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Upper airway inflammation in children exposed to ambient ozone and potential signs of adaptation

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Upper airway inflammation in children exposed to ambient ozone and potential signs of adaptation. M.V. Kopp, C. Ulmer, G. Ihorst, H.H. Seydewitz, T. Frischer, J. Forster, J. Kuehr. ©ERS Journals Ltd 1999.

ABSTRACT: In order to investigate nasal inflammation and subsequent adaptation after ambient ozone exposure, nasal lavage (NL) fluid was collected from 170 schoolchildren on 11 occasions (time points) between March and October.

Eosinophil cationic protein (ECP), albumin and leukocytes were quantified as markers of nasal inflammation. The highest half-hour outdoor O_3 concentration for each individual on the day prior to the NL was used as a measure of exposure (O_{3indiv}). To avoid confounding with exposure to common environmental allergens, the study population was restricted to children without sensitization to inhalant allergens.

In the initial period of increased O_3 levels in May (time point 4), with a median O_{3indiv} of 135 $\mu g \cdot m^{-3}$ (5th –95th percentile 100–184 $\mu g \cdot m^{-3}$), the highest medians of all 11 leukocyte and ECP measurements were observed. The highest O_{3indiv} were observed in June at time point 7 (O_{3indiv} 173 $\mu g \cdot m^{-3}$, 5th–95th percentile 120–203 $\mu g \cdot m^{-3}$). Cross-sectional analysis of all 11 time points revealed no significant association of O_{3indiv} on the one hand and ECP, albumin and leukocyte levels on the other. A multivariable model estimated using generalized estimating equations showed a statistically significant association of O_{3indiv} and leukocytes and ECP as the dependent variable, when time points 1–4 were analysed ($p<0.05$). In the same model, this association diminished continuously when time points 5–11 were added stepwise, in spite of high O_3 exposure. Not even a tendency towards an O_3 effect could be recognized when time points 1–8 were considered.

The results indicate: 1) acute inflammation of the nasal mucosa after the first increase in ambient ozone levels, with 2) a significant dose-dependent increase in leukocyte and eosinophil cationic protein levels, and 3) possible adaptation of the nasal mucosa in spite of constant high levels of ozone exposure in children during the summer season.

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In central Europe, the public is concerned about the health effects of ozone, a major air pollutant during spring and summer. Its short-term effects are well known: O_3 causes subjective complaints such as conjunctivitis, cough or shortness of breath [1] as well as reduced lung function [2–4] and an inflammatory response of the airway mucosa in animals [5, 6] and humans [7–9]. Significant increases in respiratory epithelial permeability, leukocyte count and albumin, eosinophil cationic protein (ECP) and myeloperoxidase (MPO) concentration as mediators of inflammation have been reported [8, 10, 11]. Despite a large number of studies, there is a lack of knowledge about the subchronic and chronic health effects and the time course of inflammation processes after natural O_3 exposure during the summer season. FRISCHER *et al*. [10] recently performed nasal lavage (NL) in schoolchildren and described a positive correlation between neutrophil count and ambient O_3 concentration. However, the question remains as to what degree children adapt to natural O_3 exposure. This report focuses on adaptation phenomena under high O_3 levels in a country area without industrial air pollution (the Black Forest).

Based on a repeated measurment design, 170 schoolchildren were examined at 11 time points from March to October using NL to investigate the time-dependent effects of inflammation and possible processes of adaptation of the nasal mucosa after ambient O_3 exposure.

Materials and methods

Study design

This epidemiological panel study on O_3-induced inflammation in the airways of schoolchildren comprised the children of the second and third grades of two primary schools in Freudenstadt and Villingen, two towns situated in the Black Forest (Southwestern Germany). The field study was carried out between March and October 1994. The study protocol was approved by the ethics committee of the University of Freiburg and written consent obtained from the parents.

Population

Out of a total of 201 questionnaires sent to pupils of the second and third grade of two primary schools, 181 (90%)

questionnaires completed by parents were received. The pupils were aged 8.0–10.7 yrs (Ninety per cent of median: 9.1 yrs). Of these 181 children, 170 (84.6%) participated in NL and 165 (82.1%) in skin-prick testing.

Methods

Skin-prick test. The skin-prick test was performed on the volar surface of the forearm using extracts of hazel, birch and grass pollens, dog and cat dander, dust mites (*Dermatophagoides farinae*, *D. pteronyssinus*), histamine hydrochloride (10 $mg \cdot mL^{-1}$) and sodium chloride (9 $g \cdot L^{-1}$) as control solutions. The allergens (Scherax, Hamburg, Germany) corresponded to a concentration of 10 histamine equivalent potency. All skin reactions were evaluated after 15 min. A mean weal diameter ≥2 min, together with a ratio of the allergen weal to the histamine weal >0.5, was considered to be a definite positive reaction [12]. The requirement that the allergen weal be greater than half the size of the histamine weal for positivity may have reduced the reported prevalence of atopy since, the histamine weal was shown to be highly variable in size. However, this criterion was used to compare the present data with earlier results [12].

Nasal lavage. NL was performed 11 times in each child between March and October 1994. For the NL, the protocol of KOREN *et al.* [13] was adapted. Using a syringe, 4 mL sterile phosphate-buffered saline at 37°C was instilled into each of the nasal cavities. After 10 s, the fluid was expelled into a sterile plastic cup. The procedure was performed separately for each cavity.

The specimens were stored at room temperature (~20°C) for 60 min. Then the fluid was filtered through coarse gauze (pore size 2 mm) and centrifuged (800 g twice for 10 min). The supernatant was frozen at -70°C until analysis of mediators. Cell pellets were resuspended in 100 µL albumin (20% Curasan®) and 500 µL phosphate buffer, and leukocytes were counted using a haemocytometer (Fuchs-Rosenthal chamber).

Inflammatory changes in the upper airways caused by air pollution are commonly described by analysing changes in inflammatory cells, protein markers of epithelial injury and markers of exudation. Therefore, leukocyte counts and concentrations of ECP (CAP-ECP-fluorescence-enzyme-immuno-assay (FEIA); Kabi-Pharmacia, Uppsala, Sweden) and albumin (rate nephelometry, Beckman, Dublin, Ireland) were measured. All biochemical analyses were performed in a blinded fashion. ECP, a cytotoxic protein found in the granules of eosinophils, has been suggested to be an important contributor in the pathogenesis of upper airway inflammatory diseases such as asthma and allergic rhinitis. Recently, ECP measurement in NL fluid was used as a tool in assessing the health effect of O_3 on upper airways in asthmatic as well as normal populations [10, 13–15]. MPO concentration, as an inflammation marker of activated neutrophils, was additionally measured during NL 1 and NL 4 in a total of 594 samples. Because the concentration of MPO and leukocyte counts proved to be correlated (r_s=0.7; Spearman correlation), further measurements of MPO were not taken.

Ozone monitoring. The ambient O_3 concentrations were measured at two locations at a distance of 1.3 km (Villingen) and 2.5 km (Freudenstadt) from the schools. Using the ultraviolet absorption method (Model 1008 AH; Firma Dasibi, USA, 48 half-hour mean values per day were registered. Sulphur dioxide and nitrogen dioxide were also measured using fixed monitors based on fluorescence (Model 8850; Monitor Labs, USA) and chemoluminescence (Model 8001; UPK, Bendix, BE) methods, respectively. Total suspended particles (TSP) were collected using volume samplers (digital high volume sampler, VDI 2463; Verewa) and the concentration of particles measured by means of β-absorption (Modell FH 62 IN; FAG).

The readings were taken by the regional environmental protection agency (LFU, Baden-Württemberg). As a measure of individual short-term O_3 exposure, the maximum O_3 concentration detected during the 24 h preceding NL was selected for each child.

Analysis

To avoid confounding with exposure to common environmental allergens, the study population was restricted to children with no positive reaction to any of the seven tested inhalant allergens. The data from the two communities were analysed together. Linear regression models were employed in order to evaluate the short-term effect of O_3 on inflammation markers. Inflammation markers were log10-transformed, and values below the detection limit were set to a value of 50% of the detection limit, *i.e.* 1.0 $\mu g \cdot L^{-1}$ ECP and 0.3 $mg \cdot dL^{-1}$ albumin, respectively. The influence of selecting these values on the results was investigated by generating random numbers for values below the detection limit; it was found that this did not alter the results. In the regression calculations, O_3 exposure was expressed in $mg \cdot m^{-3}$ rather than $\mu g \cdot m^{-3}$ in order to obtain parameter estimates of a reasonable size. Besides individual O_3 exposure, the models comprised sex, passive smoke exposure and the time spent outdoors on the day preceding NL by the children as independent variables as obtained from the questionnaire.

Two different models were adopted to investigate adaptation. First, the time periods were defined as seasons: "spring" (SP; time points 1–3), "early summer" (ES; time points 4 and 5), "late summer" (LS; time points 6–8), and "autumn" (AU; time points 9–11). The first model had four different O_3 variables relating to exposure during the time periods thus defined. The model equation is given by:

$$\text{Log ECP (or other variable)} = \text{Intercept} + (b_1 \times O_3SP) + (b_2 \times O_3ES) + (b_3 \times O_3LS) + (b_4 \times O_3AU) + \text{(further regressors)}$$

where O_3SP takes the corresponding O_3 concentration, if the measurement belongs to the spring period, and is defined to be zero otherwise, further regressors are adjustments for sex, passive smoke *etc.* and b_n is the parameter describing the ozone effect. O_3ES, O_3LS and O_3AU were defined in a similar manner. The purpose of this formulation was to model different O_3 effects during different times of the year in one model for all data collected.

The second method consisted of augmenting one model with one O_3 variable successively, *i.e.* starting with the data from the first three NLs, then adding the data from the 4th NL, until finally the complete data set was analysed. The model equation is:

$$\text{Log ECP}_j = \text{Intercept}_j + (b_j \times O_3) + (\text{further regressors}), \quad j=1.....T$$

The index j refers to the number of the time point, and T denotes the number of time points included in the calculation, ranging 3–11. Thus, j=1,...3 is the first regression, and j=1,...11 the last. Thus, the parameter estimates obtained at each step can be compared in order to answer the question as to whether different results (in terms of ozone parameter estimates) would have been achieved if the study had stopped earlier than it did.

Estimates for both of these regression models were obtained by applying the generalized estimation equations (GEE) method of LIANG and ZEGER [16]. This method takes the correlation of measurements coming from one individual into account by modelling a so-called working correlation matrix, which need not necessarily be correct, but intends to describe reality better than assuming independence among measurements. In the present case, it was assumed that there was equal correlation among all measurement coming from one subject. Further, by applying a quasi-likelihood approach [17] instead of likelihood methods, it was not necessary to assume an underlying error distribution. It was then possible to give robust estimates of the SEM. Since both models are linear regression models, the parameter estimate for O_3 may be interpreted as the slope, *i.e.* it gives the increase in log ECP concentration when O_3 concentration increases by 1 $mg \cdot m^{-3}$. Finally, the individual maxima of inflammation markers were calculated for each child and the time points at which these maxima were attained were investigated.

Statistical analysis was performed using the Statistical Analysis System (SAS, Cary, NC, USA) and an SAS-macro for solving the GEE of GRÖMPING [18].

Results

Study population and air pollution measurement

The distribution of the main characteristics of the study population and the nonatopic population are given in tables 1–3. All relevant variables showed a similar distribution between the two schools (data not shown). Therefore, the data of the two communities were pooled.

Table 1. – Main characteristics of the study population

Variable	Study population*		Nonatopic subpopulation$^+$	
	n	%	n	%
Subjects	170		113	
Sex: M/F	83/87	48.8/51.2	58/55	51.3/48.7
Villingen	97	57.1	60	53.1
Freudenstadt	73	42.9	43	38.1
SPT+	37	21.8	0	0.0
Tobacco smoke exposure	84	49.4	62	54.9
Age (median) yrs	9.1		9.1	

*: of 181 children with completed questionnaires, 170 participated in nasal lavage. $^+$: children with a positive reaction to any of seven tested inhalant allergens (n=37) or a doctor's diagnosis of asthma or allergic rhinitis (n=12), without skin-prick test (test refused) (n=5) and with less than six nasal lavages (n=3) were excluded; (SPT+=positive skin prick test).

Table 2. – Air pollution exposure

	Villingen			Freudenstadt		
	Mean	5%	95%	Mean	5%	95%
O_3 $\mu g \cdot m^{-3}$	64	1	140	105	45	179
NO_2 $mg \cdot m^{-3}$	14	3	35	13	4	30
SO_2 $mg \cdot m^{-3}$	3	0	9	3	0	9
TSP $\mu g \cdot m^{-3}$	24	4	54	17	8	30
PM10 $\mu g \cdot m^{-3}$	25	6	57	17	7	32

*: March 1, 1994–October 16, 1994. TSP: total suspended particles (data only available from March to August); PM10: particles with a 50% cut-off aerodynamic diameter of 10 μm (data only available from September to October); 5%: 5th percentile; 95%: 95th percentile.

The ambient O_3 concentrations in Villingen and Freudenstadt are presented as the daily maximum concentration and the daily median of half-hour means in $\mu g \cdot m^{-3}$ ($\mu g \cdot m^{-3} = 0.5 \times$ parts per billion) (fig. 1). Ambient O_3 levels started to increase at the beginning of May at time point 4. After a decrease, ambient O_3 levels again increased at the end of June.

To assess the individual ozone exposure (O_{3indiv}) of each child, the highest O_3 concentration of all half-hour means 24 h prior to NL was taken. Figure 1 shows the O_{3indiv} across all 11 time points. The O_{3indiv} shows a similar time course to ambient O_3 concentration, with the first increase in May at time point 4 (O_{3indiv} 135 $\mu g \cdot m^{-3}$, 5th–95th percentile 100–184 $\mu g \cdot m^{-3}$) and highest values in June at time point 7 (O_{3indiv} 173 $\mu g \cdot m^{-3}$, 5th–95th percentile 120–203 $\mu g \cdot m^{-3}$).

Mean ozone concentration during the whole summer season in Freudenstadt (105 $\mu g \cdot m^{-3}$) was higher compared to Villingen (64 $\mu g \cdot m^{-3}$). However, the O_{3indiv} of children living in Freudenstadt and Villingen were comparable and the NL analyses between the two communities did not differ significantly. Therefore, the data from the two communities were analysed together.

Table 2 shows the NO_2, SO_2, TSP and particles with a 50% cut-off aerodynamic diameter of 10 μm (PM10) concentrations in Villingen and Freudenstadt during the study period.

Leukocyte counts and eosinophil cationic protein and albumin concentration in nasal lavage fluid

Figure 2 shows the distribution of leukocyte counts, and ECP and albumin concentration. ECP concentration and leukocyte count showed their highest median values at time point 4, *i.e.* immediately after the first increase in ambient ozone levels. The median albumin concentration increased at time point 4 (2.39 $mg \cdot dL^{-1}$), but was not

Table 3. – Children's mean time spent outdoors on the day of the test in Villingen and Freudenstadt at time points 1–11

	Outdoor time min										
	1	2	3	4	5	6	7	8	9	10	11
Villingen	80	57	55	89	77	118	131	134	77	68	77
Freudenstadt	82	48	56	88	74	114	121	145	92	74	72

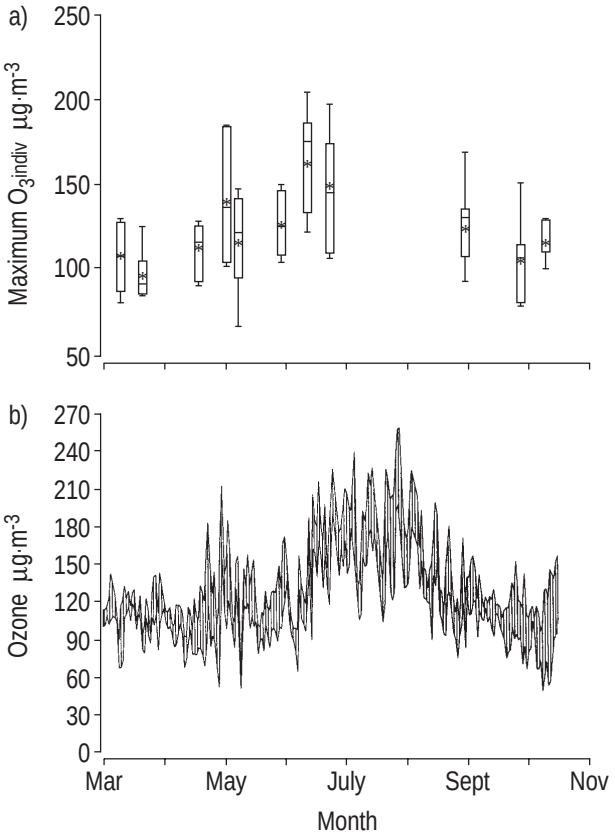

Fig. 1. – Individual ozone exposures (O_{3indiv}) across all 11 time points (a) and ambient O_3 concentrations in Villingen and Freudenstadt (b). The boxes include the interval between the 25th and 75th percentile with the extremes (5th and 95th percentile) represented by the vertical bars. the horizontal bar represnts the median; *: mean. Ambient O_3 concentrations are presented as the daily maximum concentration and the daily median of half-hour means. The 1st of each month is indicated. Mar: March; Sept: September; Nov: November.

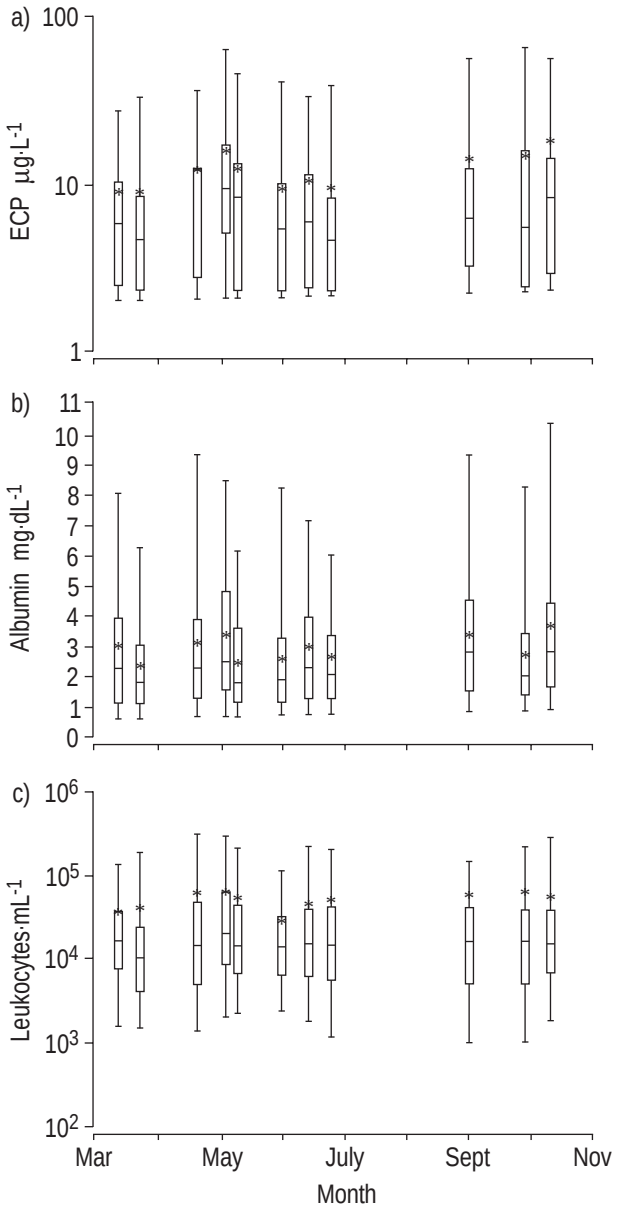

Fig. 2. – Distribution of: a) eosinophil cationic protein (ECP) concentration; b) albumin concentration; and c) leukocyte count in the nasal lavage fluid across all 11 time points. The box plots show the median and 25th and 75th percentiles, with the vertical bars representing the 5th and 95th percentiles. *: mean. The 1st of each month is indicated. Mar: March; Sept: September; Nov: November.

as high as at time point 9 (2.53 mg·dL^{-1}) or 11 (2.49 mg·dL^{-1}).

Figure 3 shows the distribution in time of individual peak leukocyte counts and albumin and ECP concentrations as well as O_{3indiv}. It is obvious that 50% of the children had their individual maximum before time point 5, whereas >50% had their maximum O_{3indiv} at time point 7 in June. These descriptive findings indicate that heights of inflammation markers are associated with the first increase in ambient O_3 and not with the maximum of ozone exposure.

Multivariate analysis

Employing multiple linear regression, the potential O_3 effect on markers of nasal inflammation were investigated cross-sectionally and with repeated measurement analysis. In each of the 11 cross-sections, no significant association of O_{3indiv} on the one hand and ECP concentration, leukocyte count and albumin concentration on the other was seen (data not shown). However, the ozone effect varied throughout the study period, with the highest parameter estimate being observed at time point 4.

In order to evaluate the repeated measurements, one GEE model was established for each marker of inflammation, each taking all 11 measurements into account (table 4). The variable of interest was O_{3indiv}, which was divided into four time-spans. During periods 1–3, a parameter estimate of -0.02 was calculated for ECP concentration, *i.e.* it describes the factor of nonsignificant decrease in log ECP concentration in cases in which ozone concentration increases by 1 µg·m^{-3}. Significant effects of ozone on ECP concentration and leukocyte count occurred in periods 4 and 5 and on leukocyte counts in periods 1–3 and 4 and 5. For ECP concentration, in periods 4 and 5, a parameter estimate of 0.97 describes the factor of significant increase in log ECP concentration in cases in which when ozone concentration increases by 1 µg·m^{-3}.

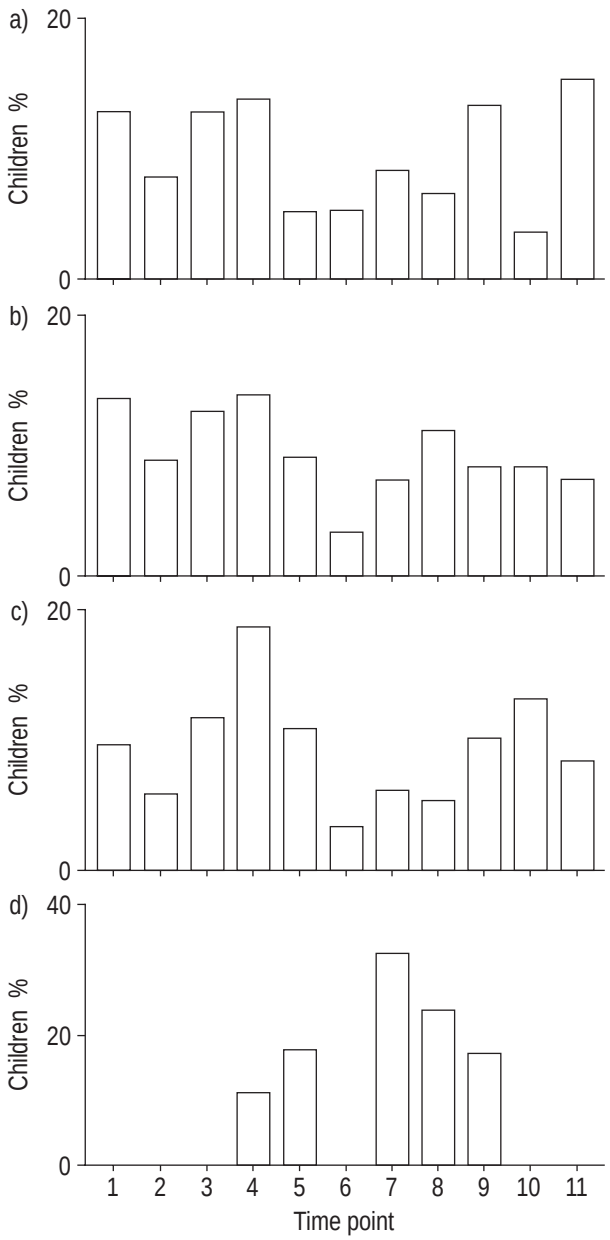

Fig. 3. – Distribution of maximal response of: a) albumin concentration; b) leukocyte count; and c) eosinophil cationic protein (ECP) concentration; as well as d) individual ozone exposure across all 11 time points. Peak responses were selected from the 11 time points of each child; the percentages of children with the maximum response are shown.

No significant effect was seen in any of the three models for periods 6–8 and 9–11. No significant association of O_3indiv and albumin concentration was seen in the model (data not shown).

In order to take chronological changes in the O_3 effects into account, a regression model including only data from period 1–3 was used and data from further time points were added stepwise (fig. 4). With regard to ECP concentration and leukocyte count, significant effects of ozone occurred when using data from periods 1–4, whereas the ozone parameter estimate became smaller in size when period 1–5 and 1–6 were added. When periods 1–11 were analysed, no tendency could be seen at all. This indicates that a significant ozone effect on nasal ECP concentration and leukocyte count can only be observed in the early summer period and that the inflammatory parameters diminish during the summer period.

Table 4. – Longitudinal analysis using the generalized estimation equations model for eosinophil cationic protein (ECP) concentration and leukocytes count

	ECP		Leukocytes	
	Parameter estimate	95% CI	Parameter estimate	95% CI
O3SP	-0.02	-1.16–1.12	1.99*	0.39–3.59
O3ES	0.97*	0.03–1.92	2.18*	0.76–3.59
O3LS	-0.43	-1.34–0.47	0.63	-0.66–1.91
O3AU	0.46	-0.82–1.63	0.96	-0.89–2.83
Sex	-0.22*	-0.35– -0.09	-0.12	-0.26–0.02
Passive smoke	0.07	-0.05–0.20	0.04	-0.11–0.18

The variable of interest was individual ozone exposure, which was divided into four time-spans or seasons: "spring" (SP, time points 1–3), "early summer" (ES, time points 4 and 5), "late summer" (LS, time points 6–8) and "autumn" (AU, time points 9–11). CI: confidence interval. *: $p<0.05$.

Discussion

By analysing data on inflammatory markers during a period of changing natural, O_3 exposure, it was possible to observe an inflammatory effect, the maximum of which followed the first increase in ambient O_3 levels in spring. This effect was most pronounced for ECP concentration, for which it gained statistical significance. The inclusion of further consecutive tests with high O_3indiv resulted in a statistical diminution of the O_3 effect, which could suggest adaptation in cases of continuous O_3 exposure.

The results could have been influenced by a number of potential biases. Other environmental factors such as airborne pollen or air pollutants, *e.g.* nitrogen oxides (NO_x), SO_2 or TSP/PM10, could have led to the observed results.

To avoid confounding with pollen exposure, all children with a positive skin-prick test were excluded. In the atopic population with at least one positive skin-prick test, the inflammatory markers of NL were separately analysed. The highest ECP concentrations were observed time points 6 and 7, when pollen counts reached their maximum, and not at time points 4 and 5, when the O_3 concentration peaked (data not shown). This argues against a possible confounding that the observed inflammatory response in the nonatopic population during spring could have been related to pollen exposure even though the skin-prick test results were negative [19].

Since a study region with very low concentrations of NO_x and SO_2 was selected, the confounding effects of these components in ambient air appeared negligible.

Only the TSP concentration and not PM10 or particles with a 50% cut-off aerodynamic diameter of 2.5 μm (PM25) data gathered during the present study are presented. As demonstrated by SPENGLER *et al*. [20], fine particle concentrations are highly correlated with O_3 exposure in the USA. The fine particles (PM10) are produced by the burning of fossil fuels or by photochemical reactions. By bypassing the mucociliary and cellular defence mechanisms, fine

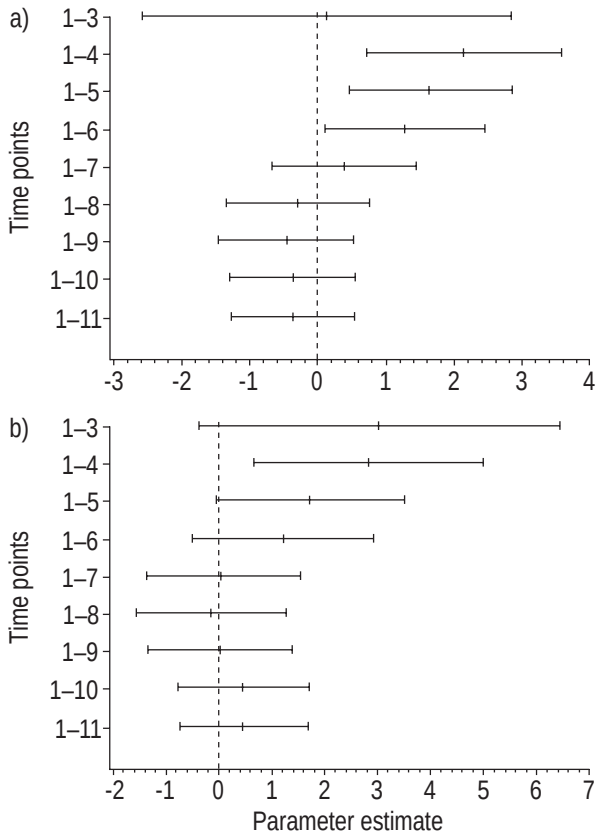

Fig. 4. – Multiple linear regression models for the assessment of parameter estimates for ozone exposure with regard to: a) $\log_{10}$ eosinophil cationic protein (ECP) concentration; and b) $\log_{10}$ leukocyte count. Initially, the data from the first three nasal lavages (NLs) was analysed, then the data from the 4th NL was added, and so on, until finally the complete data set was analysed. The ends of the horizontal bar indicate the 95% confidence interval, the central ticks indicate the parameter estimate. The horizontal bars which do not traverse the 0 represent statistically significant values.

particles can invade the lung and cause an inflammatory response [21]. Since fine particles are predominantly deposited in the alveolar space, the authors speculate that reaction of the nasal compartment reflects more an effect of O_3 than of fine particles. Nevertheless, it is known that O_3 is just one substance of a photochemical mixture during the summer season, and so the observed effect should be described as an O_3-related effect and O_3 should be regarded as a major component of summer air pollution.

The assessment of O_{3indiv} was based on the highest half-hour O_3 concentration in the 24 h before the child′s NL. This period of preceding exposure appeared appropriate, since ozone effects had previously been detected on the same basis [22, 23]. It must be emphasized that the chosen method of calculating O_{3indiv} is an "estimate" and in this sense only a surrogate parameter for the real exposure. Again, it was not possible to provide the study population of 103 children with a personal ozone sampler, which would have resulted in a more precise assessment of average exposure.

In addition to this methodological limitation, the results were also somewhat weakened in that there was no opportunity to perform NLs during the summer holiday period, when O_3 concentration peaked.

Epidemiological investigations of health effects of ambient O_3 have often lacked well-characterized exposure assessment and have not used personal O_3 sampling. To the authors′ knowledge only one small study of 12 asthmatic subjects has been carried out, showing that personal O_3 exposure but not outdoor O_3 levels were positively associated with asthma symptom severity and medication use [24]. Therefore, the authors concluded that the epidemiological effect of O_3 might be underestimated rather than overestimated by using outdoor levels.

The effective incorporated dose of ozone per time depends on the outdoor concentration of ambient O_3, the duration of time spent outdoors and the physical activity under these conditions. If mean O_3 conntration during the 24-h interval before the NL had been taken, there would have been insufficient variability in the exposure data. This was the reason for choosing the peak rather than the mean O_3 concentration. In terms of a physiologically appropriate parameter, this is in line with recently published data, namely the studies of THURSTON *et al.* [25] and ROMIEU *et al*. [26], who reported O_3 effects on medication use in asthmatics and lower respiratory illness, respectively, on the basis of a rise in the daily O_3 1-h maximum concentration.

Repeated NL was performed on the children over a whole summer season under natural exposure and environmental conditions frequently found in central Europe. At the time, most epidemiological studies focused on lung function testing to confirm functional impairment after O_3 exposure. Recently BRAUN-FAHRLÄNDER *et al.* [4] demonstrated a significant decrease in the peak flow values of children of school age following a 10-min heavy exercise period under natural outdoor O_3 exposure conditions. However, lung function measurements are an appropriate tool for assessing changes in the lower airway, where ozone effects might differ from those on upper airway reactivity. Inflammatory changes in NL fluid, therefore, promise to deliver additional information compared to changes in pulmonary function. This was demonstrated by TEPPER *et al*. [6] who revealed a progressive pattern of the inflammatory effect of inhaled O_3 in the lungs of rats, although the effects on lung function had diminished after 5 days of exposure. Increased inflammatory marker concentrations in NL fluid are detectable for $\geq$18 h after O_3 exposure, as demonstrated by KOREN *et al*. [9] and FRISCHER *et al*. [10]. However, the authors did not evaluate potential adaptation, as described in several chamber and animal studies. For this reason, the current study design focuses on adaptation phenomena.

Leukocyte count and albumin concentration have been commonly used in chamber studies to analyse inflammatory processes after O_3 exposure [7–10]. The highest median of all time points as well as the medians of the individual peak leukocyte counts and ECP concentrations were observed immediately after the first increase in ambient O_3 levels at the beginning of May (time point 4). In the respective time period, half of the exposure values exceeded an O_3 concentration of 130 $\mu g \cdot m^{-3}$, which represents exposure conditions similar to those in the earlier field study [10].

Linear regression models for both ECP concentration and leukocyte count revealed significant dose-dependent effects of O_3, based on data from time points 1–4, whereas parameter estimates of O_3 diminished continuously when

the consecutive time points were added (fig. 4). This might indicate a process of adaptation to elevated O_3 levels.

When the parameter estimates for different time periods were calculated separately, a statistically significant negative parameter estimate was obtained during periods 1–3 and a statistically significant positive parameter estimate during periods 4 and 5, whereas the values during periods 6–8 and 9–11 were -0.43 and 0.46, respectively, but not statistically significant. This might be further evidence for the observed process of adaptation.

Several articles in the literature have reported findings which could indicate an adaptation process parallel to continuous or repeated O_3 exposure. HACKNEY *et al*. [27] found less responsiveness to O_3 in people living in highly-exposed regions compared to people living in rural regions, and interpreted the reduced responsiveness as a sign of adaptation. LINN *et al*. [28] examined a small group of Los Angeles residents using repeated lung function tests and pointed out that, after high natural O_3 exposure in summer, re-exposure of former responders to 0.18 parts per million (ppm) O_3 for 2 h revealed no O_3 effect. Recently, JÖRRES *et al*. [29] exposed healthy nonsmoking volunteers to O_3 on four consecutive days. Pulmonary function values showed significant changes after the initial O_3 exposure but returned to baseline after the fourth exposure, whereas markers of inflammation in the lower airways remained altered. These findings are in accordance with previous studies, showing that the greatest effect of O_3 exposure on lung function can be observed on the second day, followed by a progressive diminution in O_3- response [30, 31].

Several authors have described mechanisms of adaptation following chronic exposure based on functional, biochemical and morphological studies in rats [32–35]. A dose-dependent (0 ppm, 0.12 ppm and 1 ppm ozone) increase in activities of antioxidant enzymes, such as glutathione transferase and peroxidase as well as superoxide dismutase, was evoked through ozone exposure for 90 days and 20 months [34], which may reflect an active reaction of airway epithelial cells [36]. Thus, inactivation of free radicals by antioxidant substances could play a key role in the development of adaptation to O_3 and could, secondarily, interrupt the inflammatory effects of O_3. An additional explanation for adaptation to continuous O_3 exposure is downregulation of inflammatory events. PENDINO *et al*. [37] were able to show that pharmacological pretreatment of alveolar macrophages abrogates the O_3-induced increases in the number of cells as well the amount of protein recovered in bronchoalveolar lavage fluid. Therefore, physiological effects on macrophages could result in diminution of inflammatory events initiated by O_3. The present data suggest that a process of restitution on the nasal mucosa was not completed in autumn. For example, albumin concentration had its maximum at time point 11. Also, ECP concentration and leukocyte count were elevated in autumn, when the ozone concentration had decreased. The present findings showing diminution of inflammatory markers in the NL of children under continuous O_3 exposure must therefore be interpreted with caution, since progressive epithelial damage was observed, even in the presence of functional adaptation [36]. Furthermore, it is not clear in how far nasal epithelium is representative of O_3-induced changes in the lower airways.

In conclusion, the present data are in accordance with most experimental findings based on animal studies. However, the natural exposure conditions might point towards the fact that adaptation mechanisms could be of relevance, even at ozone concentrations of <0.1 ppm.

The interpretation of the data, showing the attenuated inflammatory and functional response in the nasal lavage fluid of children remains difficult, as it is unclear whether potential adaptation is a sign of protection, or whether there is an ongoing process of chronic tissue damage, particularly in the lower airways.

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