxides [8]. It has been shown that O_3 molecules increase allergenic proteins in pollen, thus aggravating allergic symptoms [9, 10]. Therefore, the question arises to what extent the change in pollen proteins due to O_3 is reflected in clinical symptoms of patients with allergic rhinoconjunctivitis when exposed to specific pollen?

A validated method to study clinical symptoms upon pollen exposure is the mobile GA^{2}LEN allergen exposure chamber, which allows standardized allergen exposure assessment by delivering defined amounts of allergens in a controlled setting. We previously validated the reproducibility, sensitivity, and specificity of the clinical response to pollen exposure in allergen exposure chamber, and the reproducibility between subsequent runs in patients with grass pollen allergies could be demonstrated [11]. Furthermore, sensitivity for symptoms in pollen-allergic patients (exposure vs. placebo $p < 0.00001$) and specificity (very low clinical scores in non-allergic subjects) were confirmed [11]. The study could also rule out a priming effect between days 1 and 5 of repeated exposure tests [11]. Importantly, a dose dependency of allergic symptoms induced by grass pollen was demonstrated, indicating that induction of relevant nasal symptoms, corresponding to a change of at least three score points on the total nasal symptom score (TNSS), takes 20–30 min of pollen exposure [11]. Boelke et al. [12] evaluated the peak nasal inspiratory flow (PNIF) in the exposure chamber in a randomized, controlled, blinded study with subjects suffering from allergic rhinitis to grass pollen, birch pollen, house dust mite compared to placebo. They could show that PNIF is a valid instrument to assess nasal symptoms in provocation trials with allergens, especially grass pollen and house dust mite [12]. The GA^{2}LEN allergen exposure chamber has also been used in studies testing anti-allergy devices, such as air purifiers and facemasks, for their efficacy in pollen reduction and prevention of allergic symptoms [13, 14].

The aim of this study was to test the effect of a double exposure (DE) to O_3 and grass pollen in patients with sensitization to grass pollen and symptoms of allergic rhinoconjunctivitis, in the exposure chamber. In this study, all three barrier organ systems (nasal, skin, and lungs) were assessed with established and validated diagnostic tests to quantify the effects of allergen exposure under different environmental conditions. Thus, we tested the following hypothesis: (i) DE in a controlled exposure chamber to grass pollen and O_3 induces increased mucosal allergic symptoms in a time-dependent manner, and (ii) DE to grass pollen and O_3 induces a systemic reaction.

Materials and Methods

Study Population

Sixteen subjects, aged 21–58 years, were recruited at Charité Allergy Center over a period of 3 months: 8 subjects with allergic rhinoconjunctivitis against grass pollen and 8 non-allergic subjects (inclusion and exclusion criteria: online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000530115). The mandatory inclusion criteria for the allergic patients were clinically relevant grass pollen sensitization with rhinoconjunctivitis symptoms for at least 2 years and a positive prick test for grass pollen (grass mix, 30 HEP/mL; LETI Pharma GmbH, Ismaning, Germany), with a wheal diameter of ≥3 mm. The major exclusion criteria were specific immune therapy against grass pollen within the last 5 years, severe and/or uncontrolled asthma within 3 months before screening, and FEV1 <80% before exposure and acute infections. All patients were asked and examined regarding atopic dermatitis. None of them showed skin alterations or cutaneous signs of active atopic dermatitis. The study protocol (EA1/193/14) was approved by the Ethics Committee of Charité - Universitätsmedizin Berlin. Written informed consent was obtained from each participant prior to the inclusion and participation in the study. The study was conducted in accordance with the World Medical Association Declaration of Helsinki.

Study Design

16 subjects participated in our monocentric, single-blinded, case-control study. Two 120-min-long appointments, 48 h apart, were performed in the GA^{2}LEN allergen exposure chamber. In the first visit, the subjects were exposed to grass pollen (*Phleum pratense*), 4,000 pollen/m^3 (Allergon HB, Angelholm Sweden), for a total of 120 min; during the last 60 min, 100 µg/m^3 O_3 was added (labeling: P-O SHORT). In the second visit, the subjects were exposed for initial 20 min of 100 µg/m^3 O_3 followed by 100 min of co-exposure of 100 µg/m^3 O_3 and 4,000 pollen/m^3 grass pollen (labeling: O-P LONG).

Clinical Scores

To monitor the severity of clinical symptoms, each symptom was assessed with the following: 0 = no symptom; 1 = mild symptoms; 2 = moderate, bothersome, but tolerable symptoms; 3 = severe, bothersome symptoms that interfere with daily activity/sleep. The TNSS assesses the sum score of nasal symptoms of allergic rhinitis, including (a) rhinorrhea, (b) obstruction, (c) sneezing, and (d) pruritus (maximum 12 points) [15]. To further quantify the nasal symptoms, the used handkerchiefs were weighed to measure the amount of nasal secretions during the exposure. The ocular symptoms considered in the total eye symptom score (TESS) included (a) redness, (b) pruritus, (c) foreign body sensation, and (d) tearing in the eye (maximum 12 points) [16]. In the total bronchial symptom score (TBSS), the symptoms considered were (a) wheezing, (b) cough, (c) dyspnea, and (d) asthma (maximum 12 points). The total symptom score represents the sum of the TNSS, TESS, and TBSS (maximum 36 points). In addition, the general well-being (GWB) of the subjects was assessed on a visual analogue scale (0–10 scale points) every 10 min. A value of 0 corresponded to "very good" well-being without impairment by symptoms and a value of 10 to "very poor" well-being with severe impairment of GWB by allergic symptoms. All scores were assessed at T0 and every 10 min (except TBSS; every 30 min).

Lung Function Tests

Before and after each 120 min exposure, FEV1 was measured using EasyOne (NDD Medizintechnik AG, Zurich, Switzerland). The test was performed to ensure that the exposure did not induce potentially harmful asthma episodes.

PNIF Measurement

PNIF was measured with In-check™ Portable Inspiratory Flow Meter (Clement Clarke International, Harlow, UK). Patients were asked to inhale as hard and fast as possible through a mask while keeping their mouth closed [12].

The Skin Physiology Tests

Each subject acclimatized for 15 min prior to the measurement with an exposed forearm, the room temperature (19°–21°C) and humidity (40%). Non-invasive skin physiology measurements were performed at T0 and T120 min (according to the published guidelines [17–19]) with instruments from Courage + Khazaka (Courage + Khazaka electronic GmbH, Cologne, Germany). Epidermal barrier function was assessed in terms of trans-epidermal water loss (TEWL), recorded in g/m^2/h using the Tewameter TM300. To assess cutaneous pigmentation and skin erythema, the Mexameter MX16 was used, recording values in AU. Skin surface pH values were measured using a planar glass probe connected to a voltmeter using the Skin pH Meter PH 905 in pH units. Stratum corneum hydration was assessed with the capacitance-based Corneometer CM 825, expressed in AU.

Statistics

Statistical tests of the collected study data were performed using GraphPad PRISM 6. The applied statistical tests were *T* test with normal distribution, Mann-Whitney U test, and the Wilcoxon test. Normal distribution was assessed with D'Agostino and Pearson omnibus normality test. Repeated measurements over time were combined as an area under the curve to minimize multiple testing. Statistical significance was set at $p < 0.05$.

Due to the exploratory character of the present study, a formal power calculation was not performed. The sample size was based on previous studies with the pollen chamber [11].

Results

Participant Characterization

Eight healthy and eight grass pollen allergy patients with a gender distribution ratio of 1:1 and average age of 27.9 years (range 21–58 years) participated in the study. Prick testing was conducted in all participants for grass pollen. All allergic patients were positive for grass pollen with a mean wheal diameter of 10.7 mm, histamine wheals 4.3 mm in allergic and 4.7 mm in non-allergic volunteers. The healthy control group showed no wheal development against grass pollen. To confirm the prick testing, a blood serum test for total IgE and the specific IgE for grass pollen (grass mix) was carried out (normal value of total IgE <100 kU/L). All non-allergic subjects, except for one, revealed normal total IgE (mean [without the outlier] 44.1 kU/L),

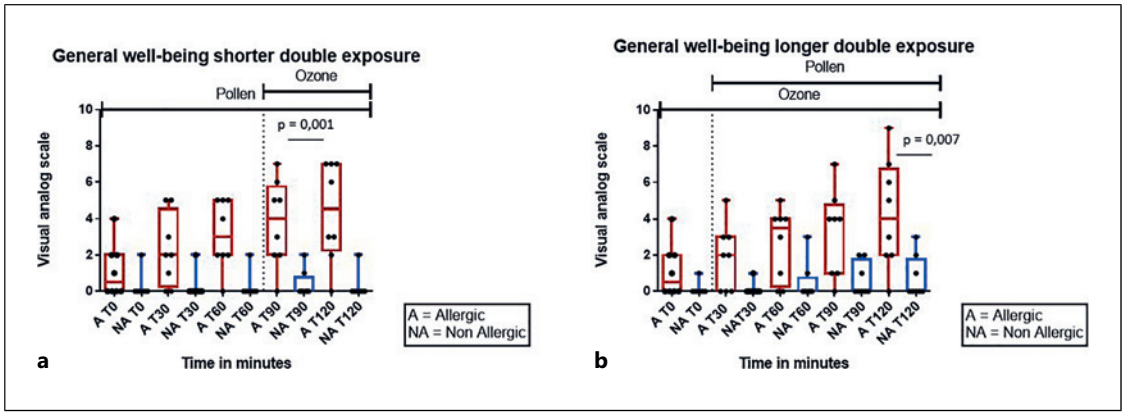

Fig. 1. A general well-being (GWB) self-assessment of allergic (red) and non-allergic (blue) patients: based on the VAS values at 30-min intervals for a total of 120 min during a DE to ozone and pollen. **a** 90-min pollen single exposure followed by 30-min DE induced a significant increase in the GWB score in the allergic group compared to non-allergic volunteers ($p = 0.001$ at the end of DE). **b** 30-min single exposure to ozone followed by 90-min DE induced a significant increase in the GWB score in the allergic group compared to non-allergic volunteers ($p = 0.007$ at the end of DE).

while all allergy sufferers had total IgE levels above normal values (mean 409.6 kU/L). One non-allergic outlier in the total serum IgE was a subject who had recently been to Southeast Asia; therefore, the increased serum IgE may have been induced by a parasitic infection. The prick test and the allergy history did not reveal any evidence of clinically relevant allergies in this patient. The specific IgE against grass pollen (grass mix) is significantly higher in the allergic patients (mean 26.6 kU/L) than in non-allergic subjects (mean 0.26 kU/L) ($p < 0.0001$).

GWB Is Impaired by Double Exposure

The GWB over the 120 min exposure period (Fig. 1) was unchanged in the healthy group, while the allergic patients experienced an increase in visual analogue scale values (corresponding to an impaired GWB) with significantly higher values at the endpoint after 120 min in the allergic group ($p = 0.001$ after P-O SHORT Fig. 1a; $p = 0.007$ after O-P LONG Fig. 1b). However, it is noticeable that also in the healthy cohort the values of well-being increased slightly during the DE time, which was seen as non-specific discomfort during the exposure.

Ocular Symptoms

TESS analysis showed steadily increase, especially during the DE (online suppl. Fig. S1A, B). Comparing the values after 60-min pollen versus 60-min DE showed slightly higher values for the O-P LONG without reaching the significant level (data not shown).

Nasal Symptoms

According to the TNSS, the allergic cohort had significantly higher values compared to the healthy cohort (Figures 2, 3 and 4a–c), both P-O SHORT ($p = 0.0005$) and O-P LONG ($p = 0.0005$) DE, without a significant difference between the shorter and longer exposure (Fig. 2d). A relevant increase of nasal symptoms started after 30 min of pollen exposure (Fig. 2a), while the increase in the initially O_3 exposed group started already after 20 min of the additional pollen exposure (Fig. 2b). The allergy sufferers produced significantly more nasal secretion than the healthy cohort in both exposure scenarios (Fig. 2e), without a significant difference (Fig. 2f). PNIF showed no relevant decrease in the allergy group over time compared to non-allergic participants (online suppl. Fig. S2).

Bronchial Symptoms

TBSS showed a significant increase in the area under the curve of P-O SHORT group ($p = 0.006$) which could not be detected in O-P LONG group (online suppl. Fig. S3) without a significant difference (online suppl. Fig. S4B). Overall, the TBSS showed relatively low values. The FEV1 test results revealed a slight decrease only in the shorter DE in the allergic group ($p = 0.03$) (online suppl. Fig. S4C, D). No asthma symptoms were reported.

Skin Physiology

The erythema index did not change in P-O SHORT (data not shown), while O-P LONG leads to a significant increase in the allergic group ($p < 0.004$) (Fig. 3a). When

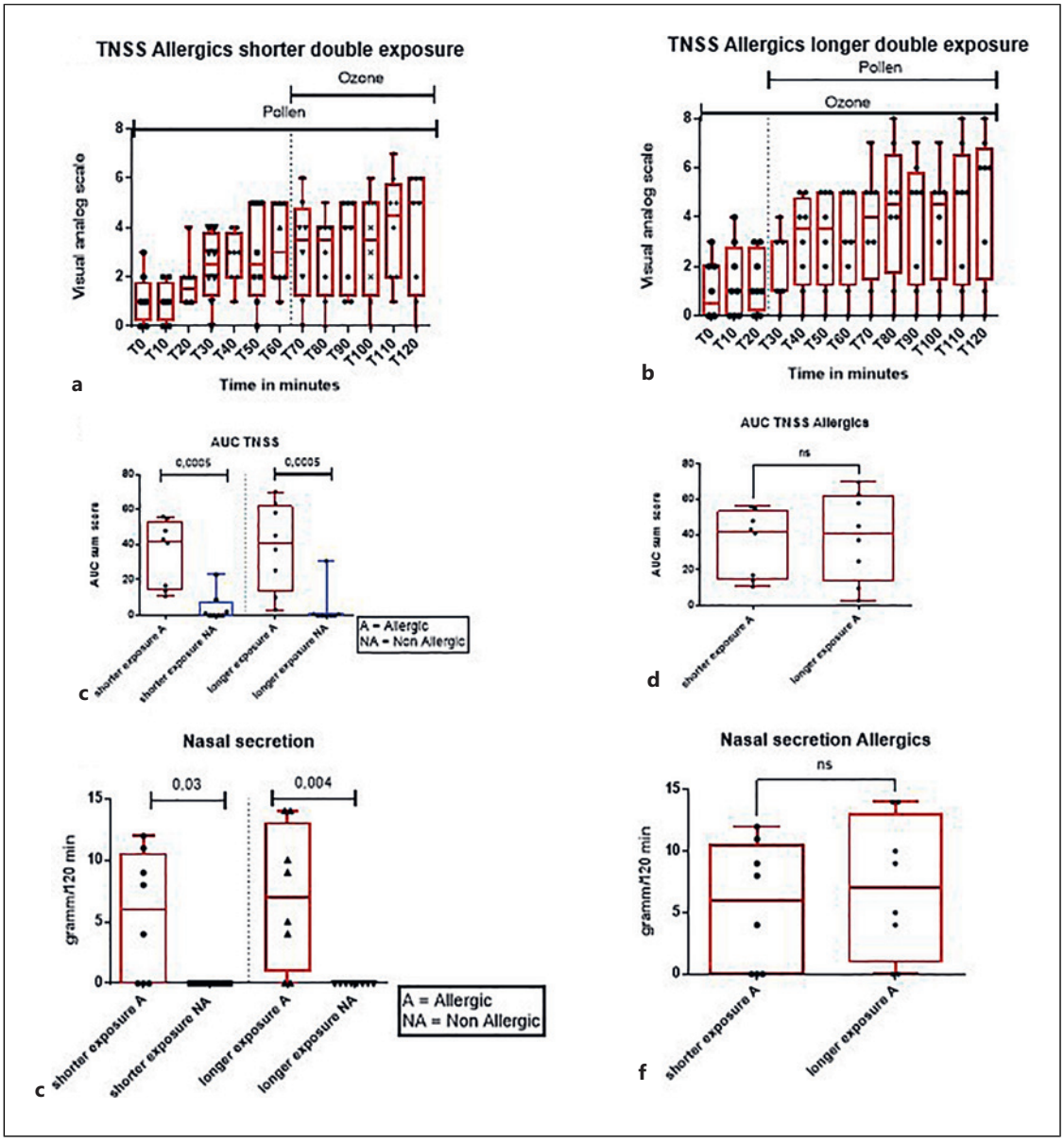

Fig. 2. Nasal symptom assessment was determined according to the VAS during the exposure time. In addition, the nasal secretion was quantified by weighing the used soft tissues. The non-allergic volunteers did not develop relevant nasal symptoms. Their values are not depicted in this graph. **a** Shorter DE shows an increase in the TNSS score values starting 30 min after initiating the pollen exposure. **b** The longer (90 min) double exposure showed a relevant increase of TNSS score values already 20 min after of pollen exposure. **c** An area under the curve (AUC) analysis of the TNSS data was conducted between allergic and non-allergic patients comparing short and long double exposure with a significant increase in the allergic group. **d** The comparison of AUC values between longer and shorter double exposure did not show a significant difference. **e** The nasal secretion in gram over 120-min exposure time showed a significant increase in the allergic group versus non-allergic volunteers. The non-allergic volunteers showed no nasal secretion. **f** The time of double exposure had no significant influence on the secretion in the allergic group.

comparing the erythema index after 120 min, the O-P LONG showed significant ($p < 0.04$) higher erythema values than P-O SHORT in allergic subjects (Fig. 3b). Epidermal barrier function (TEWL) showed significantly higher values after 120 min in the P-O SHORT ($p < 0.05$) (Fig. 4a) as well as in O-P LONG ($p < 0.04$) (Fig. 4b), while the non-allergic volunteers showed no significant alteration in TEWL values. The increase in the longer DE was

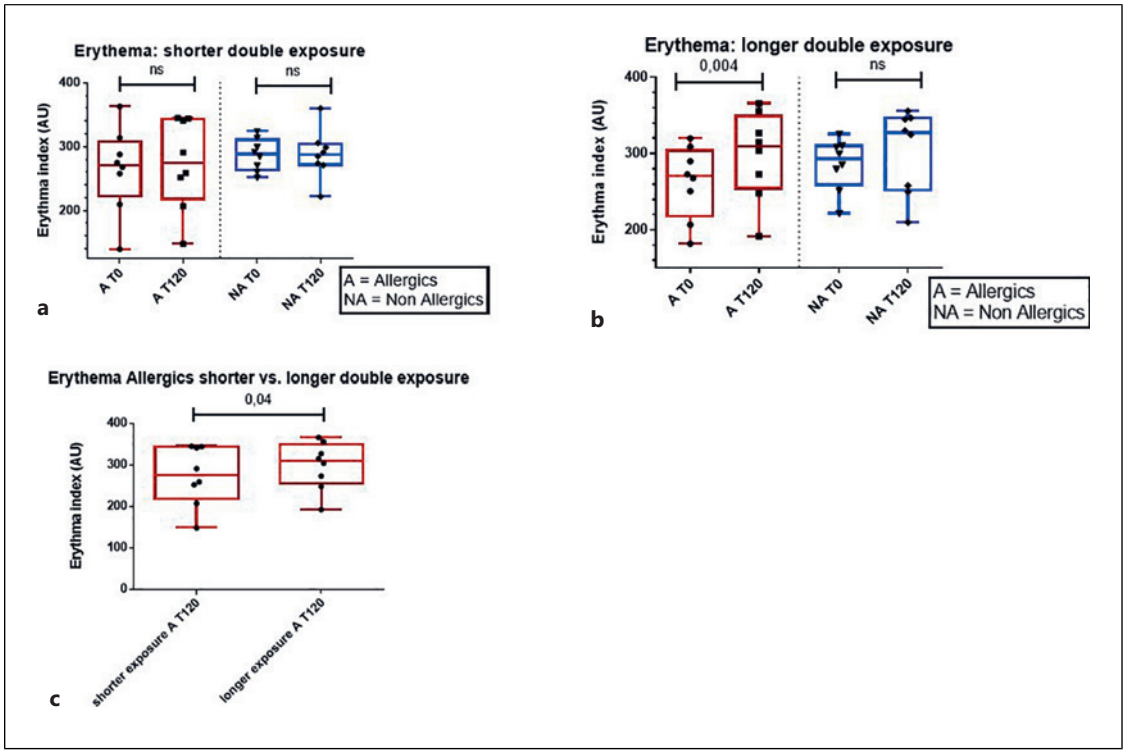

Fig. 3. a, b Skin erythema assessment in allergic versus non-allergic patients using the erythema index before and after 120 min of double exposure. **b** The longer double exposure induced a significant increase ($p = 0.004$) in skin erythema. **c** The erythema index was significantly higher ($p = 0.04$) in the longer double exposure group compared to the shorter double exposure in the allergic group.

not significantly different from the shorter DE setting (data not shown). Surface pH values showed a significant increase in allergic volunteers for both the shorter ($p < 0.02$) (Fig. 5a) and the longer DE ($p < 0.004$) (Fig. 5b), while the non-allergic subjects had no significant pH changes. The longer DE induced a significantly higher pH increase after 120 min ($p < 0.04$) compared to the shorter DE (Fig. 5c). Stratum corneum hydration measurements showed a significant decrease for allergic and non-allergic volunteers, for both shorter and longer DE (online suppl. Fig. S5A, B), without significant difference (data not shown).

Discussion

Air pollution is a global major concern for our well-being with an increased allergy prevalence. Berger et al. conducted a study to decipher which air quality parameters most impact the severity of pollen allergy symptoms [20]. They found that only O_3 significantly influences the clinical symptom severity of pollen allergy sufferers [20]. Beck et al. [21] also showed that O_3 has an impact on pollen allergenicity: skin prick tests on allergic patients with pollen extract from an area with high-O_3 versus low-O_3 concentrations revealed that wheal and flare sizes were significantly larger when patients were tested with high-O_3 pollen extracts. Additionally, analysis of pollen-associated lipid mediators revealed that O_3 modifies the lipid composition, allowing for a higher chemotaxis of cultured neutrophils toward the pollen originating from areas with high-O_3 concentration, as well as providing a higher immune stimulatory potential by an increased release of IL-12 from dendritic cells [21].

We analyzed mucosal reaction in combination with skin-related parameters to better understand the systematic effects of O_3-influenced pollen allergenicity under controlled conditions. We tested the hypothesis that (i) DE in a controlled exposure with grass pollen and O_3 induces increase in mucosal allergic symptoms in a time-dependent manner. This hypothesis was tested in ocular, nasal, and bronchial systems. For ocular symptoms, the allergic reaction was induced but not in a

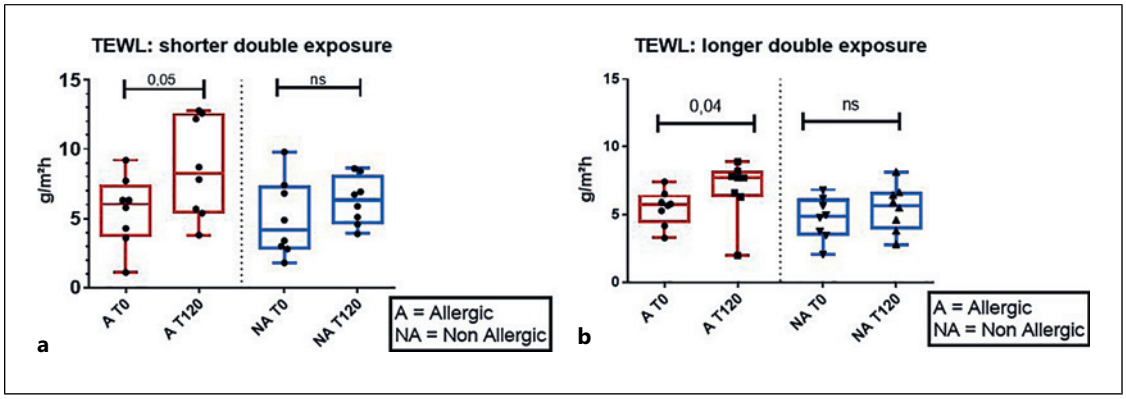

Fig. 4. Skin barrier function assessment in allergic versus non-allergic patients using the transepidermal water loss (TEWL) measurement during a double exposure to pollen and ozone. **a** The shorter double exposure induced a significant increase (p = 0.05) of TEWL values in the allergic but not in the non-allergic group. **b** The longer double exposure led to a significant increase (p = 0.04) of TEWL values in the allergic but not in the non-allergic group.

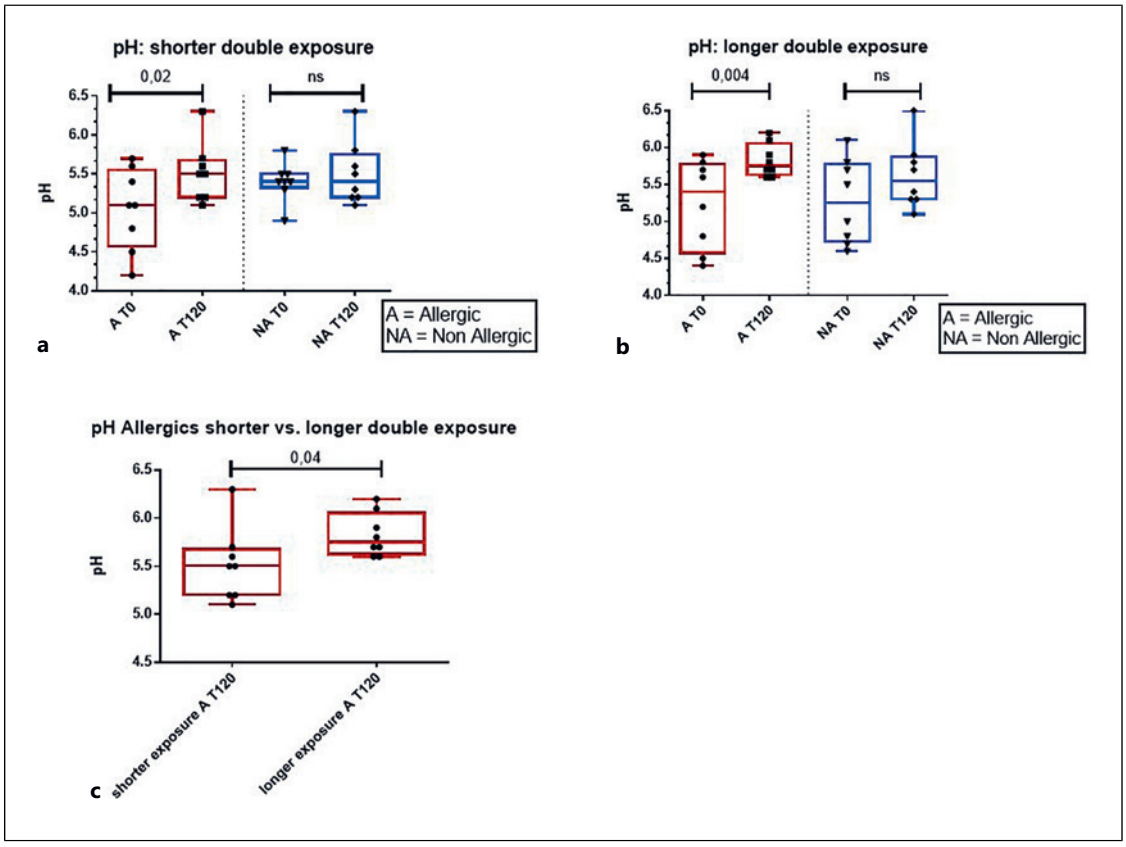

Fig. 5. Skin surface pH level assessment in allergic versus non-allergic patients after 120 min of double exposure to pollen and ozone. **a** Shorter double exposure induced a significant increase (p = 0.02) in surface pH in allergic but not in non-allergic patients 30 min. **b** Longer double exposure had a significant increase (p = 0.004) in surface pH values in allergic but not in non-allergic volunteers. **c** In allergic volunteers the skin pH levels in the longer double exposure were significantly higher in the longer double exposure group (p = 0.04).

time-dependent manner. If the nasal mucosa was pre-exposed with O_3, the nasal symptoms occurred slightly earlier upon grass pollen exposure. In the bronchial system, the shorter DE showed more symptoms than the longer DE, in both clinical symptoms and FEV1 measurements. Overall, the allergic symptoms could be induced in grass pollen allergic patients, but a relation between the duration of DE and symptom severity could not be established. It is of importance that the exposure model was developed with concentrations that induce none or only mild bronchial symptoms, to avoid the induction of severe asthma symptoms.

The second hypothesis regarding (ii) the induction of a systemic reaction was tested with the GWB score and the assessment of skin physiology parameters in allergic patients. We could demonstrate a systemic reaction, which was not time-dependent, in the GWB score. In contrast, skin erythema and surface pH increased in a dose-dependent manner in allergic subjects, which compliments the findings by Beck et al. [21]. The altered epidermal barrier function determined by TEWL measurements in allergic patients was seen for the shorter and the longer DE.

Kumamoto et al. [22] described a mechanism by which pollen allergens can disrupt epidermal permeability barrier. They reported that the peptide, the major allergen of Japanese cedar (Cry j1), interacts with the PAR-2 receptor which induces an elevation of intracellular calcium in human keratinocytes and impaired the epidermal barrier function of human skin ex vivo.

In line with the findings of Berger et al. [20] that O_3 is a major contributor of air pollutants to allergy prevalence [20], and with our findings of increased TEWL in allergic patients during O_3 and pollen DE, a review by C. Akdis also proposed that the increase in epithelial barrier-damaging agents linked to industrialization, urbanization, and modern life might be a driving factor in the rise of allergies [23].

Berger et al. [24] demonstrated that elevated O_3 concentrations had a significant impact on the symptom severity of pollen allergy sufferers. They differentiated two of their study results into two systems: one in which allergic rhinitis symptoms in the pollen-allergic patients were increased during the periods of birch, grass, and ragweed pollen flight, and in the second system where temperature and humidity were included in the analysis, revealing that O_3 led to increased symptoms in grass pollen allergy sufferer [24].

A study that elucidates a mechanistic rationale for increased allergic symptoms in pollen and O_3 DE was conducted by Ribeiro et al. [25]. They showed that pollen exposed to O_3 increased IgE reactivity to pollen proteins compared to the non-exposed pollen [25]. Zhu et al. [26] explored the reactivity of O_3 on the surface and lipidic fraction of pollen in vitro. They found a 4-fold increase in O_3 uptake for crushed pollen compared to native pollen indicating a higher susceptibility of cytoplasmic granules and broken pollen grains to O_3 [26]. They also found that the quantity of O_3 trapped in the lipidic fraction during a 15-min exposure and at 115 ppb pressure is enough to contribute to the reactivity of one-third of the alkenes, suggesting that pollen could be susceptible to an atmospheric increase of O_3 concentration even for a very short duration [26].

Celebi Sozener et al. [27] showed that O_3 has an influence on allergenicity and pro-inflammatory reactions. O_3 penetrates airways, leads to cellular stress, desquamation, cell death with oxidative stress, induces epithelial and myeloid cells to produce IL−1α and IL−33, and increases protein leakage, neutrophil, and macrophage influx.

Our study showed an induction of mild inflammation (vasodilation) in terms of an increased erythema on the volar forearm of pollen-allergic volunteers. The longer DE induced a further elevated erythema in the allergic group pointing toward a partial exposure time/symptom severity relation. While most pollen exposure was directed to the head of the subjects, the effects on the volar forearm can be seen as a predominant systemic reaction to the DE. The impaired GWB in allergic patients is indicative of a general systemic reaction. The systemic reaction included an altered epidermal barrier function in allergic patients, measured by the TEWL. The induced inflammation might have been facilitated by an altered epidermal barrier, even if the basal barrier function was normal. It is known that inflammatory skin reactions are associated with an increase in skin surface pH [28–32]. We could show a pH increase in the pollen-allergic groups. The longer DE further enhanced the pH increase significantly. The pH modulation can be seen as a regulatory mechanism in inflammation and barrier alteration. The decrease in stratum corneum hydration was observed in all groups. Thus, we interpreted this effect as a non-specific dehydration effect of the exposure chamber environment. Future studies should elucidate whether these effects are short term or a prolonged phenomenon. Patients with pollen allergies are known to present altered epidermal barrier function as part of atopy [33]. The specific effect of pollen and O_3 (as well as DE) on TEWL has not been reported so far. Exogenous factors have an important impact on both epidermal barrier function and symptom severity of allergic diseases. The exposome has a relevant

influence on the skin physiology including microbiome and barrier-related parameters [34, 35].

The link between environmental factors such as O_3 and pollution negatively impacts skin cells by upregulating xenobiotic metabolism and a subsequent activation of oxidative stress and inflammation mechanisms [36]. The consequences of our findings for allergic patients with type I allergies are that the induction of symptoms by exposure to the respective allergens has an impact to the entire organism including barrier-related functions. Subsequently, these patients are more prone to develop skin-mediated allergies including type IV allergies. Adequate skin care might be recommended to diminish the epidermal consequences of the exposure to allergens in type I allergies.

This study provided valuable insight into the impact that O_3 and pollen DE have on the mucosa and skin. However, there are some limitations of our study. The study consisted of a small cohort with limited age range (case-control study). The season in which the study was conducted was during a time where at least partially the immune system could be activated by natural exposure to pollutants and pollen (subjects adapted to high levels of O_3 in outdoor air), which may have impacted the outcome of tests. Lastly, complete double-blind implementation was not possible because the subjects could detect the smell of O_3. The present model allows studying the impact of different climatic components on symptoms of type I allergies. Future studies will address other climatic aspects such as ambient temperature and relative humidity.

In conclusion, we were able to show that DE to O_3 and pollen induces a systemic effect in grass pollen allergic patients. Longer DE affected the nasal mucosal membrane, skin erythema, and the surface pH of the skin. Future studies will focus on longer DE times to further elucidate the time-symptom relation. In addition, inflammatory markers will be studied to better understand undelaying mechanism. Studies in standardized exposure chambers will be fundamental for climate change preparation for allergic patients and patients with skin diseases.

Statement of Ethics

The study protocol (EA1/193/14) was approved by the Ethics Committee of Charité - Universitätsmedizin Berlin. Written informed consent was obtained from each participant prior to the inclusion and participation in the study. The study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

Conflict of Interest Statement

None of the authors has a conflict of interest related to this manuscript.

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The study was financed with internal funding.

Author Contributions

Joachim W. Fluhr: conceptualization and planning, data analysis and interpretation, and writing of the manuscript. Katarina Stevanovic: data analysis and interpretation, and writing of the manuscript. Priyanka Joshi: conceptualization and planning, performing the study, data analysis and interpretation, and writing of the manuscript. Karl-Christian Bergmann: conceptualization and planning, interpretation of the data, and revision of the manuscript. Leonie S. Herzog: data analysis and interpretation, and revision of the manuscript. Yasmeen Alwaheed: conceptualization and planning, performing the study, and revision of the manuscript. Shirina Al Sowaidi: conceptualization and planning and revision of the manuscript. Torsten Zuberbier: conceptualization and planning, interpretation of the data, and writing of the manuscript.

Data Availability Statement

The original data are not publicly available on legal grounds. All data generated or analyzed during this study are included in this article and its online supplementary material. Further inquiries can be directed to the corresponding author.

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