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Effect of experimental exposures to 3-D printer emissions on nasal allergen responses and lung diffusing capacity for inhaled carbon monoxide/nitric oxide in subjects with seasonal allergic rhinitis

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Abstract

3-D printers are widely used. Based on previous findings, we hypothesized that their emissions could enhance allergen responsiveness and reduce lung diffusing capacity. Using a cross-over design, 28 young subjects with seasonal allergic rhinitis were exposed to 3-D printer emissions, either from polylactic acid (PLA) or from acrylonitrile butadiene styrene copolymer (ABS), for 2 h each. Ninety minutes later, nasal allergen challenges were performed, with secretions sampled after 1.5 h. Besides nasal functional and inflammatory responses, assessments included diffusing capacity. There was also an inclusion day without exposure. The exposures elicited slight reductions in lung diffusing capacity for inhaled nitric oxide (DL_{NO}) that were similar for PLA and ABS. Rhinomanometry showed the same allergen responses after both exposures. In nasal secretions, concentrations of interleukin 6 and tumor necrosis factor were slightly reduced after ABS exposure versus inclusion day, while that of interleukin 5 was slightly increased after PLA exposure versus inclusion.

KEYWORDS
3-D printer emissions, allergic rhinitis, diffusing capacity of the lung, exposures study, nanoparticles, nasal allergen response

1 | INTRODUCTION

In several studies, emissions of 3-D printers have been linked to potential adverse health effects,[1–5] based on the fact that the emissions of volatile organic compounds and nanoparticles can reach considerable magnitude.[1,6–8] Besides commercial and industrial applications, the use of these printers is also widespread for personal use.[9] The devices are even advertised for children and adolescents who might be particularly sensitive to adverse health effects. In contrast to industrial applications, personal use is not subject to statutory regulations and printers commonly do not have encapsulations or ventilation devices reducing exposure. This might be relevant as users often observe the printing process from a short distance, in part due to curiosity, in part because intervention in case of printing failures may be required. In these settings, the inhaled air may contain high emission levels depending on the material used. For example, the use of polylactic acid (PLA) and acrylonitrile butadiene styrene (ABS) can lead to large differences in the number of emitted nanoparticles and other compounds.[1,7,10,11]

Although it is assumed that 3-D printer emissions have health effects analogous to ambient air pollutants including nanoparticles,[12–14] this has been addressed in only few studies. In fact, there is only one experimental study in human subjects thus far.[1] In this study, healthy volunteers were exposed to either PLA or ABS emissions in 1-h printing sessions, showing differences in the time course of the fractional concentration of exhaled nitric oxide (FeNO) after ABS compared with PLA. FeNO is generally considered as marker of TH2-type inflammation related to respiratory allergies in asthma and rhinitis, but may also be affected by changes in mucosal permeability impeding the transfer of endogenous nitric oxide (NO) to the airway lumen, or scavenging via oxidants.[15–17] Although subjects in the previous study were required to have no medical history of allergies, the difference in FeNO hinted at a potential effect on allergic pathways. This notion was corroborated by the observation that FeNO correlated with eosinophilic cationic protein (ECP) in nasal secretions, a marker linked to allergic responses of the respiratory tract.[18] The suspicion of a potential involvement of allergic pathways was further supported by a case report on the recurrence of previous allergic asthma after massive exposure to ABS emissions from 3-D printers, with beneficial effects of exposure reduction via ventilation and change of material.[19]

These considerations led to the hypothesis that allergic responses are enhanced by 3-D printer emissions, potentially leading to clinically relevant amplification of irritant responses, as demonstrated for other air pollutants, such as ozone,[20,21] nitrogen dioxide[22,23] and particles.[24] This could be of importance in view of the high prevalence of respiratory allergies, including allergic rhinitis.[25] There is, however, a second potentially important effect. Nanoparticles could also have non-allergic respiratory effects, especially in the alveolar space as reflected in subtle alterations of gas uptake, which comprises pulmonary membrane factor and capillary blood volume as fundamental determinants of diffusing capacity. Both can be assessed via the combined diffusing capacity for inhaled nitric oxide (DL_{NO}) and carbon monoxide (DL_{CO}). In previous studies, we found these parameters informative regarding the response to inhaled aerosols[26] or particles.[27]

In this study, we therefore examined whether the exposure to ABS compared with PLA emissions (a) led to an increase in allergen responsiveness and (b) affected the lung function including gas uptake capacity.

2 | MATERIALS AND METHODS

2.1 | Study design

The study was conducted using an experimental cross-over design. It comprised 3 days, all at least 3 weeks apart from each other. The first day (recruitment visit) was used to assess whether the subjects fulfilled the inclusion criteria and familiarize them with the measurement procedures described below. Moreover, the appropriate allergen for nasal challenges was selected for each subject. At the two exposure days, the subjects were exposed to 3-D printer emissions from either PLA or ABS in random order. The sequence of the procedure during an exposure day is illustrated in the Figure S1.

2.2 | Participants

Subjects were required to be of age 18–40 years, non-smokers and without diseases requiring the intake of corticosteroids, such as asthma, in order to avoid potential interference with comorbidities and their treatment and enhance the likelihood for a stable clinical state. Clinical history was assessed by standard diagnostic procedures. Subjects were required to show seasonal allergies to grass or tree pollen with reported nasal symptoms, to be available for three study visits, and to show sufficient cooperation in all tests. Subjects with regular contact to 3-D printers, symptomatic allergies to perennial allergens, or recent or ongoing immunotherapy, either ongoing or within the last 2 years, were excluded from the study. Subjects were asked to avoid exercise within 2 h prior to the start of the assessments and to refrain from the intake of food rich in nitrite in order to avoid potential interference with FeNO measurements.

Initially, 146 subjects were screened using these criteria; 44 were invited for the recruitment visit, and 28 were finally included and investigated in two exposure visits as described below. The study including exposures to 3-D printer emissions, functional and clinical assessments, allergen challenges and the use of commercial allergen solutions was approved by the Ethical Committee of the Ludwig Maximilian University of Munich (file reference #19-059) and performed in accordance with the Declaration of Helsinki. All subjects gave their written informed consent.

2.3 | Determination of the appropriate allergen for nasal challenge

Information regarding the presence of allergic rhinitis, allergen exposures, and associated symptoms was collected at the recruitment visit. For screening and identification of suitable allergens, a skin prick test was performed following the guidelines of the German Society of Allergology and Clinical Immunology (DGAKI).[28] It comprised 20 common allergens (seasonal and perennial; Allergopharma; ALK-Abelló Arzneimittel GmbH) routinely used in the diagnosis of allergic asthma and rhinitis. Based on the results and the medical history on symptoms and need for medication, the most promising allergen for nasal challenges was chosen. Subjects with a mild (<3 mm) positive response to perennial allergens in skin prick testing but without any corresponding history of nasal symptoms were not excluded, as we considered these sensitizations to be irrelevant in the experimental challenges. However, subjects with stronger (≥3 mm) prick test responses to perennial allergens were excluded even if they did not report a corresponding history and symptoms. This procedure and the fact that challenges were performed outside the individual allergen season intended to ensure that the experiments were (a) performed with the individual allergen eliciting the strongest responses and (b) undisturbed by interference with perennial allergen exposures.

2.4 | Functional assessments and questionnaires

The assessments comprised questionnaires, vital function (blood pressure and heart rate), the measurement of exhaled biomarkers and lung function, as well as rhinomanometry. As questionnaires, we used the self-reported multiple chemical sensitivity (sMCS) questionnaire[29] for the determination of odor sensitivity,[30] comprising eight questions with 1–5 possible score points for each question. Furthermore, a symptom questionnaire comprising 22 general questions and four additional post-exposure questions. Both questionnaires have been used previously.[1,27,30]

The assessment of exhaled biomarkers comprised the determination of the fractional concentration of exhaled nitric oxide (FeNO) at an expiratory flow rate of 50 ml/s,[31] using a Vivatmo *pro* device (Bosch Healthcare Solutions GmbH). This device has been shown to yield FeNO values comparable with those of other devices.[32] Furthermore, the concentration of exhaled carbon monoxide (eCO) was assessed via the BreathCO Exhaled Carbon Monoxide Analyzer (Vitalograph GmbH) according to the manufacturer's instructions.

Spirometry (SpiroScout, LFX, Ganshorn Medizin Electronic GmbH) was performed following international recommendations,[33] determining forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC) and their ratio FEV_1/FVC.

The combined diffusing capacity for inhaled NO (DL_{NO}) and CO (DL_{CO}) was assessed as described previously[27] in two consecutive measurements separated by 4 min, using the MS-PFT Analyzer Unit (SentrySuite Version 3.00; Vyaire Medical Inc.). The inhaled gas (Linde GmbH) contained 9.3% helium, 0.28% CO, and 40 ppm NO. After complete expiration, subjects inhaled the test gas until total lung capacity was reached and held their breath for 8 s, followed by a deep expiration during which the gas was sampled. As done in a previous study,[27] the value of eCO was used to correct DL_{CO} for CO backpressure in the blood due to inhalation of CO in previous measurements of diffusing capacity.[34] The dilution of helium was used to estimate alveolar volume (V_A) and to compute values of diffusing capacity per liter of alveolar volume (K_{NO}, K_{CO}). Pulmonary capillary volume (V_C) and membrane factor (D_M) were derived from DL_{CO} and DL_{NO} values via standard procedures.[35,36]

2.5 | Allergen challenges

For nasal allergen challenges, commercial solutions containing lyophilized allergens extracted from the respective plants and dissolved in the provided solvent were used (concentration: 5000 standardized biological units per ml for grass and birch solutions, 5000 biological units per ml for ragweed solution; Allergopharma). The grass solution contained a mixture of allergens relevant in Germany extracted from six grass species (*Holcus lanatus*, *Dactylis glomerata*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *and Festuca pratensis*), while birch and ragweed solutions were extracted from *Betula pendula* and *Ambrosia artemisiifolia*, respectively. Administration of these solutions was performed using a spray flask provided by the manufacturer, which sprayed 0.04–0.05 ml of fluid per nebulization. All challenges comprised initial administration of saline solution followed by allergen administration. The allergen chosen for each subject was given in up to three consecutive steps at the recruitment visit, and as a single cumulative dose at the exposure days.

Nasal responses were measured via rhinomanometry (Merz Rhino, Merz Medizintechnik GmbH), using the flow rate achieved at a pressure difference of 150 Pa between nasopharynx and ambient air as the primary response parameter. Additionally, the flow rate for a pressure difference of 300 Pa was recorded. As recommended by the German Society for Allergology and Clinical Immunology,[37] prior to each challenge, the nostril showing the highest flow rate, that is, least resistance, was identified. In each challenge, solutions were sprayed only into the selected nostril, however always both nostrils were measured to determine the responses.

Assessments performed after each nebulization step comprised (a) rhinomanometry and (b) the determination of allergic symptoms using a standardized score addressing nasal secretion, nasal irritation and a set of non-nasal symptoms (conjunctivitis, urticaria, and breathlessness), each of them evaluated in a 3-point score (0, 1, 2 points). After administration of allergen, the criterion for a positive response was (a) a reduction in the 150 Pa flow rate in the challenged nostril by ≥40% irrespective of symptoms, or (b) in case of a reduction by only ≥20% an increase in the sum score of symptoms by at least 3 points, as proposed in the recommendations.[37,38] If none of these criteria was satisfied, the response was considered negative.

A different criterion was used for saline (2 sprays of 0.9% NaCl) which was given prior to the first allergen administration. If the flow rate at 150 Pa showed a reduction by at least 10% or symptoms occurred, this was considered as non-specific irritant response. We preferred the change of 10% over that of 20% proposed in a previous guideline[37] in order to ensure a stable baseline prior to allergen administration. For this purpose, the administration of saline and the subsequent assessments were repeated, until there was no response.

The protocol of the allergen challenges is illustrated in the Figure S2. On the recruitment day, the initial administration of allergen comprised two spray doses. If the response was not sufficient according to the criteria described above, the same allergen solution was administered in two further spray doses, and the response in terms of flow rate and symptoms was measured again. If this still was not sufficient, two further spray doses of the same allergen solution were given. If there was no sufficient response after a total of six sprays, the subject was excluded. A final reduction of flow rate by ≥40% was achieved in 27 of 28 subjects, and the case that the response was based on a reduction by <40% but ≥20% in combination with symptoms occurred in only one subject.

At the exposure visits, saline solution was administered first, followed by the cumulative dose of allergen that had resulted in a sufficient response at the recruitment day, that is, either two, four, or six nebulizations in one step. Responses were assessed via rhinomanometry and symptom scores in the same manner as at the recruitment visit but the criteria for a positive response to allergen did not apply, although the criteria for responses to saline were the same.

2.6 | Nasal secretions

Nasal secretions were sampled 90 min after the allergen challenge. The procedure was performed as described previously[1] and nasal fluid was analyzed for protein content, ECP, interleukins IL-5 and IL-6, interferon-gamma (IFN-γ) and tumor necrosis factor (TNF). These biomarkers were selected as indicators of allergic (IL-5, ECP) and non-allergic (IL-6, TNF, IFN-γ) inflammatory responses. Information on their determinations is given in the Appendix S1.

2.7 | Exposures

Exposures to 3-D printer emissions and monitoring of particles were performed analogous to our previous study[1] using the same devices but were modified to comprise a second 1 h printing session. For further information on printing and exposure measurement, please see the Appendix S1.

After the first printing, subjects remained seated in the same position for 30 min, but the ventilation of the exposure chamber was switched on to eliminate particles and gases from the printing process and the exhaled carbon dioxide accumulated. Then, printing of the same object using the same material was repeated, again without ventilation of the chamber. This duplicate exposure was chosen to mimic situations, in which printing may be repeated after examination of the first printed object.

2.8 | Data analysis

For data presentation, numbers and percentages, or mean values and standard deviations (SD) were computed, depending on the type of data and their distribution. Values of FeNO or ratios of values for diffusion capacity were logarithmically transformed to derive geometric mean values and SD (to be interpreted as dimensionless variability factor). The same was applied to the compounds in nasal secretions. Comparisons between conditions were performed with contingency tables and chi-squared statistics, or the nonparametric Wilcoxon matched-pairs signed-ranks test. Correlations were analyzed via Spearman rank correlation coefficients.

Cytokine concentrations in nasal secretion showed large differences between subjects and tests, resulting in a combination of very large and very small effect sizes. These differences were likely to be the result of different overall concentrations of biochemical material, that is, different dilutions, in the cotton pads. We therefore attempted to normalize cytokine (IL-5, IL-6, TNF) values in the samples, using two approaches and checking for the consistency of results. IFN-γ was omitted based on the fact that the majority of values (66.3%) was below the detection limit.

The first, direct normalization involved the computation of ratios of cytokine concentrations over protein concentration. As cytokines are likely to be only a minor part of protein content, we developed a second procedure based on the concentrations of cytokines only, with the aim to improve statistical power by reduction of variability. The approach was motivated by the observation that extreme values in one cytokine in a sample tended to be linked to extreme values of other cytokines in the same sample, probably due to common dilution factors. In accordance with multiplicative factors, analyses used logarithmically transformed values.

In the first step, values were averaged over subjects as usual, thereby determining mean responses for each of the three samples and cytokines. Then, the residuals from this average were computed. We now averaged these residuals over the three cytokines for each subject and sample separately, thus deriving an average residual value common to all cytokines of one sample in order to account for common variations in concentration. We then subtracted these average residuals from the previously computed residuals of each cytokine. As a consequence, all values now showed a similar range of variation, without affecting mean values, as we still operated with residuals of mean zero. We then added the raw average values over subjects for each cytokine to re-establish the mean values and the differences between them. In this manner, variability was reduced without affecting the differences in mean values or the rank order of values when comparing the three samples. A detailed flowchart of this procedure is given in Figure S3. Data with these reduced standard deviations were used for statistical comparisons, while in the

data description both the directly measured and normalized geometric standard deviations are shown for comparison.

All analyses were performed using the software package SPSS (Version 26; IBM). Statistical significance was assumed for *p*-values (errors of the first kind) of less than 0.05. We did not implement corrections for the multiplicity of testing but preferred to provide *p*-values explicitly as far as feasible.

3 | RESULTS

3.1 | Study population

Baseline characteristics of the 28 participants are shown in Table 1. The mean age was 25 years, and there were 14 female and 14 male subjects. Lung function was within the normal range, and only four participants had concomitant mild asthma not requiring medication with bronchodilators or corticosteroids at the time of the study. Most challenges (79%) were performed using grass pollen allergen, and in most subjects (89%), 2 or 4 nebulizations of the allergen were sufficient to elicit the required response. Only two of the 28 subjects reported previous exposure to 3-D printer emissions. The sMCS showed low values (Table 1), with a mean value of the sum score of 10.5 points compared to a maximum value of 40.

Polylactic acid exposure was performed first in 14 subjects, ABS exposure in the other 14 subjects. The mean (±SD) time between recruitment visit and first exposure was 29.2 ± 10.6 days, and between first and second exposure 28.8 ± 8.5 days.

TABLE 1 Baseline characteristics at the recruitment visit.

Baseline characteristics	Value	Range
Sex (male/female)	14/14 (50%/50%)	—
Age (years)	25.0 ± 4.2	20–37
Height (cm)	175.9 ± 9.3	160–193
Weight (kg)	71.1 ± 12.4	53–105
BMI (kg/m^2)	22.9 ± 2.8	18.2–33.4
FEV_1 (% predicted GLI)	99.2 ± 12.0	81.2–127.7
FVC (% predicted GLI)	102.0 ± 13.7	81.7–131.3
FEV_1/FVC (%)	82.5 ± 5.8	71.4–96.1
FeNO (ppb, geometric mean and SD)	14.0 (1.81)	4–60
History of nasal allergy (yes)	28 (100%)	—
History of mild asthma (yes)	4 (14.3%)	—
Previous exposure to 3-D printer emissions (yes)	2 (7.1%)	—
Sum sMCS (8 items with scores 1–5)	10.5 ± 3.2	8.00–21
Results of nasal challenge		
Chosen allergen (grass pollen/birch/ragweed)	22/5/1	—
Final allergen dose, # of nebulizations (2/4/6)	15/10/3	2–6
Nasal flow (ml/s) at final dose and Δp of 150 Pa	96.6 ± 35.4	42–187
Nasal flow (ml/s) at final dose and Δp of 300 Pa	180.4 ± 61.4	95–340

Note: The table shows either numbers (percentages) or mean values ± standard deviations (SD). In case of FeNO, the geometric mean is shown and the geometric SD that is to be understood as variability factor.

Abbreviations: BMI, body mass index; FeNO, fractional concentration of exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; sMCS, self-reported multiple chemical sensitivity questionnaire.

3.2 | Results of exposures

Individual exposure characteristics are given in the Table S1, demonstrating that in nearly all cases exposure levels in terms of LDSA were markedly higher with ABS compared to PLA exposure, while temperature and CO_2 concentration of room air were comparable. The Figure S4 provides information on the symptoms reported by the subjects prior to and after exposure. In the majority of cases, symptoms did not change over exposures, while they slightly improved in some cases and slightly deteriorated in other cases, but without apparent difference between PLA and ABS exposure. Post-exposure heart rate, diastolic and systolic blood pressure were lower compared to pre-exposure. This was probably a result of resting for about 2.5 h, but the difference was not significant. The sMCS score prior to exposures was not different from that determined at the recruitment visit indicating that there were no changes in the sensitivity to chemicals.

3.2.1 | Lung function and FeNO

Values assessed at the recruitment visit are included in Table 1, and baseline values prior to exposures are shown in Table 2. Values of FEV_1, FVC, DL_{CO}, DL_{NO}, K_{CO}, K_{NO}, V_C, D_M, and FeNO prior to exposures were not significantly different from each other, but there was a slightly elevated value of V_A prior to ABS compared with PLA exposure ($p = 0.047$, see Table 2).

Immediately after PLA exposure, statistically significant ($p < 0.05$ each) increases occurred in FEV_1 and FeNO, as demonstrated by arithmetic and geometric mean values, respectively (Table 2). Such changes were not seen after ABS exposure. However, when comparing the changes occurring over exposures, only those regarding FeNO showed a significant difference between the two materials, as indicated by a small increase after PLA and a small decrease after ABS exposure. The changes in FeNO are summarized in the box plots of Figure 1.

After both PLA and ABS exposure, there were significant reductions in DL_{NO}, the ratio DL_{NO}/DL_{CO}, K_{NO} and D_M (Table 2). There were no significant changes in DL_{CO} corrected for CO uptake from previous measurements. Uncorrected DL_{CO} decreased by 1.2% on

TABLE 2 Responses of lung function and FeNO immediately after PLA and ABS exposure versus values prior to exposure.

	PLA exposure				ABS exposure				
Variable	Pre	Post	Δ	*p* Value pre vs. post	Pre	Post	Δ	*p* Value pre vs. post	*p* Value between values of Δ
FEV_1 (L)	4.04 ± 0.81	4.09 ± 0.78	0.05 ± 0.11	**0.037**	4.08 ± 0.81	4.13 ± 0.84	0.05 ± 0.16	0.130	0.955
FVC (L)	5.02 ± 1.00	5.04 ± 0.96	0.02 ± 0.19	0.699	5.02 ± 1.01	5.01 ± 1.05	−0.01 ± 0.16	0.690	0.538
DL_{CO} uncorrected (mmol/min/kPa)	10.16 ± 2.36	10.03 ± 2.24	−0.14 ± 0.32	**0.042**	10.29 ± 2.61	10.10 ± 2.48	−0.18 ± 0.39	**0.021**	0.706
DL_{CO} corrected (mmol/min/kPa)	10.24 ± 2.40	10.29 ± 2.35	0.06 ± 0.32	0.424	10.35 ± 2.64	10.37 ± 2.58	0.01 ± 0.39	0.949	0.819
DL_{NO} (mmol/min/kPa)	42.87 ± 9.09	41.46 ± 8.77	−1.41 ± 1.96	**0.001**	42.89 ± 9.19	41.5 ± 8.35	−1.38 ± 2.07	**0.003**	0.484
DL_{NO}/DL_{CO}[a]	4.2 (1.08)	4.04 (1.08)	0.96 (1.04)	**<0.001**	4.17 (1.09)	4.04 (1.09)	0.97 (1.05)	**0.004**	0.367
V_A (L)	5.84 ± 1.13	5.84 ± 1.12	0.01 ± 0.12	0.903	5.88 ± 1.17	5.88 ± 1.2	0.002 ± 0.12	1.000	0.809
K_{CO} corrected (mmol/min/kPa/L)	1.76 ± 0.24	1.77 ± 0.23	0.01 ± 0.07	0.751	1.76 ± 0.24	1.77 ± 0.25	0.01 ± 0.08	0.949	0.716
K_{NO} (mmol/min/kPa/L)	7.37 ± 0.75	7.11 ± 0.70	−0.25 ± 0.29	**<0.001**	7.32 ± 0.71	7.10 ± 0.59	−0.22 ± 0.35	**0.003**	0.536
D_M	21.8 ± 4.6	21.1 ± 4.5	−0.8 ± 1	**<0.001**	21.9 ± 4.7	21.1 ± 4.2	−0.8 ± 1.1	**0.001**	0.581
V_C	80.8 ± 21.6	81.3 ± 20.7	0.5 ± 5	0.354	82.6 ± 25.9	82.3 ± 25.8	−0.2 ± 6	0.949	0.484
FeNO[a] (ppb)	12.5 (1.66)	13.4 (1.61)	1.07 (1.18)	**0.026**	13.4 (1.62)	13.1 (1.62)	0.98 (1.20)	0.737	**0.022**

Note: Corrected values of DL_{CO} were obtained from uncorrected values via the values of exhaled CO (eCO) that were used to estimate the backpressure from CO in the blood (see Section 2). Mean values ± SD are given. The values of D_M (membrane factor) and V_C (pulmonary capillary blood volume) were estimated from those of DL_{CO} and DL_{NO} via formulas describing the corresponding physiological relations.[27,34] Comparisons with *p*-values <0.05 are marked in boldface.

Abbreviations: ABS, acrylonitrile butadiene styrene; DL_{CO}, diffusing capacity for inhaled carbon monoxide; DL_{NO}, diffusing capacity for inhaled nitric oxide; FeNO, fractional concentration of exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; K_{CO}, diffusing capacity for inhaled carbon monoxide per alveolar volume; K_{NO}, diffusing capacity for inhaled nitric oxide per alveolar volume; PLA, polylactic acid; V_A, alveolar volume estimated from helium dilution.

[a]Geometric mean and SD and geometric mean and SD of ratio post over ratio pre. Comparisons pre vs. post were performed with the Wilcoxon matched-pairs signed-ranks test, and the same was true for the comparisons between the changes (Δ).

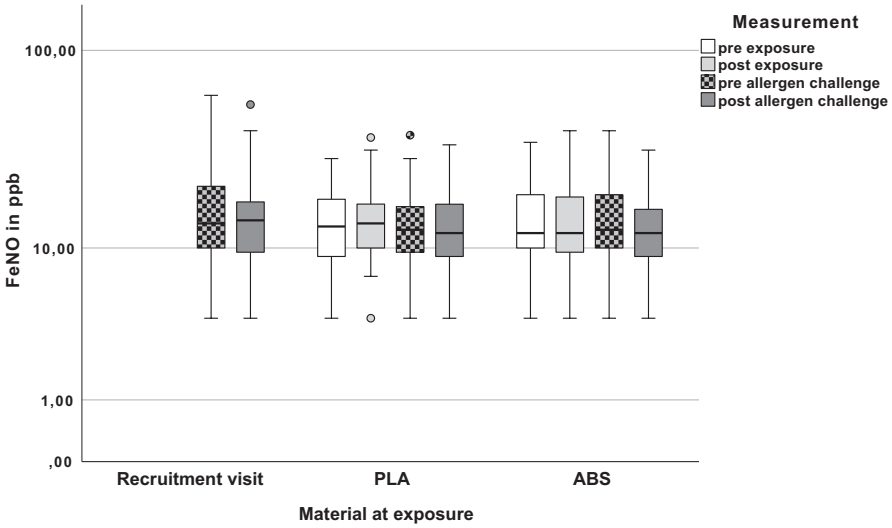

FIGURE 1 Box plots of FeNO, either post vs. pre allergen challenge at the recruitment visit, or post vs. pre allergen challenge after previous PLA and ABS exposure, or post vs. pre PLA and ABS exposure, as indicated by the different shadings of bars. ABS, acrylonitrile butadiene styrene; PLA, polylactic acid.

average after PLA, and by 1.6% after ABS, without significant difference between exposures. The correction via eCO resulted in average increases of DL_{CO} values post-exposures by 2.6% after PLA and by 2.6% after ABS. Correspondingly, corrected DL_{CO} increased by 0.75% and 0.29%, respectively. Figure 2A shows the individual values of DL_{CO} corrected, and Figure 2B those of DL_{NO}, demonstrating the reduction of post- compared with pre-exposure values, without significant difference between PLA and ABS exposures. The reductions in DL_{NO} amounted to 3.2% for PLA and 2.9% for ABS.

Spirometry and the assessment of FeNO were repeated 1.5 h after exposure prior to allergen challenges as well as 1.5 h after allergen challenges. As shown in Table 3, we observed a slight, but statistically significant reduction in FEV_1 ($p < 0.05$) after the allergen challenge preceded by PLA exposure, and correspondingly a difference between the changes after PLA and ABS exposure. There were no significant changes or differences in FVC. FeNO tended to be lower after allergen challenges compared with the values measured before, and this was significant if the challenge was preceded by ABS exposure.

3.2.2 | Nasal challenges and rhinomanometry

Prior to allergen administration, flow rates at a pressure difference of 150 Pa showed marked differences between the values of the control nostril and the allergen-challenged nostril (Table 4 and Figure 3), thereby reflecting the adequate choice of the nostril with larger lumen for challenge. Figure 3 summarizes the results obtained for 150 Pa. In the challenged nostril, the flow rate was significantly ($p < 0.001$ each) reduced after both PLA and ABS exposure, as well as at the recruitment visit (Table 4 and Figure 3). There was also a reduction in the control nostril to which no allergen had been given. This was statistically significant ($p < 0.05$) after PLA but not after ABS exposure, but the changes in the control nostril were much smaller compared to those of the challenged nostril. In line with this, the differences between the responses of challenged minus control nostril were highly significant for both exposures ($p < 0.001$ each).

When comparing any of these response parameters between PLA and ABS exposure, no significant differences occurred (Table 4). Flow rates for 150 Pa on the recruitment visit showed a significant higher decrease in nasal flow (−118 ± 62 ml/s) compared to that measured after PLA (−88 ± 52 ml/s; $p = 0.047$) and ABS exposures (−90 ± 53 ml/s; $p = 0.013$) (Figure 3). Flow rates corresponding to a pressure difference of 300 Pa changed in parallel with those measured for 150 Pa for values of PLA and ABS exposures (Table 4) but there were no significant differences observed between the recruitment visit and the exposures.

3.2.3 | Nasal secretions

Samples were obtained 1.5 h after the allergen challenges at the recruitment visit and following either PLA or ABS exposure. Geometric mean values and SD (to be interpreted as variability factor) of cytokine and protein concentrations are given in Table 5. As described in the Section 2, values were either normalized for cytokine content (upper part) or for protein content (lower part). In the upper part, the geometric SD of the cytokine-normalized data and that of the raw data is shown in the parentheses, demonstrating the marked reduction of variation by cytokine normalization without affecting mean values.

When comparing the raw data of the cytokines between the three samples, there were no significant differences, due to the large scatter. However, after normalization for cytokine content, statistically significant differences occurred ($p < 0.05$ each). Compared with the recruitment visit, the concentration of IL-5 increased after PLA exposure, and the concentrations of IL-6 and of TNF decreased after ABS exposure. Correspondingly, the levels of IL-5 and IL-6 tended to be different between PLA and ABS exposure as indicated by p-values being only slightly above 0.05 (Table 5). The relationship of cytokine levels between exposures is illustrated in Figure 4, showing PLA and ABS results versus those at the recruitment day (left panels), and those of PLA versus ABS results (right panels).

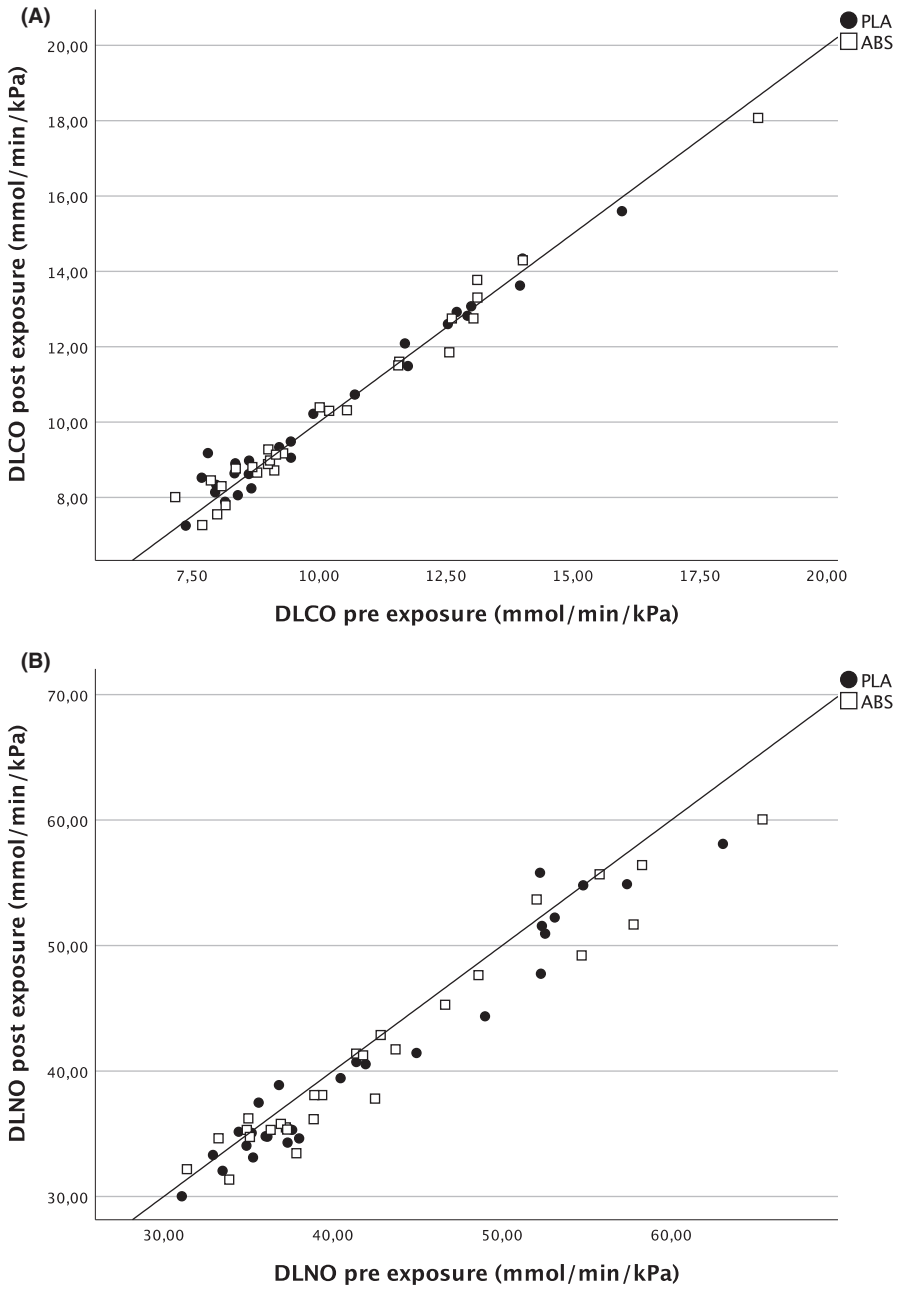

FIGURE 2 Values of DL_{CO} (A, upper panel) and DL_{NO} (B, lower panel) assessed prior to exposures. In case of DL_{CO}, the values corrected for CO in the blood via the measurement of exhaled CO are shown. The line is that of identity and has been inserted to show the differences. For statistical results see Table 2 and the text. ABS, acrylonitrile butadiene styrene; DL_{CO}, diffusing capacity for inhaled carbon monoxide; DL_{NO}, diffusing capacity for inhaled nitric oxide; FeNO, fractional concentration of exhaled nitric oxide; PLA, polylactic acid.

On average, there were no significant differences in protein content between samples, but we used the individual values to normalize cytokine levels via the protein content. The results are shown in the lower part of Table 5. Compared to the recruitment visit, the normalized levels of IL-6 and TNF were reduced ($p < 0.05$ each) after ABS exposure. Neither for PLA nor for ABS, IL-5 levels differ from those of the recruitment visit, but there was a significant ($p = 0.039$) difference between PLA and ABS exposure. In terms of mean values, the results of the two approaches chosen to deal with the large variation of cytokine concentrations in nasal secretions, appeared consistent with each other.

Eosinophilic cationic protein values did not show significant differences between the three samples, neither in terms of directly measured values nor after normalization via protein content. Neither for the recruitment visit nor for ABS or PLA exposure, there was a statistically significant correlation between the levels of IL-5 and those of ECP in the nasal secretions, independent of the type of normalization.

4 | DISCUSSION

The present study addressed potential health effects of 3-D printer emissions in subjects with allergic rhinitis and sensitization against seasonal pollen allergens using a randomized, cross-over experimental design. As source of emissions, a standard 3-D printer for personal use and a typical printing job were chosen in order to mimic everyday conditions as closely as possible. Based on our previous study,[1] our main goal was to investigate the effect of PLA and ABS emission on the modulation of allergen responses. As a second outcome measure,

TABLE 3 Responses of spirometric lung function and exhaled nitric oxide after nasal allergen challenge and previous exposure to PLA or ABS.

	Allergen challenge after previous PLA exposure				Allergen challenge after previous ABS exposure				
Variable	Pre	Post	Δ	*p* Value pre vs. post	Pre	Post	Δ	*p* Value pre vs. post	*p* Value between Δ
FEV_1 (L)	4.17 ± 0.72	4.04 ± 0.75	−0.08 ± 0.17	**0.017**	4.07 ± 0.84	4.14 ± 0.83	0.06 ± 0.20	0.211	**0.014**
FVC (L)	5.09 ± 0.91	5.02 ± 0.95	−0.07 ± 0.20	0.121	5.04 ± 0.97	5.08 ± 1.01	0.05 ± 0.25	0.810	0.136
FeNO[a] (ppb)	13.0 (1.64)	12.1 (1.61)	0.93 (1.20)	0.068	13.0 (1.68)	12.2 (1.60)	0.94 (1.18)	**0.045**	0.904

Note: Pre values refer to the time point 1.5 h after the end of exposures to 3-D printer emissions, after which allergen challenges were initiated, while post values were assessed 1.5 h after the end of allergen challenges. Mean values ± SD are given. Comparisons with *p*-values <0.05 are marked in boldface.

Abbreviations: ABS, acrylonitrile butadiene styrene; FeNO, fractional concentration of exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; PLA, polylactic acid.

[a]Geometric mean and SD. Comparisons pre vs. post were performed with the Wilcoxon matched-pairs signed-ranks test, as well as comparisons of the changes (Δ) between the two exposures.

we assessed the combined lung diffusing capacity for CO and NO,[26,27] with the aim to identify potential non-immunological effects on the lung periphery suggested by previous observations.

Previous studies described amplification of bronchial allergen responses for various air pollutants such as ozone,[20,21] nitrogen dioxide,[22,39] and fine particulate matter.[40–42] Neither regarding the functional responses to nasal allergen application nor regarding lung diffusing capacity, we observed significant differences between PLA and ABS exposure. Nasal responses of flow rate at Δ150 Pa after allergen challenges were higher on the recruitment visit without previous exposure to 3-D printer emissions (Table 4 and Figure 3). This was probably due to the titration process that was performed on the recruitment visit for the identification of the individual allergen dose needed to elicit a sufficient response. The fact that nasal flow rate prior to the allergen challenges did not significantly differ between the 3 days suggests that subjects were in a comparable clinical condition. When compared to values obtained at Δ300 Pa, there were no such changes between nasal responses of the recruitment day and the exposures. Unfortunately, the number of subjects having always only two nebulizations of allergen was too low to allow for meaningful, statistically reliable comparisons between recruitment and exposure visits. We therefore would not infer an attenuating effect of PLA or ABS exposure.

In contrast to the previous study in which ECP in nasal secretion increased after both exposures and correlated with FeNO, there was no correlation between ECP levels and FeNO values measured at different time points in the present study. This could be due to the fact that ECP values were affected by the preceding allergen challenges in a different manner in different subjects. The same could be true for FeNO, which could differently respond in allergic subjects compared to non-allergic subjects.

The differences in cytokine levels between allergen challenges were small and mainly referred to differences between exposure days and the recruitment day. The reduction in IL-6 and TNF after ABS exposure might be tentatively interpreted as suppression of non-TH2-responses, in contrast to the increase in TNF levels observed in cell culture experiments[43] but without allergen challenge. The increase in IL-5 levels after PLA exposure might be seen as enhancement of TH2-responses, although it did not correspond to changes in ECP levels. Cell culture experiments showed an increase in the level of IL-13 after exposure to ABS-based emissions.[43] This cytokine is known to be linked to IL-5, but IL-5 did not change with ABS exposure, only with PLA exposure. We believe that these interpretations can be considered no more than a hint, but still they might be useful for future research. Furthermore, it might be worthwhile to replace IFN-γ by IL-13 in future investigations.

The combined CO/NO lung diffusing capacity has been used in several studies to assess potential effects of challenges such as inhaled hypertonic aerosols[26] or laser printer emission.[27] From the measured values of NO and CO diffusing capacity, estimates of membrane factor and pulmonary capillary blood volume could be derived, but these derived values were not more informative than the directly measured values of diffusing capacity. There were no changes in DL_{CO}, indicating that no relevant changes in pulmonary capillary blood volume occurred. Regarding the observed changes in DL_{NO}, several potential mechanisms might have been involved. For a detailed discussion of these findings, please see the Appendix S1.

5 | LIMITATIONS

The time interval of 1.5 h between the end of the exposure to 3-D printer emissions and the allergen challenges might have been too short to affect allergen responsiveness, although time intervals of this magnitude have been found to be sufficient for other air pollutants such as ozone.[21] In addition, the time interval of 1.5 h between the end of the allergen challenge and the sampling of nasal secretions might also have been too short to detect effects of previous exposures on allergen responses, although the acute response to allergen renders it likely that changes occur within

TABLE 4 Responses to nasal allergen administration as measured by rhinomanometry.

		Previous PLA exposure				Previous ABS exposure				
Variable		NaCl	Allergen	Δ	*p* Value NaCl vs. allergen	NaCl	Allergen	Δ	*p* Value NaCl vs. allergen	*p* Value between values of Δ
Challenged nostril	Flow (ml/s) at Δp 150 Pa	182.7 ± 55.3	91.4 ± 38.5	−91.3 ± 49.2	**<0.001**	191.1 ± 65.1	96.4 ± 44.9	−94.7 ± 48.9	**<0.001**	0.603
	Flow (ml/s) at Δp 300 Pa	321.5 ± 87.6	177.3 ± 59.0	−144.2 ± 76.5	**<0.001**	331.6 ± 86.3	184.2 ± 71.2	−147.4 ± 63.7	**<0.001**	0.551
Control nostril	Flow (ml/s) at Δp 150 Pa	99.8 ± 51.8	81.4 ± 38.9	−18.4 ± 33.7	**0.006**	97.5 ± 52.2	87.9 ± 42.9	−9.6 ± 33.9	0.182	0.182
	Flow (ml/s) at Δp 300 Pa	194.2 ± 85.6	167.4 ± 58.9	−26.8 ± 58.1	**0.020**	199.1 ± 81.1	173.9 ± 69.6	−25.1 ± 54.7	**0.021**	0.648
Challenged minus control	Flow (ml/s) at Δp 150 Pa	82.9 ± 60.5	10.0 ± 42.2	−72.9 ± 56.2	**<0.001**	93.6 ± 86.1	8.5 ± 61.3	−85.1 ± 54.8	**<0.001**	0.134
	Flow (ml/s) at Δp 300 Pa	127.3 ± 93	9.9 ± 64.1	−117.4 ± 88.7	**<0.001**	132.6 ± 120	10.3 ± 95.9	−122.3 ± 75.4	**<0.001**	0.525

Note: Challenges were performed 1.5 h after previous exposure to either PLA or ABS, using the same type of allergen and the same dose. For challenges, the nostril with the higher flow rate was chosen as recommended in guidelines (for details see text). Initial NaCl application served as reference, and allergen was administered in a single dose that had been found at the recruitment visit. Mean values ± SD are given for flow rates at two standardized pressure differences across the nostrils, as well as for the differences between values of the challenged nostril and the control nostril. Comparisons pre vs. post were performed with the Wilcoxon matched-pairs signed-ranks test, as well as comparisons of the changes (Δ) between the two exposures. Comparisons with *p*-values <0.05 are marked in boldface.

Abbreviations: ABS, acrylonitrile butadiene styrene; PLA, polylactic acid.

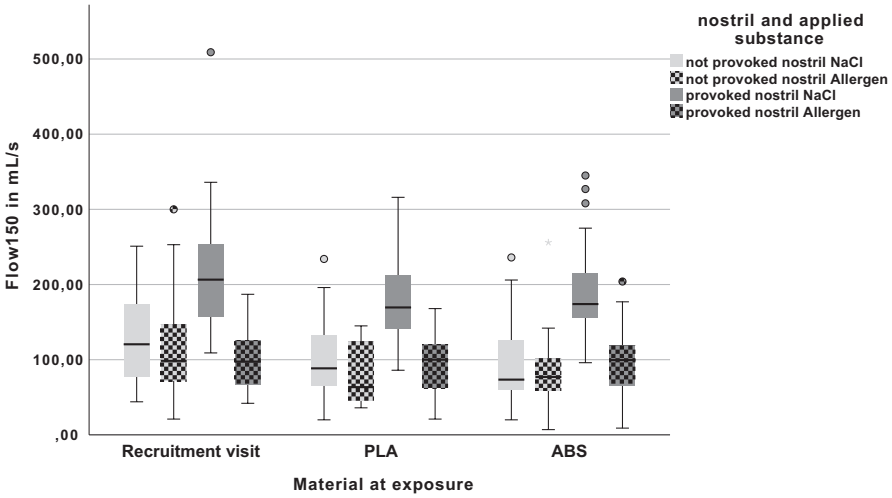

FIGURE 3 Box plots of flow rates at a pressure difference of 150 Pa assessed via rhinomanometry during nasal allergen challenges. Results are shown for the recruitment visit (left), PLA exposure (middle) and ABS exposure (right). The left two bars of each block show the values before and after allergen administration in the unchallenged control nostril, the right two bars the values before and after allergen application in the challenged nostril. For numerical data and statistical comparison see Table 4 and the text. ABS, acrylonitrile butadiene styrene; PLA, polylactic acid.

TABLE 5 Measured values of nasal secretion samples at the recruitment visit and the two exposure visits.

	Test			Comparison and *p* value		
Variable	Recruitment visit	Post PLA	Post ABS	Recruitment vs. PLA	Recruitment vs. ABS	PLA vs. ABS
Measured values						
IL-5 (pg/ml)[a]	6.53 (2.54; 4.54)	9.68 (2.82; 6.80)	6.95 (2.05; 4.40)	**0.026**	0.509	**0.062**
IL-6 (pg/ml)[a]	90.57 (1.81; 3.05)	76.38 (1.85; 2.69)	59.98 (1.59; 3.00)	0.106	**<0.001**	**0.065**
TNF (pg/ml)[a]	6.27 (1.82; 3.35)	5.71 (1.88; 2.51)	5.16 (1.69; 2.81)	0.362	**0.036**	0.274
ECP (ng/ml)	35.85 (1.22)	35.51 (1.28)	36.64 (1.29)	0.829	0.469	0.657
Protein (µg/ml)	6152.3 (1.86)	6061.2 (1.97)	5998.7 (1.87)	0.675	0.517	0.981
Values relative to protein concentration						
IL-5/Protein (fg/µg)	0.97 (4.23)	1.46 (5.63)	1.16 (3.76)	0.182	0.337	**0.039**
IL-6/Protein (fg/µg)	14.01 (2.22)	12.29 (2.17)	10.00 (2.30)	0.889	**0.032**	0.349
TNF/Protein (fg/µg)	0.99 (2.21)	0.91 (1.74)	0.86 (1.90)	0.439	**0.039**	0.428
ECP/Protein (µg/mg)	5.67 (2.06)	5.95 (2.09)	6.12 (2.25)	0.737	0.439	0.989

Note: Nasal secretion samples were taken 90 min after nasal allergen provocation. Geometric mean values and SD (in parentheses) are given, the latter being interpreted as dimensionless variability factors. The first SD in the parentheses describes the variance after variance reduction as described below, the second value the variance obtained with the raw values. To account for the dilution of samples, values relative to protein concentration are also given. Comparisons between values obtained at different days were performed with the Wilcoxon matched-pairs signed-ranks test. In the upper three rows ([a]), statistical comparisons were performed using the procedure of unbiased variance reduction using cytokine levels as described in the Section 2. The values of the protein content and ECP in the middle lines of the table were analyzed as raw values. The lower part provides the values of the ratio of cytokine and ECP levels to protein content, as an alternative method of normalization. Different from Tables 2–4, comparisons with *p*-values <0.10 are marked in boldface in order to emphasize the similarity between the results for the two normalization approaches.

Abbreviations: ABS, acrylonitrile butadiene styrene; ECP, eosinophil cationic protein; IL-5, interleukin 5; IL-6, interleukin 6; PLA, polylactic acid; TNF, tumor necrosis factor.

this time interval. From our previous study,[1] we knew that sampling of nasal secretions per se elicits effects on the nasal mucosa resulting in alterations of subsequent samples even several hours later. This was the reason why we collected secretions only once after the allergen challenges and not additionally prior to them or several hours later.

It also might be argued that particle deposition in the nose is much lower than that in the bronchial tract, thus bronchial allergen responses would have been more sensitive toward enhancement by 3-D printer emissions. The fact that we did not observe changes in spirometric values does not exclude this possibility as it has been demonstrated that the bronchial allergen responsiveness can be enhanced by ozone inhalation even in the absence of any other effects on function and symptoms.[20] Our study also did not address the question, whether multiple, long-term exposures would enhance allergen responses. In addition, the possibility of

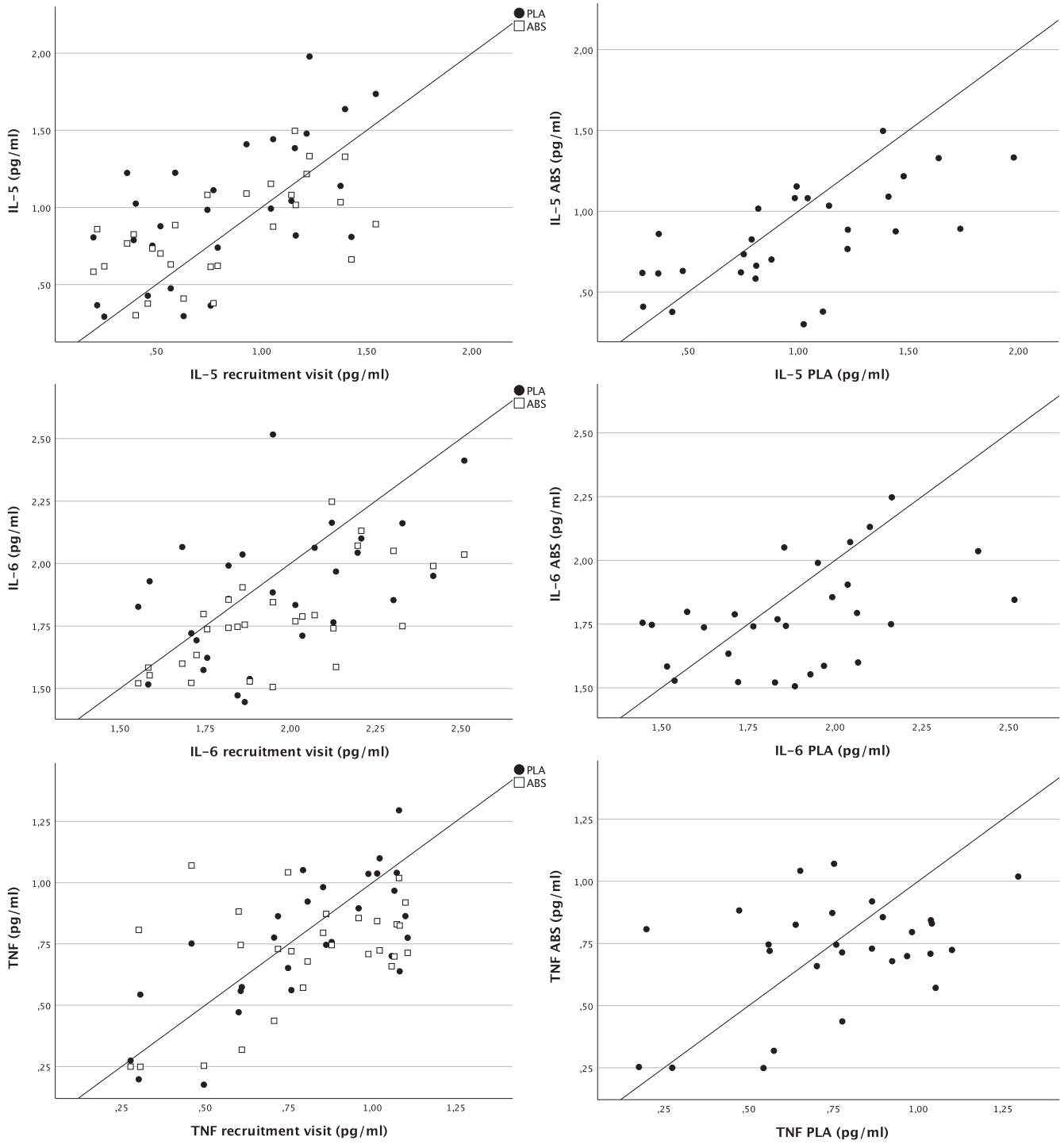

FIGURE 4 Cytokine concentrations in nasal secretions sampled after allergen challenges. Data have been normalized for cytokine content (see Section 2). The panels on the left side (IL-5, IL-6, TNF) show the values obtained at the recruitment visit on the horizontal axis, and the values obtained at the PLA (filled circles) and ABS exposure days (open rectangles) on the vertical axis. To illustrate the relationship between PLA and ABS results, the panels on the right side show the same values with PLA data on the horizontal axis and ABS data on the vertical axis. The lines are of identity. ABS, acrylonitrile butadiene styrene; IL-5, interleukin 5; IL-6, interleukin 6; PLA, polylactic acid; TNF, tumor necrosis factor.

enhanced bronchial allergen responses in patients with a history of asthma has to be kept in mind, particularly after multiple exposure to 3-D printer emissions. This has been shown for pollutants such as ozone[20] and would be in line with the existing case report.[19] As the potential alveolar responses assessed by the combined diffusing capacity and the nasal responses assessed by allergen challenges rely on different mechanisms in different compartments and there are no hints on a mutual relationship, at least

not in subjects with allergic rhinitis and normal lung function, we consider it valid to have addressed both study questions within one set-up. In the nasal tests, we used commercially available allergen solutions for grass, birch, and ragweed that were approved by German authorities for clinical routine and are in widespread use. These solutions contained mixtures of allergens extracted from the respective plants but not purified allergen molecules (for details, see Section 2). We used these extracted mixtures in order to better match the spectrum of allergens encountered in the environment. When selecting different allergen solutions, the potential differences between solutions would probably require to follow the dose recommendations for clinical use provided by the manufacturer in order to reproduce our results.

Compared with our previous study,[1] particle emissions showed differences, which we could not explain. The handling of the printer was the same, printing temperatures were within the recommended ranges, and filaments were of the same type and from the same manufacturer but from different batches. There were no statistically significant correlations between individual exposure levels, either quantified as mean, median, upper quartile, or 90th percentile values of LDSA over time, and individual lung function or nasal responses.

The small amount of material in nasal secretions prevented us from including a broad panel of cytokines and other mediators. It might have been of advantage to include cytokines such as IL-4, IL-10 and IL-13 but only four cytokines could be reliably measured. We considered IL-6 and TNF indispensable as markers of non-specific inflammation that have been assessed in numerous studies on air pollutants. Moreover, we preferred IL-5 compared with IL-4 and other cytokines because of its assumed link to eosinophil activation and thus ECP levels. In the present data, IL-5 and ECP levels after nasal allergen challenges were not associated with each other, probably due to the variability in biochemical allergen responses. It also has to be kept in mind that the differences in allergen responses between the exposure days and the recruitment day might be, at least partially, attributed to the difference in allergen administration. However, the comparison of PLA and ABS, that is, the primary aim of our study, was not affected by this. Thus, our results regarding the comparison between PLA and ABS can provide methodological insight that could be relevant for the design of further experiments.

6 | CONCLUSION

We studied potential health effects of emissions from a 3-D printer in volunteers with a history of seasonal allergic rhinitis. For this purpose, nasal allergen challenges were performed after experimental short-term exposures to emissions from two 3-D printing materials (PLA and ABS). Compared with control data from the recruitment visit, functional allergen response was not different between PLA and ABS, while the slight attenuation compared with the recruitment visit was likely to be due to methodological differences. The changes in the cytokine content of nasal secretions sampled after allergen challenges were small and difficult to interpret. Moreover, the diffusing capacity to inhaled nitric oxide but not that to inhaled carbon monoxide slightly declined after both exposures. The present data do not provide solid evidence that short-term exposures to 3-D printer emissions elicit health effects in subjects with allergic rhinitis that may be considered as clinically relevant, especially regarding potential amplification of nasal allergen responses. However, the effects of long-term exposure to 3-D printer emissions still require further research in view of the increasing use of 3-D printers in young people, including children, many of whom may be allergic. Until then, users of 3-D printers should follow recommendations by environmental agencies[44] and avoid the exposure to 3-D printer emissions by sufficient room ventilation and absence during the printing process.

AUTHOR CONTRIBUTIONS

Philipp Würzner was involved in data curation, formal analysis, investigation, software, writing—original draft and review & editing. **Rudolf A. Jörres** was involved in conceptualization, formal analysis, methodology, writing—original draft and review & editing. **Stefan Karrasch** was involved in conceptualization, supervision, investigation. **Caroline Quartucci** was involved in supervision and investigation. **Stephan Böse-O'Reilly** was involved in supervision and investigation. **Dennis Nowak** was involved in conceptualization and supervision. **Stefan Rakete** was involved in conceptualization, methodology, project administration, resources, funding acquisition, supervision, investigation.

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CONFLICT OF INTEREST

The authors declare they have no actual or potential competing financial interests. No conflict of interest declared.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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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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