Coarse Fraction Particle Matter and Exhaled Nitric Oxide in Non-Asthmatic Children

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Abstract: Coarse particle matter, PM_{coarse}, is associated with increased respiratory morbidity and mortality. The aim of this study was to investigate the association between short-term changes in PM_{coarse} and sub-clinical airway inflammation in children. Healthy children aged 11 years from two northern Swedish elementary schools underwent fraction of exhaled nitrogen oxide (FENO) measurements to determine levels of airway inflammation twice weekly during the study period from 11 April–6 June 2011. Daily exposure to PM_{coarse}, PM_{2.5}, NO_{2}, NOx, NO and O_{3} and birch pollen was estimated. Multiple linear regression was used. Personal covariates were included as fixed effects and subjects were included as a random effect. In total, 95 children participated in the study, and in all 493 FENO measurements were made. The mean level of PM_{coarse} was 16.1 µg/m³ (range 4.1–42.3), and that of O_{3} was 75.0 µg/m³ (range: 51.3–106.3). That of NO_{2} was 17.0 µg/m³ (range: 4.7–31.3), NOx was 82.1 µg/m³ (range: 13.3–165.3), and NO was 65 µg/m³ (range: 8.7–138.4) during the study period. In multi-pollutant models an interquartile range increase in 24 h PM_{coarse} was associated with increases in FENO by between 6.9 ppb (95% confidence interval 0.0–14) and 7.3 ppb (95% confidence interval 0.4–14.9). PM_{coarse} was associated with an increase in FENO, indicating sub-clinical airway inflammation in healthy children.

Keywords: exhaled NO; respiratory inflammation; coarse particle matter; air pollution; children

1. Introduction

Studded tires wear the asphalt surface and contribute to particle matter (PM), specifically the coarse fraction PM_{coarse} between 2.5–10 microns in aerodynamic diameter (PM_{2.5-10}). Short-term exposure to PM attributed to mechanical wear has been associated with emergency room visits for asthma [1] and increased mortality [2]. Stronger effects of short-term exposure to coarse particles PM_{2.5-10} were found on respiratory outcomes as asthma admissions compared to other endpoints [3] and increased respiratory morbidity and mortality in relation to higher short-term PM_{2.5-10} concentrations [4]. However, the findings have been heterogeneous and stronger associations have been reported from arid regions [3] and in European studies [4].

Nitric oxide (NO) is a signalling molecule produced by epithelial cells in the airways. In airway inflammation the fraction of exhaled nitric oxide (FENO) is increased [5]. Increased levels of FENO have also been shown to predict new-onset asthma in children [6] and new-onset wheeze in adults [7]. It has also been suggested to be a predictor for asthma in children with virus-induced wheeze [8].
Taken together, these data indicate that FENO is a relevant biomarker to assess early sub-clinical inflammation, as elevated FENO levels are present before the onset of symptoms.

In northern Sweden, the exposure to coarse particles is unusually high during April and May when streets and highways are cleared from sand and particles produced by wintertime driving with studded tires and the sanding of roads from October, resulting in lots of wear particles which are present until the roads are cleaned in late spring. This is therefore an ideal time point to better elucidate the effect of coarse particles. We were especially interested in the effect of exposure in children, as they are potentially more vulnerable.

FENO has been used in children to assess the effects of traffic exposure in several studies; exposure to black carbon and PM$_{2.5}$ has been associated with increases in FENO in both healthy [9,10] and asthmatic subjects [11]. In children, PM$_{2.5}$ was associated with elevated FENO in both healthy [12] and allergic children [13]. PM$_{10}$ from traffic as well as biomass burning has also been associated with increased FENO in healthy children [14,15] as well as in a panel of urban- and suburban-dwelling children [16], and with PM$_{coarse}$ in asthmatic children [17]; however, the study was smaller ($n=58$) and the setting radically different. A European study found associations between background exposure to PM$_{coarse}$ and increased risk of pneumonia in children at ages two to three [18], whereas no association was found with lung function in children in another study [19]. The role of PM$_{coarse}$ from studded tires on airway inflammation in healthy children has not been investigated.

The aim of this study was to investigate if there was an association between airway inflammation (FENO$_{50}$) and PM$_{coarse}$ in a northern Swedish town in healthy children.

2. Materials and Methods

2.1. Study Population

The study population was sampled from grades 4, 5 and 6 (age 11–12) in two different schools in Umeå, Västerbotten, Sweden. The study participants were recruited from classes which had schedules compatible with the clinical research staff’s. All eligible children were invited to participate through a letter to their parents, who gave informed consent for the participation. The study protocol was approved by the regional ethical review board (reference number: 2010-345-31M).

2.2. Exposure

The children’s air pollution exposure at school was estimated based on data from measuring stations located within 1500 m of the schools. PM$_{10}$ and PM$_{2.5}$ and ozone (O$_3$) were measured at the Mården continuous measuring station using a TEOM instrument (Monitor Europe 9810, Casella Measurement, Bedford, UK) for PM. Coarse particles, PM$_{coarse}$, were defined as the fraction between PM$_{2.5}$ from PM$_{10}$ and calculated by subtracting PM$_{2.5}$ from PM$_{10}$. Nitrogen dioxide (NO$_2$), nitrogen oxides (NOx), and nitrogen oxide (NO) was measured at Västra Esplanaden measuring station using a chemilluminiscence instrument (ML 9841 B, Teledyne Monitor Labs, Englewood, CO, USA). Temperature was measured at Umeå airport, approximately 5 km away from the schools, and provided as 30 minute means and recalculated into daily means when 75% of the data were available; otherwise, it was coded as missing. Pollen was measured as daily birch pollen using a Burkard trap at the roof the University Hospital. Pollen counts were recoded into categories from the daily value (0 = 0, 1–10 = 1, 11–100 = 2, 101–1000 = 3).

2.3. FENO Measurements

FENO measurements were performed on each participant twice each week during spring 2011 (11 April to 6 June) if the child was present and did not have respiratory symptoms.

Fraction of exhaled nitric oxide was measured at the flow rate 50 mL/s (FENO$_{50}$) using Niox Mino (Aerocrine AB, Solna, Sweden). One measurement was performed at each occasion, according to the manufacturer’s instructions. The parents of the participants answered questionnaires about
respiratory health, use of asthma medication, and rhinitis (allergies to furred animals and pollen). At each measuring occasion, children were asked about cold symptoms. If possible the measurements were performed at the same hour on each occasion.

If the FENO value was less than 5 ppb \((n = 32)\), i.e., the lower limit of the measurement device, it was set to 2.5 ppb. As FENO is skewed to the right, log transformed values were used in the model, the estimate was then log-transformed back after the modelling.

### 2.4. Statistical Methods

Cumulative means of pollution exposure 24, 48 and 72 h prior to the FENO measurement were calculated and used in the model. Same-day temperature and pollen counts were included in the model. In the statistical analysis, MLR (multiple linear regression) was used to examine personal covariates related to FENO as a first step. If the \(p\)-value was <0.25 for a variable, it was included in the initial models. Then the personal covariates of interest, sex, allergy, pollen, day of week and meteorological variables, were included in the linear mixed models, together with the pollutants, to examine if the pollutants had any effect on FENO. Subjects were included as a random effect to account for personal differences, and all other variables were included as fixed effects.

First each pollutant’s effect on FENO was examined individually in single-pollutant models. Since PM mass and nitrogen oxides (reflecting vehicle exhaust) were represented by several variables, the multi-pollutant models were constructed for each pairwise combination of \(\text{PM}_{2.5}\) and \(\text{PM}_{\text{coarse}}\) and \(\text{NO}, \text{NO}_2\) and \(\text{NOx}\) which were included in the multi-pollutant models with \(\text{O}_3\). Each pollutant’s effect on FENO was examined with exposure windows of 24, 48 and 72 h. If a covariate changed any pollutants’ effect on FENO by more than 10% or lowered Akaike’s information criteria (AIC) it was considered to be an effect modifier and was included in the model. Model selection was based on minimizing AIC.

Sensitivity analysis of sex, allergy status and background residential exposure by quartile of modeled annual mean NOx exposure at each participant’s home address was performed.

The results are reported as change FENO (ppb) per interquartile range (IQR) of the pollutants with a 95% confidence interval (CI). All statistical analysis was performed using PASW 18 (SPSS, Chicago, IL, USA).

### 3. Results

In total, 240 children from 11 classes in the two schools were invited to participate. One hundred and four children accepted the invitation and the participation rate was 43%. Nine children had asthma or were treated with anti-inflammatory medication and were excluded from the present analysis. In total, 95 children free of asthma were included in the study group of which 46 (48%) were females, and in all, 23 (24%) had allergic rhinitis. In total, 973 FENO measurements were made on non-asthmatic children free of respiratory symptoms, five measurements per individual on average. All participants were 11 years old at the beginning of the study period. The mean FENO level in the population was 13.3 ppb (standard deviation (SD) = 10.7), higher in boys than girls (Table 1).

| FENO_{SO2} (ppb) | N Subjects | Mean | SD | Min./Max. |
|------------------|------------|------|----|-----------|
| All              | 95         | 13.3 | 10.7| 3.0/71.0  |
| Male             | 49         | 15.4 | 13.0| 3.0/71.0  |
| Female           | 46         | 11.2 | 7.3 | 3.0/66.0  |

SD: Standard deviation; ppb: Parts per billion.

The study period was 62 days. PM measurements were missing the first three days and one day had missing data for NO and \(\text{PM}_{2.5}\), so these days were excluded from the analysis. The mean level of
PM\textsubscript{coarse} was 16.1 µg/m\textsuperscript{3}, the mean \text{O}_3 was 75.0 µg/m\textsuperscript{3}, the mean \text{NO}_2 was 17.0 µg/m\textsuperscript{3}, the mean \text{NO}_2 was 82.1 µg/m\textsuperscript{3}, and the mean \text{NO} was 65 µg/m\textsuperscript{3} (Figure 1, Table 2).

**Table 2.** Exposure data (µg/m\textsuperscript{3}) for the study period.

| Exposure  | N  | Mean | SD  | IQR  | Min./Max. |
|-----------|----|------|-----|------|-----------|
| PM\textsubscript{2.5} | 60 | 5.6  | 2.6 | 2.6  | 2.3/16.7  |
| PM\textsubscript{coarse} | 49 | 16.1 | 9.8 | 9.6  | 4.1/42.3  |
| NO\textsubscript{2}    | 61 | 17.0 | 7.3 | 12.8 | 4.7/31.3  |
| NO\textsubscript{x}    | 61 | 82.1 | 41.5| 77.6 | 13.3/165.3|
| NO     | 61 | 65.0 | 34.8| 65.6 | 8.7/138.4 |
| O\textsubscript{3}     | 59 | 75.0 | 12.3| 17.0 | 51.3/106.3|

SD: Standard deviation.
3.1. Descriptive Statistics

There were no significant correlations between 24 h means of PM$_{\text{coarse}}$ and other pollution variables or temperature. PM$_{2.5}$ was positively correlated with NO$_2$, O$_3$ and temperature. O$_3$ was positively correlated with temperature. There were high correlations (>0.9) between nitrogen oxide, nitrogen dioxide and NOx (Table 3).

Table 3. Pearson correlation coefficients for the for the 24 h pollutant concentrations ($\mu$g/m$^3$) and weather covariates.

| Exposure | Ozone | PM$_{2.5}$ | PM$_{\text{coarse}}$ | NOx | NO$_2$ | NO | Temp. |
|----------|-------|------------|---------------------|-----|--------|----|-------|
| Ozone    | 1     |            |                     |     |        |    |       |
| PM$_{2.5}$ | 0.415* | 1          |                     |     |        |    |       |
| PM$_{\text{coarse}}$ | −0.139 | 0.008 | 1                   |     |        |    |       |
| NOx      | −0.041 | 0.257 | 0.150 | 1 |        |    |       |
| NO$_2$   | 0.158 | 0.363* | 0.126 | 0.938** | 1 |    |       |
| NO       | −0.083 | 0.232 | 0.153 | 0.997** | 0.909** | 1 |       |
| Temp.    | 0.423 ** | 0.414* | −0.117 | −0.228 | −0.143 | −0.242 | 1 |

*: $p < 0.05$; **: $p < 0.01$.

3.2. Analysis Results

Temperature increased the model fit, pollen and week day were the effect modifiers and gender was a significant predictor; these were all included in the models. Reported rhinitis did not improve the model fit and was not an effect modifier; thus it was not included in the models.

In the single-pollutant models, there were no statistically significant associations between air pollutants and FENO. Only exposure to PM$_{\text{coarse}}$ during the previous 24 h period was near statistical significance with an estimated relative change in FENO of 6.3 ppb (95% CI: 0.5; 13.5%) per IQR change in pollutant concentration (Table 4).

Table 4. Change in FENO (ppb) and 95% CI associated with an IQR change in pollutant concentration from a single-pollutant model adjusted for sex, temperature, pollen and day of week.

| Exposure     | NOx       | NO$_2$    | NO    | PM$_{\text{coarse}}$ | PM$_{2.5}$ | O$_3$ |
|--------------|-----------|-----------|-------|----------------------|------------|-------|
| 24 h average | 2.8 (−1.1, 6.8) | 0.1 (−3.7, 4.1) | 0.3 (−0.4, 6.6) | 6.3 (−0.5, 13.5) | 0.5 (−1.5, 2.6) | −3.7 (−8.3, 1.2) |
| 48 h average | 2.4 (−1.6, 6.6) | 1.4 (−2.3, 5.3) | 3.0 (−1.7, 7.8) | −1.8 (−6.7, 3.2) | 1.4 (−0.8, 3.5) | 0.1 (−3.4, 3.7) |
| 72 h average | 2.0 (−0.9, 5.0) | 2.0 (−1.5, 5.5) | 2.4 (−1.1, 6.2) | −2.0 (−6.1, 2.3) | 0.9 (−1.2, 3.1) | 0.1 (−3.8, 4.1) |

In the multi-pollutant models, 24 h PM$_{\text{coarse}}$ exposure was associated with statistically significant increases in FENO from 6.9 ppb (95% CI 0.0; 14) to 7.3 ppb (95% CI 0.4; 14.9) per IQR, depending on whether the regression was adjusted for for NO$_x$, NO$_2$ or NO. Seventy-two-hour NO$_2$ was associated with a significantly increased FENO of 7.3 ppb (95% CI 0.6; 14.6) in the models adjusted for PM$_{\text{coarse}}$ and O$_3$. Twenty-four-hour O$_3$ was, on the other hand, associated with statistically significant decreases in FENO of between −6 ppb (95% CI −12.0; −0.2) to −7.3 (95% −13.2; −1.2) in all models except in the model adjusted for PM$_{2.5}$ and NO. The effect estimates associated with O$_3$ for the previous 72 h in models adjusted for NO$_2$ were −6.6 ppb and −6.7 ppb per IQR but these estimates did not reach statistical significance (Table 5). There were no significant associations between FENO and any pollutant in the 48 h pollutant models. There were no significant associations with PM$_{2.5}$, NOx or NO in any models in any exposure window (Table 5). The daily pollen level was an effect modifier, but was not itself significantly associated with FENO and the results are not shown.

In sensitivity analyses stratified by allergy status there were no changes in the association between FENO and pollutants. The long-term residential exposure quartile of (modelled NOx) was not a significant predictor of FENO and did not modify the association with short-term exposure (data not shown).
which limits the available outcome. The monitors are located near the schools, but the children’s effects from coarse PM in urban settings could be due to variation in regional background PM (e.g., desert dust) which is reflected in concentrations measured at a single monitor rather than variation changes present as large relative changes. Newly and locally generated particles thus become a larger fraction of PM in urban settings where most central monitors are located. Previous reports of health increases present as large relative changes. Newly and locally generated particles thus become a larger fraction of PM in urban settings where most central monitors are located. Previous reports of health effects from coarse PM in urban settings could be due to variation in regional background PM (e.g., desert dust) which is reflected in concentrations measured at a single monitor rather than variation from nearby local sources, e.g., a construction site.

A possible limitation of the current study is assigning exposure from a single, central monitor which limits the available outcome. The monitors are located near the schools, but the children’s residence may not be very close and other daytime activities may modify the children’s daily exposure. However, assigning PM coarse exposure from a single, central monitor is considered to be representative for large urban areas, especially in studies of temporal variation. Several studies have determined

| Models | NOx, PM coarse, O3 | NOx, PM2.5, O3 | NO2, PM coarse, O3 | NO2, PM2.5, O3 | NO, PM coarse, O3 | NO, PM2.5, O3 |
|--------|--------------------|----------------|--------------------|----------------|--------------------|----------------|
| Exposure time | NOx | NOx | NO2 | NO2 | NO | NO |
| 24 h average | 3.8 (−1.1, 8.9) | 2.9 (−1.9, 8.1) | 1.9 (−3.7, 7.8) | 1.1 (−3.9, 6.4) | 3.6 (−0.6, 7.9) | 2.9 (−1.3, 7.4) |
| 48 h average | 4.4 (−1.1, 9.8) | 2.1 (−4.8, 9.4) | 2.9 (−4.2, 11.0) | 2.3 (−6.0, 11) | 5.0 (−0.9, 11) | 2.0 (−5.2, 9.7) |
| 72 h average | 4.2 (−0.2, 8.8) | 3.1 (−15.8, 0) | 7.3 (0.6, 14.6) | 9.1 (−0.5, 20) | 4.5 (−0.6, 10.0) | 2.9 (−2.0, 8.2) |
| PM coarse | 24 h average | 7.0 (0.1, 14.3) | 1.0 (−1.9, 4.0) | 7.3 (0.4, 14.9) | 1.8 (−0.9, 4.5) | 6.9 (0.0, 14) * | 0.9 (−2.1, 3.9) |
| PM2.5 | −0.7 (−5.7, 4.6) | 1.6 (−22.3, 55) | −0.7 (−5.8, 4.7) | 1.8 (−17.5, 54) | −0.8 (−5.8, 4.5) | 1.7 (−2.1, 5.6) |
| PM coarse | 72 h average | −0.4 (−5.5, 5.0) | 0.1 (−33.3, 3.5) | −2.2 (−68.2, 27) | −1.3 (−51.2, 17) | −0.2 (−54.5, 4.0) | 0.4 (−28.3, 3.7) |

* p < 0.05.

4. Discussion

In this panel study of repeated FENO measurements in 95 schoolchildren over two months, we found significant within-individual increases of FENO after exposure to PM coarse during the previous 24 h and NO2 during the previous 72 h in models adjusted for other pollutants. Exposure to O3, on the other hand, was associated with decreased FENO levels after adjusting for other pollutants. The results indicate that PM coarse, derived mainly from mechanical wear, can induce sub-clinical airway inflammation in healthy children, and it would appear that exposure 24 h before the clinical examination is most relevant. This could be due to very low background levels of PM, so even moderate exposure levels affect FENO in the study group.

FENO is mainly associated with T-helper cell type 2 (Th2) driven airway inflammation, where an increase in airway eosinophils is a major characteristic even if the association between FENO and eosinophils is not very strong [20,21]. It seems likely that eosinophils in induced sputum and FENO reflect parallel processes in the inflamed airways. Traffic exposure, on the other hand, is merely inducing neutrophilic inflammation [22]. Nevertheless, FENO has been shown to be elevated in children with asthma living close to major roads [23], as well as after ozone exposure [24,25], and hence seems a biomarker of interest. In the current study, children with colds were excluded; nevertheless, the maximum FENO value was 71 ppb, but FENO has high variability.

PM coarse is a general problem in cities in northern Sweden where sand and studded tires are used to increase driving safety on icy roads. The entire road network is an important source of PM coarse in winter and spring, especially during periods of dry and windy weather where the European 24 h air quality guideline values are exceeded in the central part of Umeå, the current study setting [26]. However, regional background levels of PM are very low, which is why small absolute concentration changes present as large relative changes. Newly and locally generated particles thus become a larger fraction of PM in urban settings where most central monitors are located. Previous reports of health effects from coarse PM in urban settings could be due to variation in regional background PM (e.g., desert dust) which is reflected in concentrations measured at a single monitor rather than variation from nearby local sources, e.g., a construction site.

A possible limitation of the current study is assigning exposure from a single, central monitor which limits the available outcome. The monitors are located near the schools, but the children’s residence may not be very close and other daytime activities may modify the children’s daily exposure. However, assigning PM coarse exposure from a single, central monitor is considered to be representative for large urban areas, especially in studies of temporal variation [27]. Several studies have determined...
that there is a high correlation between personal exposure in children and exposure from central
monitors in children and that classroom PM$_{10}$ exposure was highly correlated with these metrics [28],
and similar trends have been found for smaller particles in studies of the relationship between ambient
and classroom measures [29].

For NO$_2$, which increases during stagnation and cold temperatures, the cumulative 72 h exposure
in models adjusted for PM$_{coarse}$ and O$_3$ yielded a higher effect estimate than at shorter lags.

Twenty-four-hour O$_3$ levels were associated with reduced FENO in the multi-pollutant models.
O$_3$ levels correlated with levels of PM$_{2.5}$ and temperature, but no other pollutants. O$_3$ levels in this
region are positively correlated with temperature. During stagnation O$_3$ levels fall as nitrogen species
build up. This happens especially in places where NOx levels are dominated by local sources due
to a high NO/NO$_2$ ratio from NO from local exhaust emissions. Higher O$_3$ levels could indicate
lower levels of exhaust components that are not measured such as ultrafine particles and aldehydes.
This result is in contrast with results from other studies in settings with shorter exposure windows and
higher O$_3$ levels [24]; however, the exposure time window was different and O$_3$ levels were
lower in the current study. In previous studies FENO levels were positively associated with mean O$_3$
levels of the previous eight hours in healthy children [24,25], and with those of the previous day in asthmatics [30]. Other epidemiological studies in adults have found associations between five-day cumulative O$_3$ exposure levels and increased inflammation in the distal airways (FENO$_{270}$), but at shorter lags, the association was not significant [31]. However, our effect estimates are similar to those
reported in asthmatic children [32], where same-day and two-day average O$_3$ levels were associated
with significant decreases in FENO$_{30}$. Other studies found no association between O$_3$ and FENO$_{30}$ in
healthy children [12], and chamber studies of healthy adults also found no association with exposure
to O$_3$ [33]. A possible explanatory factor for this unexpected protective effect of O$_3$ could be related to
behaviour, as O$_3$ levels tend to be higher during meteorological conditions with little wind, sun and
relative warmth which could prompt people to spend more time outdoors and be physically active.
Physical exercise was associated with lower FENO in adults even in settings with high exposure to
traffic-related air pollutants [34]. The present study has a strong advantage to assess the effects as the
study design, where both exposure and FENO were measured over a two-month period, allowing
for effect estimates based on within-individual variation independent of variation between different
schools or class rooms.

Most previous studies were cross-sectional or cohort studies which evaluated the effects of chronic
exposures. The exceptions are the studies of children in summer camps [24,25] where repeated FENO
measurements were associated with eight-hour O$_3$ means. Sarnat and colleagues [17] measured FENO
in a panel of school children for 16 weeks and found that particle matter from traffic and other sources,
rather than NO$_2$, was associated with FENO. Greenwald and colleagues [35] measured FENO in
a panel of elementary school students were measured weekly for 13 weeks to estimate the effects
of exposure to diesel truck traffic, but no association was found in healthy children. Koenig and
colleagues [36] measured children for 10 days while monitoring indoor and outdoor PM$_{2.5}$. Steerenberg
and colleagues [16] measured FENO and several other biomarkers in children in an urban school and
a suburban school weekly and found associations with black smoke, PM$_{10}$, NO, and NO$_2$.

PM$_{coarse}$ originating from biomass has been associated with same-day increased FENO in
children [15] but the effect estimates were lower than for the current study. However, in a study
of same-day PM$_{10}$, NO$_2$ and black smoke [37], much higher effect estimates were found for PM$_{10}$ than
in the current study. Barraza-Villarreal and colleagues [11] reported that eight-hour exposure to PM$_{2.5}$,
NO$_2$ and O$_3$ was associated with increased FENO in healthy subjects by 1.16 ppb per IQR (17.5 µg/m$^3$)
PM$_{2.5}$. However, the study set in Mexico City experienced much higher levels of PM$_{2.5}$ and NO$_2$ , and
lower levels of O$_3$. Other studies found no association between exhaled NO in healthy children and
two-week NO$_2$ or 48 h PM$_{2.5}$ and elemental carbon measured at the school [38].

Long-term exposure to PM$_{coarse}$ at the residence was not associated with FENO in 9–11 year-old
children [39]. In a study of oxidative stress and airway inflammation in children, black carbon (BC)
from combustion sources was associated with same-day measures of oxidative stress whereas 24 h and weekly exposure was associated with airway inflammation measured by FENO [9], so our observation could be due to the involvement of different mechanisms.

Finally, other factors affect FENO levels, e.g., gene-environment interactions, which have been described for the association between FENO_{50} and fine PM_{2.5} [40]. Among atopic rather than non-atopic children, associations between FENO and exposure to pollen [13] and PM_{2.5} have been found [10,11]. In our study, allergies were not a significant predictor of FENO and did not improve the model fit, and were thus discarded from the models. However, the current study setting had low levels of pollution, and the pollen levels were unusually low during the study period with a maximum 24 h mean concentration of 117 grains per m^{3}.

As the few asthmatic children in the recruited population were all treated with anti-inflammatory medication that may attenuate FENO response [35], these children were excluded from the study population. Some children with rhinitis were, however, included, and we lack the information about the daily use of nasal steroids, but this seems unlikely to confound the results as we studied the effects of short-term fluctuations in air pollution. The lack of information about daily use of medication against rhinitis is not likely to be a confounding problem as we study short-term effects of fluctuations in air pollution.

Participation was dependent on parental consent, but it is unlikely that this would influence the result as all analysed children were healthy. Also, we lack information on time spent outdoors and physical exercise which could depend on weather conditions and influence both outdoor and indoor exposure, which could also affect FENO levels.

5. Conclusions

Exposure to PM_{coarse} and NO_{2} is associated with an increase in FENO in healthy children in the present study where both exposure and FENO were followed over a two-month period in a low-exposure setting. Exposure to O_{3}, on the other hand, was associated with decreased FENO levels after adjusting for other pollutants. The results indicate that PM_{coarse} derived mainly from mechanical wear can induce sub-clinical airway inflammation in healthy children. The clinical significance of these results remains unclear, but is of interest for follow-up, as an increase in FENO has been associated with new-onset asthma and the role of PM_{coarse} is not well-studied.

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Author Contributions: Bertil Forsberg conceived and designed the study; Bodil Björ performed the study; Peter Boman analyzed the data; Hanne Krage Carlsen, Bertil Forsberg; and Anna-Carin Olin wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

| Abbreviation | Description |
|--------------|-------------|
| FENO         | Fraction of exhaled nitric oxide |
| NO           | Nitrogen oxide |
| NO_{2}       | Nitrogen dioxide |
| NO_{x}       | Nitrogen oxides |
| O_{3}        | Ozone |
| PM           | Particle matter |
| ppb          | Parts per billion |
| SD           | Standard deviation |
| Th2          | T-helper cell type 2 |
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