INCREASE OF EXHALED NITRIC OXIDE IN CHILDREN EXPOSED TO LOW LEVELS OF AMBIENT OZONE

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ABSTRACT

Ozone (O_3) is known to induce lung function impairment and airways inflammation during episodes of photochemical smog. The aim of the present study was to assess the inflammatory effect of ambient O_3 in healthy children using nitric oxide in exhaled air (eNO) as a non invasive test. The study was performed on six groups of children (n = 11-15), aged 6.5 to 15 years who attended summer camps in rural areas of the South of Belgium in 2002. Ambient O_3 concentrations continuously monitored in the camps ranged from 48 to 221 µg/m^3 (1-hour maximal concentration). Children remained outdoors during the experimental days doing various recreational activities but no sports. Lung function tests (FEV_1 and FVC) and eNO were measured twice in each child in the morning and in the evening. Whilst lung function tests did not show any consistent pattern of decrease at these O_3 levels, a highly significant increase in eNO was found in all subjects from an ambient 1-hour O_3 level of 167 µg/m^3. A multivariate analysis did not reveal any influence of age, gender, height, weight and BMI of the children. The threshold for this O_3-induced increase in eNO estimated benchmark dose analysis was 135 µg/m^3 for 1-hour exposure and 110 µg/m^3 for 8-hour exposure. These observations suggest that ambient ozone produces early inflammatory changes in the airways of children from levels slightly below current air quality standards.

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INTRODUCTION

Ozone (O_3), the main oxidant of photochemical smog can reach high concentrations during hot summer days in industrial countries. Episodes of O_3 pollution affects non urban as well as urban areas, O_3 peaks tend even to be lower in large cities because of the scavenging of other pollutants. Depending on the inhaled dose and the sensitivity of subjects, O_3 produces a variety of adverse effects, including decreased lung function, inflammatory reactions, increased airways permeability and resistance, and aggravation of pre-existing respiratory diseases (Bylin et al., 1996; Committee of the Environmental and Occupational Health Assembly of the American Thoracic Society, 1996). Several air quality guidelines and standards are currently in application to protect the population from these harmful effects. The World Health Organisation has established a guideline value of 120 $\mu g/m^3$ for O_3 in ambient air for a period of 8 hours/day below which acute effects on public health are likely to be small (WHO, 2000). The National Ambient Air Quality Standards (NAAQS) of the US Environmental Protection Agency for O_3 are 0.12 ppm (235 $\mu g/m^3$) for 1-hour exposure and 0.08 ppm (157 $\mu g/m^3$) for 8-hour exposure (US EPA, 1997), respectively.

Research into the biomarkers field has much progressed during the last years with the development of several non-invasive tests or approaches allowing to evaluate lung inflammation or damage without specific restriction imposed by exposure conditions and the characteristics of examined subjects. Some of these tests such as serum specific Clara cell protein have been validated to monitor airways permeability changes during O_3 exposure (Blomberg et al., 2003; Broeckaert et al., 1999). Other tests using mediators measurable in exhaled air or breath condensate are also available to assess lung inflammation. Among these tests, one of the most validated is based on determination of nitric oxide in exhaled air (eNO). Nitric oxide produced by the inducible NO-synthase (iNOS) in bronchial epithelium is considered as a sensitive marker of proinflammation and oxidative stress in the lung (Barnes and Kharitonov, 1996; Kharitonov and Barnes, 2000; Kharitonov and Barnes, 2002; Saleh et al., 1998).

The aim of the present study was to evaluate whether exhaled NO can be used to monitor non invasively O_3-induced lung inflammation, by comparing morning and evening exhaled NO levels of children exposed to increasing concentrations of ambient O_3 during summer camps.

METHODS

Subjects and Study Design

A total of 72 children (17 girls) aged 6.5 to 15 years (mean, 10.5 years) were recruited for the study. All subjects were healthy (non asthmatic) and participated in the study after a written approval by their parents. The protocol of the study was approved by the Ethical Committee of the Faculty of Medicine of the University.

The study was conducted in six groups of children (n=11-15) attending different summer camps in rural areas in the South of Belgium between 26th July and 14th August 2002. Children were studied under varying O_3 exposure conditions that included cloudy days and episodes of photochemical smog. They were examined twice, first in the morning between 10 and 12 am prior to the ozone peak and a second time in the evening between 6 and 8 pm. Children remained outdoors doing normal outdoor recreational activities with no sport and running. Examination included a spirometric and an exhaled NO test and the measurement of height and body weight. The six groups of children were referred hereafter by a letter of A to F corresponding to increasing O_3 exposure levels.

Lung Function and Exhaled Nitric Oxide

The NO concentration in exhaled breath was measured on-line by chemiluminesence using the NIOX™ analyser (Aerocrine AB, Sweden). The test was performed in compliance with the guidelines of the American Thoracic Society (Slutzky et al., 1999). The FVC and FEV_1 were then measured with a Vitalograph-Compact (Vitalograph Ltd., Buckingham, England) according to the American Thoracic Society standards (ATS, 1995). All these tests were performed indoors to avoid confounding by weather conditions.

Air monitoring

Ambient O_3 was continuously monitored in each camp using an UV photometric O_3 analyser Model 427 (Signal Instrument Company Limited, England). The equipment was calibrated just before the beginning of study by the official laboratory of the national air monitoring network (Interregional cell for the environment, Belgium). Hourly concentrations of O_3 and other air pollutants (NO, NO_2, SO_2, $PM_{2.5}$ and PM_{10}) were obtained from the nearest local monitoring station. Temperature and relative humidity were also recorded hourly by nearest meteorological stations.

Statistical Analysis

All statistical tests were performed after checking the normality of data which were normalised by log transformation when necessary. Differences between morning and evening exhaled NO values were assessed by the paired Student's test (two-sided). One-way analysis of variance (ANOVA) followed by the Dunnett's multiple comparison test was used to compare the diurnal variations of exhaled NO or lung function parameters between the six studied groups exposed to increasing ambient O_3 levels. The associations between exhaled NO concentration and possible explanatory variables (age, height, weight, BMI, sex and ambient O_3 concentration) were tested using multiple stepwise regression. The benchmark dose (BMD) analysis of the increased of eNO was performed using the Weibull Model of the US-EPA Benchmark Dose Software, version 1.3.2. In this analysis, the percentages of children with an increased eNO were calculated using a cut off level of 4.30 ppb defined as the 95th percentile of the absolute diurnal eNO changes observed in children of groups A, B and C with the lowest exposures to O_3. Results were expressed as the mean ± standard error (SEM). The statistical package StatView® 5, Release 5.0.1, a business unit of SAS, third edition, Cary, NC: SAS Institute Inc., 2001 was used for all analyses. The level of significance was assigned at $p < 0.05$.

RESULTS

Meteorological data and concentrations of O_3 and other air pollutants during the six study days are summarised in Table 1. The maximum 1-hour concentrations of O_3 varied from 48 to 221 µg/m^3 and the 8-hour O_3 concentrations varied from 37 to 159 µg/m^3. Levels of other air pollutants remained low and stable, as illustrated in Figure 1 for NOx, even decreased during the studied days. Figure 1 also shows that O_3 concentrations peaked between approximately 14:00 and 18:00.

Table 1. Meteorological data and concentrations of air pollutants during the studied days.

	A	**B**	**C**	**D**	**E**	**F**
Date	07/26/2002	08/09/2002	08/14/2002	08/08/2002	07/29/2002	07/30/2002
Camp location	Pessoux	Marche-en-Famenne	Mozet	Marche-en-Famenne	Graide	Grandglise
Mean temperature (°C)	19.4	16.1	24.9	17.9	26.1	28.8
Max. temperature (°C)	22.1 (16 h)	17.1 (15 h)	26.4 (18 h)	20.4 (16 h)	27.8 (17 h)	32 (15 h)
Mean relative humidity (%)	77	81	64	75	40	50
Max. relative humidity (%)*	92 (10 h)	93 (10 h)	77 (10 h)	99 (10 h)	49 (10 h)	67 (10 h)
1-hour [O_3] (µg/m³)	48.3 (15 h)	71 (16 h)	96.2 (15 h)	127.3 (14 h)	166.6 (16 h)	221.2 (15 h)
8-hour [O_3] (µg/m³)	37.2	65.9	78.7	109.9	135	159
1-hour [NO] (µg/m³)*	10 (14 h)	5 (10 h)	43 (10 h)	3 (11 h)	3 (10 h)	6 (10 h)
8-hour [NO] (µg/m³)	5.3	3.1	10.9	2.2	2	3
1-hour [NO_2] (µg/m³)	26 (12 h)	23 (10 h)	42 (10 h)	13 (11 h)	6 (10 h)	35.3 (10 h)
8-hour [NO_2] (µg/m³)	20.1	13.7	19.4	9.8	4	17.4
1-hour [SO_2] (µg/m³)	4	4	8 (10 h)	4	4	17.6 (12 h)
8-hour [SO_2] (µg/m³)	4	4	4.9	4	4	11.6
8-hour PM 2.5 (µg/m³)	16.1	9.3	23.5	17.9	14.9	25.6
8-hour PM 10 (µg/m³)	17.9	14.8	44.9	30.1	34.6	47.6

*between brackets: time in the day corresponding to the maximum concentration (peak)

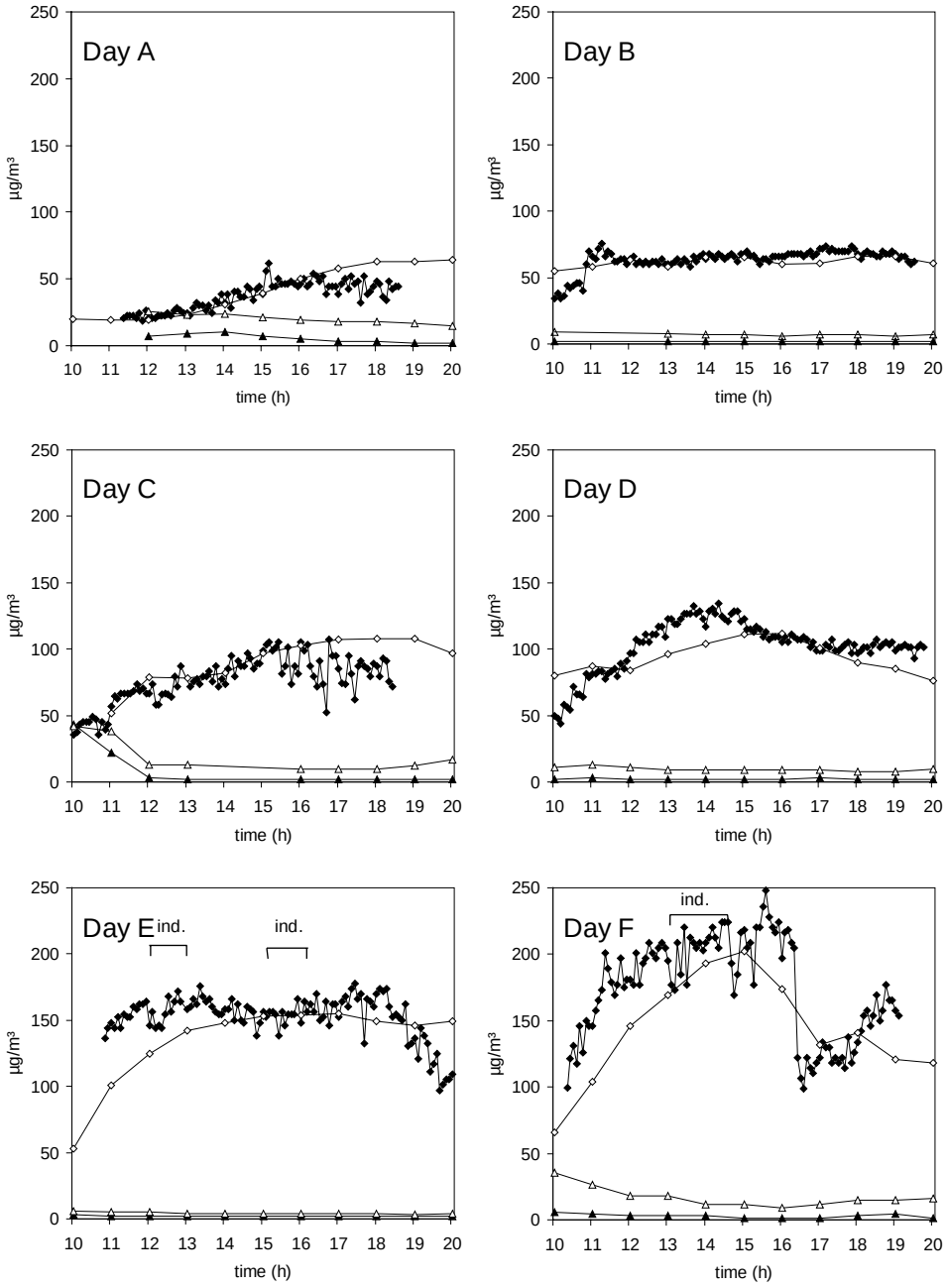

Figure 1. Diurnal variations of O_3 (dark diamonds for in-house measurements and white diamonds for station measurements), NO (dark triangles) and NO_2 (white triangles).

As shown in Table 2, the six groups were similar with regard to age and anthropometric parameters (ANOVA test, age, p = 0.4889; height, p = 0.0889; weight, p = 0.1461; BMI, p = 0.2016). Spirometric parameters (FVC, FEV_1 and FEV1/FVC ratio) did not show any consistent change with the increase of O_3 exposure levels. No significant difference in the diurnal variations of these lung function parameters was found between the studied groups by ANOVA. By contrast, as shown in Figure 2, the diurnal variation of eNO shows clearly distinct patterns between children exposed to low O_3 concentrations and those exposed to the highest O_3 levels. At O_3 levels below 100 µg/m^3, a statistically significant decrease of eNO is observed in the three studied groups (A, B

and C) whereas those exposed to the highest levels O_3 showed a marked elevation of their eNO levels (groups E and F). The breakpoint between the two patterns appears to lie around O_3 leve1s of 110 (8-hour) or 127 (1-hour) µg/m^3 when the diurnal decrease of eNO appears abolished by the rising O_3 exposure. No correlation was found between diurnal variations of exhaled NO and the concentrations of other air pollutants (PM_{10}, $PM_{2.5}$, SO_2, NOx).

Table 2. Clinical characteristics, lung function and exhaled NO concentrations of children in the morning and in the evening of the studied days. For spirometric parameters we discarded the results of three children (two in group A and one in group F) who did not perform properly the tests.

Group		**A**	**B**	**C**	**D**	**E**	**F**
Number, male/female		11/0	14/1	8/3	14/0	5/6	6/4
Age, yr		10.5 ± 0.2	10.6 ± 0.3	11.8 ± 0.9	9.7 ± 0.3	10.3 ± 0.7	11.1 ± 0.9
Height, m		1.46 ± 0.02	1.43 ±0.03	1.52 ± 0.05	1.37 ± 0.02	1.43 ± 0.04	1.43 ± 0.04
Weight, kg		34 ± 1	37 ± 2	43 ± 4	31 ± 1	34 ± 2	37 ± 4
BMI, kg/m²		15.8 ± 0.5	17.9 ± 0.5	17.9 ± 0.9	16.6 ± 0.5	16.4 ± 0.6	17.7 ± 1.5
FVC, % pred	AM	101 ± 3	97 ± 2	89 ± 3	98 ± 2	93 ± 3	91 ± 3
	PM	97 ± 4	102 ± 4	85 ± 4	90 ± 2*	97 ± 3†	88 ± 3
FEV_1, % pred	AM	90 ± 2	90 ± 3	85 ± 3	94 ± 3	91 ± 3	85 ± 3
	PM	86 ± 4	91 ± 3	79 ± 3‡	87 ± 3†	94 ± 3*	81 ± 3
FEV_1/FVC, % pred	AM	90 ± 1	93 ± 2	96 ± 2	97 ± 3	99 ± 2	94 ± 4
	PM	89 ± 3	94 ± 2	93 ± 2	96 ± 2	97 ± 2	92 ± 3
eNO, ppb	AM	13.7 ± 2.5	11.9 ± 1.7	14.2 ± 2.2	12.8 ± 2.8	9.1 ± 1.3	13.0 ± 2.5
	PM	12.1 ± 2.3*	9.9 ± 1.5‡	11.7 ± 2.0*	13.1 ± 2.5	29.2 ± 3.7‡	37.6 ± 2.1‡

Values are expressed as absolute numbers or as means ± SEM.
Significant difference between morning and evening values by paired Student't test: * $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$.

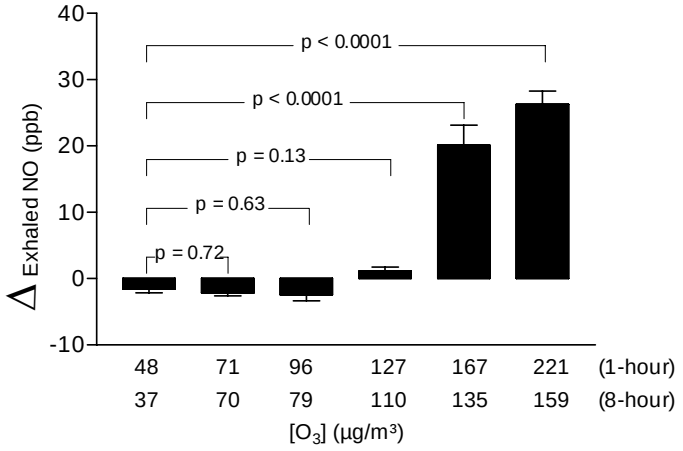

Figure 2. Diurnal variations of exhaled NO in children exposed to increasing concentrations of ambient ozone. Bars represent SE.

Multiple stepwise regression analysis shows that the increase of eNO induced by ambient O_3 in groups of children D, E and F is independent of age, sex and BMI but is only closely correlated with the maximal 1-hour ($r'' = 0.659$, $p < 0.0001$) or 8-hour O_3 concentrations ($r'' = 0.694$, $p < 0.0001$ for 8-hour). No predictor of the diurnal decrease of eNO in groups A, B and C could be identified. The O_3 threshold for increase of eNO was more precisely estimated by the calculation of the benchmark dose (BMD) and its lower-bound confidence limit (BMDL). A BMD of 134.5 µg/m^3 was derived for the maximal 1-hour O_3 concentration (BMDL, 119.2 µg/m^3) whilst the BMD for the maximal 8-hour O_3 concentration was estimated at 110.4 (BMDL, 101.5 µg/m^3).

DISCUSSION

The present study is the first to provide evidence of airways inflammation in children exposed to ambient ozone under field conditions. The inflammation induced by O_3 was detected by applying the eNO measurement, a non invasive test used to monitor inflammatory reactions in asthma and other lung diseases (Kharitonov and Barnes, 2002). At O_3 levels below 100 µg/m^3, the exhaled NO concentration shows a diurnal decrease confirming previous observations on the circadian variations of this indicator (Mattes et al., 2002). At higher O_3 concentration, the increase of eNO was not accompanied by lung function decrements, which is not really surprising given the relatively low O_3 levels in our study compared to previous reports and the well established lack of correlation between the inflammatory and functional responses of the lung to O_3 (Kinney et al., 1996; Balmes et al., 1996). These results are in agreement with the observations made in bleachery workers exposed to high peaks of O_3. Levels of eNO were found to be significantly increased among workers exposed to ozone gassings compared with those not exposed to such incidents. Like in our study, these changes were not associated with a significant decrease of FVC or FEV_1, suggesting that eNO is an early marker of airway inflammation produced by O_3 (Olin A.C. et al., 2004; Olin et al., 1999).

Although children were not exercising, the increase of eNO was observed from levels which are lower than current standards. The increase was already statistically significant from a maximum 1-hour concentration of 167 µg/m^3, which is lower than both the US and EU population information levels (235 and 180 µg/m^3, respectively, EU, 2002; US EPA, 1997). The corresponding 8-hour concentration was 135 µg/m^3, a concentration lower than the US National Ambient Air Quality Standard (8-hour ozone concentration of 157 µg/m^3). The BMD estimated (134 and 110 µg/m^3 for the 1-hour and 8-hour O_3 concentrations, respectively) from the dose-response relations suggest that the inflammatory response is triggered by even slightly lower concentrations. The 8-hour BMD is even lower than the air quality guideline recommended by the WHO and the European Union (120 µg/m^3).

In conclusion, the present study indicates that ambient O_3 induces in children an early lung inflammation that passes undetected with spirometric tests. This non invasive test, easily applicable under field conditions, represents an efficient tool which undoubtedly should improve the assessment of health risks of O_3 and the subsequent derivation of health-based air quality standards.

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