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Acute Exposure to Environmental Tobacco Smoke: A Controlled Study in Adults with Asthma

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Keywords
Allergic asthma · Environmental tobacco smoke · Lung function · Inflammation · Respiratory symptoms

Abstract
Background: Short-term, indoor exposure to environmental tobacco smoke (ETS) is still highly prevalent; however, little is known about the acute lung response in adult asthma. ***Objectives:*** We investigated whether acute, experimental ETS exposure influences symptoms, lung function, and inflammatory parameters. ***Methods:*** Human subjects with asthma (n = 23) were exposed for 180 min to either room air or ETS at 250, 450, or 850 µg/m^3. Respiratory symptoms, lung function, and exhaled nitric oxide (FeNO) were measured. Additionally, blood samples were analyzed for pro- and anti-inflammatory cytokines. ***Results:*** Humans with asthma demonstrate an increase in respiratory symptoms at all levels of ETS exposure, while the forced expiratory volume in 1 s (FEV_1) and FeNO decrease with increasing ETS. The anti-inflammatory cytokine interleukin (IL)-10 increases at intermediate ETS concentrations, whereas tumor necrosis factor (TNF)-α and IL-8 increase only at the highest ETS concentration. ***Conclusion:*** Following 180 min of acute, experimental ETS exposure, we observed a significant increase in respiratory symptoms, a decrease in lung function, and an increase in inflammatory cytokines, indicating an acute lung response in asthma.

Introduction

Asthma is one of the most common chronic diseases worldwide, with 300 million people affected [1, 2], and in many countries the asthma prevalence is still rising. Therefore, understanding environmental tobacco smoke (ETS) and its influence on asthma is important and has clinical and public health implications [3].

Despite stricter smoking policy in many countries, the prevalence of smoking worldwide remains at extremely

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high levels. It is well known that, besides other risk factors like obesity, active smoking in subjects with asthma leads to poor disease control, more frequent exacerbations, and increased respiratory symptoms [4, 5]. Additionally, at least one third of asthmatics is involuntarily exposed to ETS, a complex mixture of over 4,000 chemicals, particles and heavy metals with the potential to exacerbate airway inflammation [6]. The toxic substances of ETS can alter the structure and function of the mucosal surface and affect epithelial integrity as well as immune networks [7]. Furthermore, proinflammatory cytokines like interleukin (IL)-4, IL-8, and tumor necrosis factor (TNF)-α increase epithelial permeability and thereby promote airway inflammation [8, 9].

ETS is a major contributor to indoor air pollution and it is responsible for up to 90% of fine particulate matter (PM2.5) exposure. PM2.5 measurements in restaurants and bars have shown PM2.5 values of up to 474 μg/m^3 [10]. These values strikingly exceed the mean outdoor 24-h PM2.5 threshold of 25 μg/m^3 as currently defined by WHO guidelines [11].

A large body of evidence indicates that indoor ETS exposure is a serious health risk, and it has been shown to be related to incident asthma and impairment of lung function [12]. However, the potential harm of acute, high-level indoor ETS exposure as still occurring in many public environments worldwide still needs to be studied.

ETS is associated with a number of respiratory outcomes, especially in subjects with asthma [13]. The association between ETS and asthma, indicating a potentially highly toxic ETS effect, is observed for respiratory symptoms, exacerbations, emergency department visits, hospitalizations, asthma severity, and a decreased lung function [13]. However, many of these data are based on cross-sectional studies and therefore subject to bias; many involve only children, known to be especially vulnerable to ETS due to immature lungs and an immature immune response [14]. Only a small number of studies on ETS and asthma report repeated observations in cohort settings but still are observational in nature. To overcome some of these shortcomings with regard to causality, we designed a pilot study to experimentally expose adults with allergic asthma to room air and ETS at different concentrations. The objective of the present pilot study was to investigate the effect of experimental, short-term indoor ETS exposure on respiratory symptoms, lung function, and the lung inflammatory response in asthma. The experimental set-up of our study should allow a clearer definition of the temporal, and therefore most likely causal, sequence of ETS exposure und lung response.

Table 1. Baseline characteristics of study subjects

	Ambient air (n = 7)	ETS (n = 16)	p value
Age, years	30	34	0.137
Male/female ratio	3/4	5/11	
BMI	24	25	0.318
FEV_1, % predicted	87	89	0.387
FEV_1 post-bd, % predicted	84	86	0.379
FEV_1/FVC ratio	0.74	0.76	0.244
Asthma control test	20	20	0.497

Values are presented as means unless otherwise stated. bd, bronchodilator.

Materials and Methods

Study Subjects and Study Design

Twenty-three nonsmoking adults with mild to moderate well-controlled allergic asthma [1] were randomized to ETS (n = 16) or room air (n = 7). The subjects were exposed for 180 min either to room air or to different levels of ETS for up to 3 times in weekly intervals. However, some participants with asthma declined to complete all planned 3 ETS exposures. Some subjects were exposed to a certain concentration only twice, and not all subjects started their exposure with the lowest ETS concentration. ETS was produced by a custom-made humidified smoking device capable of burning up to 5 cigarettes in parallel to generate indoor PM2.5 concentrations of 250 μg/m^3 (ETS250), 450 μg/m^3 (ETS450), and 850 μg/m^3 (ETS850).

All of the participants were asked to avoid ETS exposure within at least 12 h prior to the study exposure. All of the experiments and analyses were performed between December 2008 and February 2010.

Evaluation of Asthma Symptoms and Lung Parameters

According to the study protocol asthma symptoms were evaluated using a visual analog scale (VAS) ranging from 0 (indicating no symptoms) to 100 (indicating an almost intolerable severity of symptoms) at 0, 90, and 180 min of exposure [15]. Spirometry (forced expiratory volume in 1 s [FEV_1]), forced vital capacity [FVC], and peak expiratory flow [PEF]) was performed according to the joint guidelines of the European Respiratory Society and the American Thoracic Society (ERS/ATS) using a hand-held spirometer (EasyOne™ 2001; ndd, Zürich, Switzerland) at 0, 15, 60, 90, 120, and 180 min of exposure as well as 60 min thereafter (240 min) [14]. The fraction of exhaled nitric oxide (FeNO) using a hand-held NO analyzer (NIOX MINO® system; Aerocrine, Solna, Sweden) and exhaled CO were measured using a Smokerlyzer device (Bedfont Scientific Ltd., Kent, UK) at 0, 90, and 180 min of exposure [16, 17]. Blood samples were drawn at 0 and 180 min, i.e., at the beginning and the end of exposure.

Luminex Analysis of Cytokines

Human cytokine panels were used for the simultaneous quantification of IL-8, IL-10, IL-13, and TNF-α in human sera (Milli-

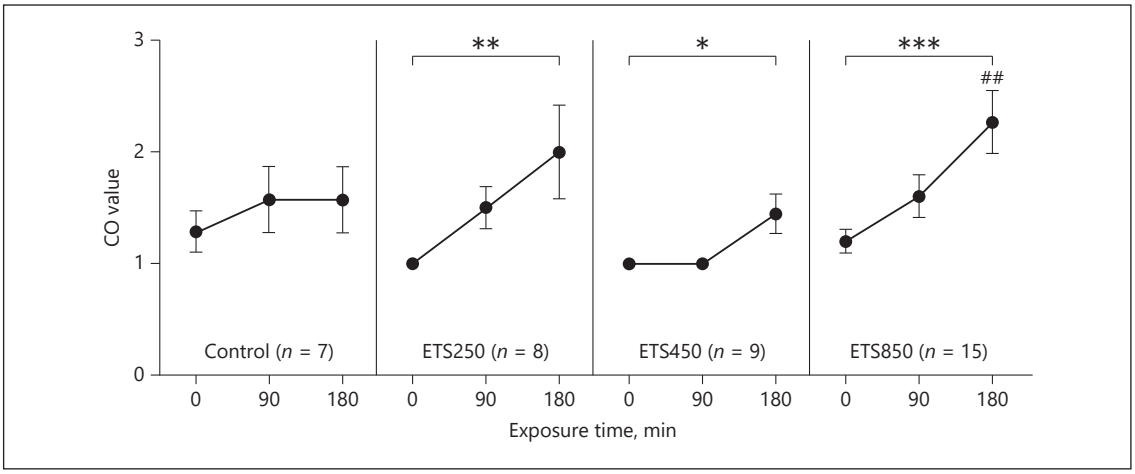

Fig. 1. Exhaled CO over time for room air (control; $p = 0.058$), ETS250 ($p = 0.002$), ETS450 ($p = 0.019$), and ETS850 ($p < 0.001$). * $p \leq 0.05$, **/## $p \leq 0.01$, and *** $p \leq 0.001$, where # indicates significance compared to 0 min of exposure, corrected with Dunn's test for multiple comparisons. Data are shown as means ± SEM.

pore Corporation; Billerica, MA, USA) using a Luminex 100 system (Luminex Corporation, Austin, TX, USA).

Statistical Analyses

Statistics was performed using GraphPad Prism (GraphPad Software, La Jolla, CA, USA). Parametric and nonparametric tests were used, and $p \leq 0.05$ was considered statistically significant. Statistical differences were calculated using a paired *t* test or the Wilcoxon signed-rank test, and for intergroup comparisons an unpaired *t* test or the Mann-Whitney rank-sum test was used. The Friedman test, which is a nonparametric test for comparing 3 or more related samples, was performed with time rows within one group.

Results

Clinical Evaluation of Lung Parameters and Asthma Scores

Twenty-three adults with allergic asthma were enrolled into this study. Their baseline characteristics are shown in Table 1 and indicate no significant difference between those randomized to the room air group and those randomized to the ETS group. All of the subjects randomized to the room air group completed all 3 exposures; however, only 7 subjects randomized to the ETS group completed all 3 exposures (ETS250, ETS450, and ETS850); the remaining 9 subjects declined to complete all 3 ETS exposures, reporting extreme unpleasantness of the exposure, and completed only exposure at 2 levels (ETS250 and ETS450) or a single level (ETS450).

To validate ETS exposure, exhaled CO was measured. Following 180 min of ETS exposure, CO values significantly increased in all ETS groups, whereas no significant increase was seen with room air (Fig. 1).

Following 180 min of ETS exposure, asthma symptoms (VAS) significantly increased at each concentration, while no changes were observed after exposure to room air (Fig. 2). The level of ETS exposure apparently had no effect on symptom scores at different time points. Both the kinetics and total scores were similar at each ETS concentration. As expected, the room air exposure group showed no significant changes at different time points.

Spirometry revealed a significant decrease in FEV_1 at ETS450 and a similar trend in PEF measurements. No significant lung function changes were measured at ETS250 and ETS850 (Table 2).

As shown in Table 3, exposure to ETS resulted in a reduction of FeNO at all 3 ETS concentrations, with similar values at different time points. Due to high SD, the ETS250 group showed no statistically significant change. FeNO did not change after room air exposure (Table 3).

Cytokine Analysis from Human Blood Serum

Cytokine analysis from blood serum revealed a trend toward elevation of both the proinflammatory cytokines IL-8 and TNF-α and the regulatory cytokine IL-10, with statistical significance for IL-10 ($p = 0.048$) in the ETS450 group and for IL-8 ($p = 0.007$) and TNF-α ($p = 0.008$) in the ETS850 groups. A similar trend could be seen with the asthma-related proinflammatory cytokine IL-13 ($p = 0.106$). Exposure to room air or ETS850 showed no effect (Fig. 3).

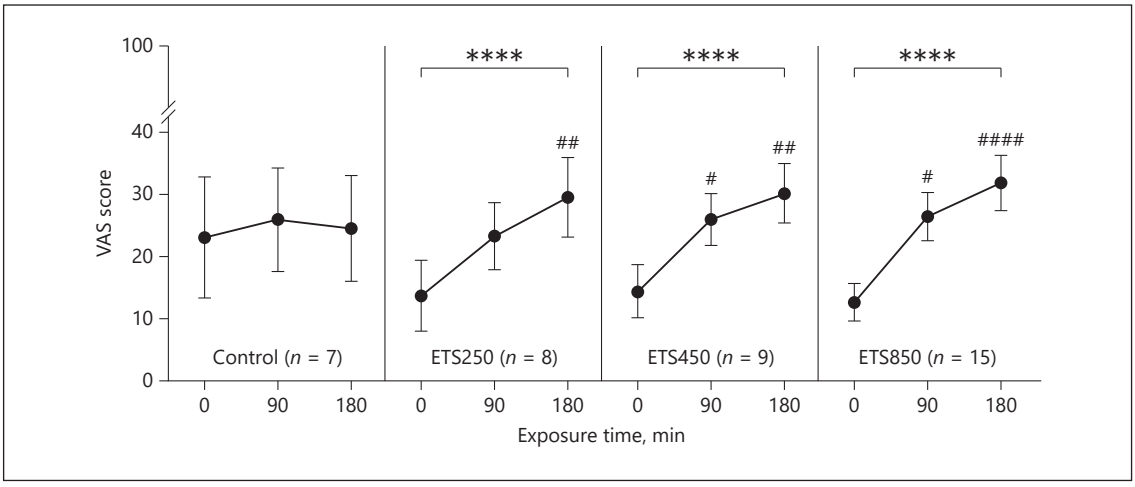

Fig. 2. Asthma symptom scores at different time points and ETS concentrations. A significant increase can be observed after 180 min of exposure to ETS250 ($p < 0.001$), ETS450 ($p < 0.001$), and ETS850 ($p < 0.001$). No differences between time points were detected after exposure to room air ($p = 0.667$). $^{\#}$ $p \leq 0.05$, $^{\#\#}$ $p \leq 0.01$, and $^{****/\#\#\#\#}$ $p \leq 0.0001$, where $^{\#}$ indicates significance compared to 0 min of exposure, corrected with Dunn's test for multiple comparisons. Data in are shown as means ± SEM.

Table 2. Lung function in subjects exposed to ETS and room air (control)

	Group	0 min	90 min	180 min	240 min	*p* value
FEV_1	Control (*n* = 7)	3.20	3.19	3.27	3.30	0.815
	ETS250 (*n* = 8)	3.24	3.21	3.21	3.28	0.489
	ETS450 (*n* = 9)	3.22	3.16	3.12	3.22	0.007
	ETS850 (*n* = 15)	3.22	3.15	3.17	3.22	0.882
FVC	Control (*n* = 7)	4.44	4.37	4.48	4.59	0.692
	ETS250 (*n* = 8)	4.32	4.31	4.18	4.32	0.861
	ETS450 (*n* = 9)	4.22	4.13	4.15	4.35	0.215
	ETS850 (*n* = 15)	4.18	4.16	4.18	4.31	0.392
PEF	Control (*n* = 7)	7.97	7.87	7.91	8.16	0.563
	ETS250 (*n* = 8)	7.98	8.22	8.01	8.31	0.136
	ETS450 (*n* = 9)	8.11	7.79	7.68	8.11	0.204
	ETS850 (*n* = 15)	7.98	7.84	7.96	8.04	0.650

p values are presented as intergroup comparisons using the Friedman test.

Discussion

ETS is considered a health hazard. Evidence comes from studies on chronic tobacco smoke exposure correlating exposure with incidence of lung cancer or cardiovascular disease [18–20]. While regulatory standards concerning outdoor air pollution are based on measureable exposure limits, indoor air pollution arising from acute ETS has not been measured in that way. Guidance

Table 3. Exhaled FeNO

	0 min	90 min	180 min	*p* value
Room air (*n* = 7)	46.3	45.0	42.7	0.100
ETS250 (*n* = 8)	25.1	21.9	21.9	0.619
ETS450 (*n* = 9)	23.4	20.7	21.1	0.028
ETS850 (*n* = 15)	33.7	32.1	29.1	<0.001

Values are presented as means.

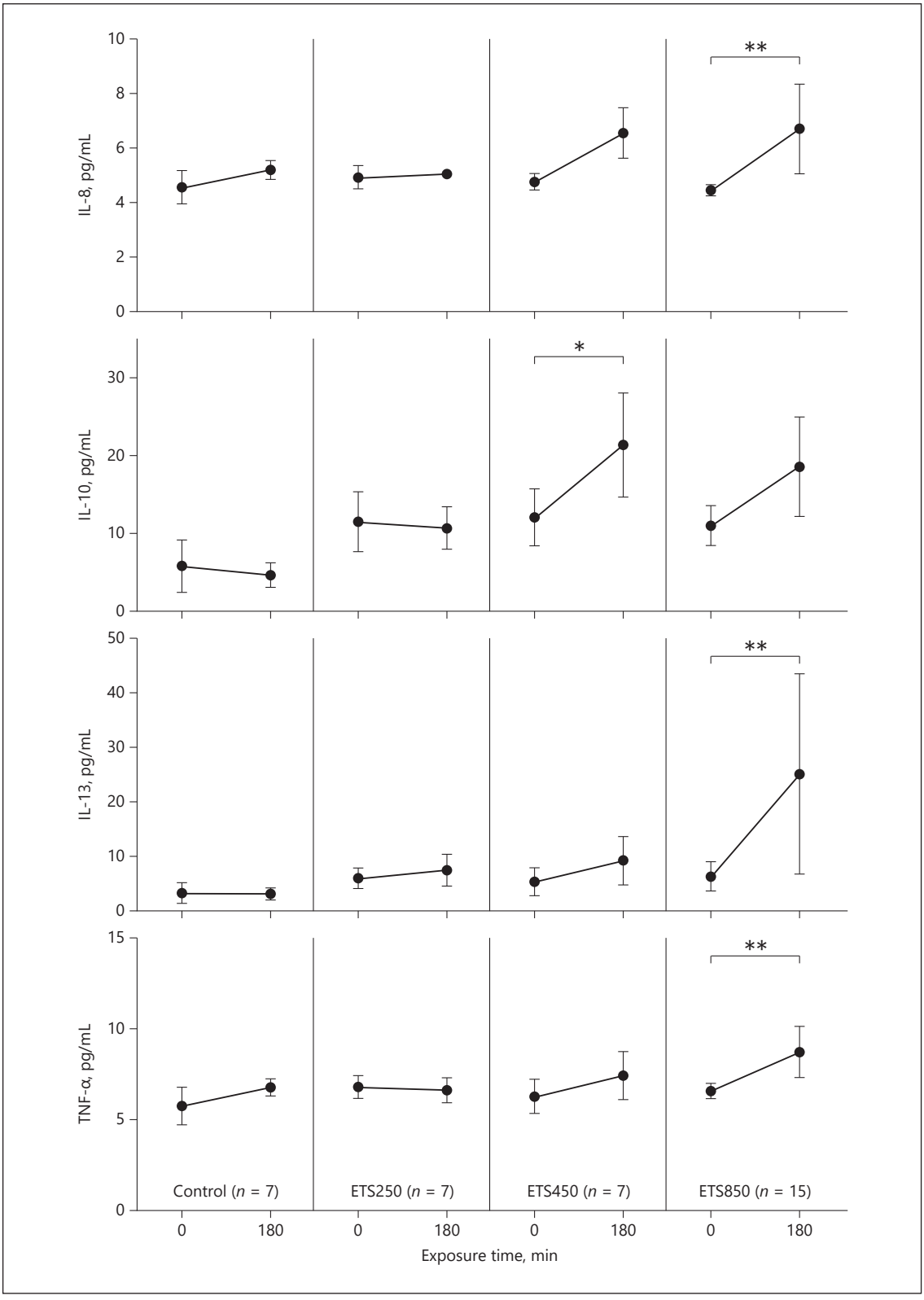

Fig. 3. The level of proinflammatory (IL-8, IL-13, and TNF-α) and anti-inflammatory cytokines (IL-10) was measured before (0 min) and after (180 min) exposure to ambient air, ETS250, ETS450, and ETS850. * $p \leq 0.05$ and ** $p \leq 0.01$. Data are shown as means ± SEM.

to minimize ETS exposure stems from observations made in exposed children, when ETS exposure was defined either by questionnaire or by cotinine measurement [21–23].

The focus of the present pilot study was to characterize short-term indoor exposure to ETS in a quantifiable and measureable way, comparable to the measurement of outdoor air pollution, and investigate acute effects in humans with allergic asthma. The experimental approach and the exposure set-up ensured provision of a defined concentration of PM2.5 from ETS. ETS exposure was further validated by measuring exhaled CO levels in the exposed study participants.

The data collected from our experiments in humans with allergic asthma clearly point to an increase in respiratory symptoms at all measured ETS concentrations when compared to room air. For some parameters a dose-dependent response was observed, e.g., for exhaled nitric oxide and serum cytokines, whereas the VAS symptom scores showed no such dose dependence. The presence of symptoms 180 min after exposure to ETS850 (and for some parameters also ETS250 and ETS450) fulfills the criterion of a relevant health hazard [24–26].

The observed lung function response indicates that short-term ETS is a risk for an acute and transient lung function decrease [27]. The latter is assumed to be caused by neurally mediated airways obstruction, the mechanisms of which include activation of vagal afferents causing airway irritation, irregular breathing patterns, and bronchoconstriction [28]. The nicotine contained in cigarette smoke triggers bronchoconstriction via the stimulation of rapidly adapting pulmonary receptors (RAR) and, together with bronchopulmonary C-fiber endings, they are responsible for defense reflexes, including coughing. The sensitivity of these sensory receptors has been shown to increase with airway mucosal inflammation [29]. The strong activation of vagal afferents under inflammatory conditions may be a possible explanation for the increased asthma symptoms as shown in VAS and the decrease in FEV_1 after ETS exposure. It also indicates a higher susceptibility to bronchoconstriction induced by cigarette smoke. This is in line with the evidence that airway hyperreactivity and airflow limitation are closely associated with an excess of reactive oxygen species and a diminished antioxidant capacity in the airways, as triggered by active and passive smoking [30].

Exhaled FeNO is considered a surrogate for eosinophilic airway inflammation in asthma and a helpful readout in asthma management [31–34]. Interestingly, we could observe a decrease in FeNO values after exposure to ETS, compared with exposure to room air. This finding is in line with published data demonstrating a short-term transient FeNO decrease in healthy subjects and asthmatics as a consequence of short-term ETS exposure [35–37]. Increased catabolism of NO or inhibition of inducible nitric oxide synthase (iNOS) has been postulated as the underlying mechanism of these findings. Therefore, an ETS-induced decrease in FeNO levels may also be associated with an increased risk of respiratory infection, bronchoconstriction, and vasoconstriction of pulmonary vessels.

Analysis of the proinflammatory cytokines TNF-α (T helper [TH] 1 type related), IL-13 (TH2 type related), IL-8 (chemokine CXCL8), and the regulatory cytokine IL-10 from human blood revealed a clear trend towards an increase in all 4 immune mediators after exposure to ETS450 and ETS850 (with statistical significance for IL-8, TNF-α, and IL-10 in at least 1 of the ETS450 and ETS850 groups). No trend could be seen after exposure to room air or ETS250. To summarize, the consistent increase in TH1 and TH2 type proinflammatory cytokines clearly points to an acute ETS-induced inflammatory lung response [14]. In view of this, the concurrent increase in the anti-inflammatory cytokine IL-10 may reflect a feedback mechanism to counterbalance the inflammatory response.

Strengths and Limitations of Our Study

Our study followed an experimental, randomized approach to expose subjects with well-controlled allergic asthma to either room air or different levels of ETS. Moreover, a number of highly relevant outcomes, i.e., respiratory symptoms, lung function, FeNo, and cytokines indicating lung inflammatory response were studied. In this way, our study uniquely adds to the abundance of literature on asthma and indoor ETS exposure as defined by a questionnaire or cotinine measurement [13].

We precisely defined acute ETS exposure and, immediately following exposure, studied outcomes, thereby minimizing bias from potentially confounding long-term exposure. This step-up allowed us to clearly delineate the causal, temporal association between ETS and health outcomes in adult, allergic asthma [38].

The most important limitation of our study is the unexpected differential drop out of study subjects who experienced the first ETS exposure as extremely unpleasant and therefore were unwilling to proceed with further exposure. This differential nonparticipation of subjects, likely sensitive to ETS, might have precluded a more significant and clear picture of the acute effects of ETS [39]. Further, confounding of a controlled ETS study exposure

by ETS exposure occurring in between study exposures cannot be ruled out, and cotinine measurements could have helped to quantify it.

Conclusion

In summary, the data of the present pilot study indicate that 180 min of exposure to an ETS trigger increased respiratory symptoms and decreased respiratory function in adults with asthma; this was demonstrated with respect to FEV_1, FeNO, and inflammatory cytokines. Due to the small number of participants and the unexpected, differential nonparticipation of asthmatics during this study (the majority of asthmatics declined to complete all 3 ETS exposures) significant changes following different level of ETS were not seen in all groups and/or concerning all read-out parameters.

Nevertheless, the data justify stating that acute indoor exposure to ETS constitutes a health risk for asthmatic individuals, and legislation worldwide should eliminate public ETS exposure and create smoke-free environments.

Statement of Ethics

The study protocol was approved by the Ethics Committee of Salzburg (E945/2–2008) and participants provided written informed consent.

Conflict of Interest Statement

The authors have no conflict of interests to declare.

Author Contributions

M.G., R.E.W., F.G-M, H.D., and B.L. performed the experiments. M.G, R.E.W, G.W., and B.K. analyzed the data. A.H. and M.S. designed the experiments. M.G., R.E.W., M.S. and A.H. wrote this paper.

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