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Health effects of a subway environment in mild asthmatic volunteers

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Summary
Particle exposure is known to have negative health effects. In Stockholm the environment in the subway has been reported to have higher particle exposure levels, measured as $PM_{2.5}$ and PM_{10}, than roads with intense traffic in the inner city area. We have recently shown that healthy volunteers exposed to subway environment had statistically significant increase of fibrinogen and CD4 cells expressing regulatory T-cell marker $CD25^{bright}$/FOXP3 in blood. The aim of the present study was to find out whether a more vulnerable population, asthmatics, would demonstrate similar or other changes in the lungs or in the peripheral blood.

Sixteen mild asthmatics were exposed to a subway and a control environment for 2 h while being monitored by measurements of lung function, and inflammatory response in the lower airways evaluated by bronchoscopy and in peripheral blood. An attempt to standardize the exposures was done, by letting the volunteers alternate 15 min intervals of moderate exercise on a bicycle ergometer with 15 min of rest.

We found a statistically significant increased frequency of CD4 cells expressing T-cell activation marker CD25 in bronchoalveolar lavage fluid, but no significant increase of regulatory T-cells in blood as was found in healthy volunteers. Our study shows that airway inflammatory responses after exposure in subway environment differ between asthmatic and healthy humans.
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Introduction

Epidemiological associations between air pollution of particulate matter and health effects has been widely discussed over the last years. Particular matter (PM) has been associated with mortality and hospital admission due to respiratory and cardiovascular diseases.[1–6] The mechanisms are not fully understood, but small particles generated by combustion are suspected to influence the heart via inflammation/oxidative stress, accelerated atherosclerotic processes and changes in the heart rhythm regulation.[7,8] Differences in size and chemical composition of particles need to be considered in association with mortality in the general population,[9] which was shown during exposure to Saharan dust.[10]

Allergic respiratory diseases such as asthma have increased in frequency, putting focus on changes in environmental factors in air pollution as genetic predisposition alone cannot explain the increase. Individuals with asthma have inflamed airways and are more vulnerable to air pollutants than a healthy population. Inhalation of particles may exacerbate the already on-going lung inflammation.[11] Asthmatic symptoms and increased need for medication for asthmatic symptoms are strongest associated with ultrafine particles and with $PM_{2.5}$, rather than PM_{10}.[12–15]

In Stockholm (Sweden) the environment in the subway has been reported to have 5–10 higher concentration levels of particles than roads with intensive traffic in the inner city area.[16] $PM_{2.5}$ and PM_{10} mass concentrations levels in the subway change little from day to day with mean average of 260 and 470 $\mu g/m^3$ respectively. The levels of particles are within levels reported world-wide, with higher concentrations of particles in subway than in ambient air such as in Amsterdam,[17] Helsinki,[18] London,[19] New York,[20] Rome[21] and Seoul.[22] The present study was performed in a subway station with lower particle level, with mean average of 77 $\mu g/m^3$ for $PM_{2.5}$ and 242 $\mu g/m^3$ for PM_{10},[23] but in the same concentration range as in a road tunnel were we have previously performed exposure studies.[24–26]

In the subway particles are rather large, more than 1 μm in diameter, and mainly originate from railway tracks with a high content of iron. We have recently shown that healthy volunteers showed no cellular response in the airways after exposure to the subway environment. However, in blood we found a statistically significant increase of fibrinogen and of regulatory T-cells expressing $CD4^{pos}CD25^{bright}$/FOXP3.[23]

The aim of this study was to study acute health effects on individuals with asthma after exposure to a subway environment, and compare these results with the outcome of the previous findings in healthy individuals in the same environment.

Methods

Volunteers

Sixteen non-smoking mild asthmatic volunteers (11 women) with a mean age of 26 years (range 18–52) participated in the study. None of the volunteers used the subway on regular bases for at least two months before the inclusion. All volunteers underwent a routine physical examination. All had normal chest X-ray. After a baseline registration of forced expiratory volume during the first second (FEV_1), each volunteer inhaled diluent followed by inhalation of increasing doses of methacholine, starting at a dose of 14.2 μg, with doubling doses up to an accumulated dose of 7256 μg. The results are expressed as the cumulative dose causing a $\geq$20% decrease in FEV_1 ($PD_{20}FEV_1$) compared to the baseline FEV_1. All subjects were hyperresponsive to methacholine, defined as $\geq$20% fall in FEV_1.

None of the volunteers were allowed to be medicated with inhaled corticosteroids, or with other anti-inflammatory drugs, for the last 3 months before participation in the study. Short-acting β2-agonist treatment could be used when needed. An *in vitro* screening for the presence of specific IgE antibodies against common inhaled allergens (Phadiatop®, Pharmacia-Upjohn, Uppsala, Sweden) showed that 14 of the volunteers (88%) had IgE antibodies, and they also had a positive test for a number of radioallergosorbents (RAST, Pharmacia-Upjohn). For basal characteristics of the study population see Table 1.

All participants had given their informed written consent to participate in the study, which was approved by the Regional Ethical Review Board, Stockholm, Sweden (2007/748-31/3).

Study design

In order to avoid allergens such as grass and birch, the study was carried out in Stockholm between mid November and the beginning of April. The study had a randomized cross-

Table 1 Basal characteristics for 16 non-smoking asthmatics.

Volunteer no	Age	Phadiatop positive	Sex	FEV_1% (pred* %)	$PD_{20}FEV_1$ (μg)
1	25	+	M	97	99.0
2	52	−	F	116	628.0
3	25	+	M	95	183.0
4	18	+	F	91	<14.2
5	21	+	F	92	585.0
6	23	+	M	109	1889.0
7	18	+	M	127	462.0
8	40	−	F	124	581.0
9	31	+	F	113	628.0
10	27	+	F	120	541.0
11	20	+	F	83	256.0
12	21	+	F	95	53.0
13	22	+	M	119	<14.2
14	19	+	F	89	326.0
15	20	+	F	88	54.0
16	33	+	F	103	87.0
Summary	Mean 26 (18–52) years	14 Phadiatop positive	11 F; 5 M	Mean ± SD 104 ± 14	Mean ± SD 400 ± 463

Abbreviations: *pred = predicted; F = female; M = male; FEV_1 = Forced Expiratory Volume in 1 s; $PD_{20}FEV_1$ = the cumulative dose of methacholine provoking a 20 percent decline in FEV_1.

over experimental design that involved exposures to both a subway environment and an office environment (control) with at least three weeks interval. For detailed description, see a previous study performed on healthy subjects with the same study design.[23] Fourteen hours after each exposure bronchoscopy and blood sampling were performed.

Asthmatic volunteers served as their own controls. During participation in the study the volunteers were told to perform ordinary daily activities. However, no transportation using the subway system was allowed during the whole study period. The volunteers were also encouraged to avoid heavy physical activities during the days of measurements and to avoid staying in areas with heavy air pollution. Participants did not have airway infections within six weeks before exposure occasions.

Location of exposures

The subway exposures were performed during the afternoon rush hours (4–6 p.m.) at the platform of a subway station (Odenplan) in the central part of Stockholm. Control exposures were performed during corresponding hours in an office environment.

For details of the subway location see the article of Klepczynska Nyström et al.[23]

During the exposure sessions the volunteers alternated 15 min intervals of moderate exercise on a bicycle ergometer with 15 min of rest. The ergometer resistance was adjusted in order to achieve an individual ventilation rate of 20 L air per minute and m^2 of the body surface.

Environmental exposure measurements

Exposure levels were monitored during each exposure session. Sampling of $PM_{2.5}$ and PM_{10} particles (with an upper 50% cut-off aerodynamic diameter of 2.5 respectively 10 μm) in the subway environment, was performed using Harvard impactors (Air Diagnostics and Engineering Inc., Maine, USA) equipped with Teflon filters (Air Diagnostics and Engineering Inc., Maine, USA) with a pore size of 2 μm and at a flow rate of approximately 10 L/min. The filters were weighed after 24 h of conditioning using a Mettler Toledo MT5 scale (Mettler, Greisensee, Switzerland), but also analyzed for a range of metals using an inductively coupled plasma mass spectrometry (ICP-MS). The number concentration of airborne UF particles was determined using an upgraded Scanning Mobility Particle Sizer (SMPS) system, which can detect ultrafine particles with diameter of 10–100 nm. For comparative reasons we chose to state particles in the same particle size range as in previous study (18–100 nm). SMPS consists an Electrostatic Classifier model 3080 (TSI, Shoreview, MN, USA) in combination with a Condensation Particle Counter (CPC) model 3010 (TSI). Nitric oxide (NO_x) levels were monitored using a chemiluminescent instrument (AC 31M, Environnement, Poissy, France).

Also portable logging instruments were used: a DataRAM (MIE pDR1000), that measures the mass of particles between 0.1 and 10 μm in diameter, and P-Trak, a particle counter (TSI) estimating the number concentration of particles with a diameter of 20–1000 nm. For measurements in the control environment only portable instruments were.

Self-reported symptoms

During the exposure sessions self-reported symptoms of irritation from eyes, nose and lower airways, as well as experience of disturbing noise and smell, were recorded before and every 30 min throughout the exposures. The intensity was graded from 0 to 10, where 0 corresponded to no symptoms and 10 to severe symptoms, according to a modified Visual Analogue Scale (VAS).[27]

Lung function tests

Immediately prior to premedication before bronchoscopies measurements of lung function were performed with spirometry (Jaeger Masterscope, Würzburg, Germany), which included vital capacity (VC), forced vital capacity (FVC) and forced expiratory volume during the first second (FEV_1) with a ECCS reference[28] as well as measurements of exhaled NO levels (Aerocrine, Niox Mino).

Measurements of peak expiratory flow (PEF) were performed with a portable lung health monitor (PIKO-1, Ferraris, Louisville, US) before the exposure session and every hour during the exposure session. In addition, each volunteer was instructed to repeat PEF measurements after 2, 4 and 12–13 h after the exposures, as well at the clinic the following morning approximately 14 h after the exposures.

Peripheral blood

Peripheral blood was sampled in connection to bronchoscopy. Cell differential counts were performed using an autoanalyser (Advia 120 Hematology System, Bayer). Fibrinogen in plasma was analyzed by a kinetic fibrinogen assay. Plasminogen activator inhibitor-1 (PAI-1) was analyzed using Enzyme-Linked ImmunoSorbent Assay (ELISA) (Chromolize PAI-1, Biopool, Trinity Biotech, Bray, Ireland). Blood was also used for immunostaining and flow cytometric analysis (see below).

Bronchoscopy, bronchoalveolar lavage and bronchial wash

Bronchoalveolar lavage (BAL) was performed by inserting a flexible fiberoptic bronchoscope, (Olympus, Olympus Optical Co. Ltd., Tokyo, Japan) under local anesthesia, as described in detail by Eklund and Blaschke[29] 250 mL sterile phosphate buffered saline was instilled into the middle lobe in 5 aliquots of 50 mL each. After each installation the fluid was gently aspirated and collected and kept on ice.

The BAL fluid (BALF) was filtered through a single layer of Dacron net (Millipore, Cork, Ireland), centrifuged at 400 g for 10 min at 4 °C. The pellet was resolved in RPMI (Roswell Park Memorial Institute) 1640 medium (Sigma–Aldrich, UK). Cells were counted and total cell viability (mean 94.4%) was determined by trypan blue exclusion, using a Bürker chamber. BALF cell pellet was also used for immunostaining and flow cytometric analysis (see below).

The supernatant was analyzed for inflammatory cytokines interleukin IL-1B, IL-6, IL-8, IL-10, IL-12p70 and tumor necrosis factor-α (TNF-α). Cytokine analyses were done by flow cytometry (FACSCalibur, Becton Dickinson, North Carolina, US) using CBA (Cytometric Bead Array) technique (Becton Dickinson, North Carolina, US).

Bronchial wash was done in a segmental bronchus in an upper lobe by installing 2 $\times$ 10 mL sterile phosphate buffered saline. Each installation was followed by aspiration of fluid.

Immunostaining and flow cytometric analysis

Lymphocyte subsets were determined in BALF and erythrocyte lysed human peripheral blood using the TBNK 6 color Multitest. TBNK consists of a combination of fluorochrome conjugated monoclonal antibodies for T-cells, B-cells and NK-cells. We also used a set of monoclonal antibodies specific for markers of T-cell activity and of T-cell regulatory functions. The monoclonal antibodies that were used for phenotypic characterization of lymphocyte subsets was as follows: CD3 (T-cell marker), CD4 (T-helper cells/regulatory T-cells), CD8 (T-cytotoxic/suppressor), CD25 (Anti-IL-2R-activation), CD45 (white blood cells), CD69 (early activation), $CD56^{pos}CD16^{pos}$ (NK-cells or NK-T-cells), CD19 (B-cells), HLA-DR (MHC II activation), Forkhead box P3 (FOXP3) (T-regulatory cell). All monoclonal antibodies were from BD Biosciences, San Jose, CA, USA, with the exception of FOXP3 which was obtained from eBiosciences, San Jose, CA, USA.

For each analysis, 1×10^6 BALF cells were incubated with antibodies for 30 min in the dark and kept on ice at 4 °C, while blood was kept at room temperature. Blood samples were lysed with lysing solution and washed twice with cell wash (BD Biosciences, San Jose, CA, USA). All samples were analyzed by a flow cytometer using an 8 color FACSCanto II (BD Franklin Lakes, NJ USA). Approximately 2.5×10^5 cells were collected from each sample. Lymphocytes were gated by forward and side scatters. For the TBNK Multitest a gate was set for CD45 (white blood cells). For the T-cell activation and T-regulatory panels a gate was set for CD3 cells (T-cells). Isotype matched negative control antibodies always stained less than 1%.

Statistical analysis

Statistical analysis was carried out with SPSS version 17.0 on a Windows based PC platform (SPSS Inc, Chicago, Illinois, USA). Individual changes in different parameters for subway and control exposure were analyzed using Wilcoxon's non-parametric rank sum tests. A paired *t*-test was performed for lung function data and exposure measurements. Values of $p < 0.05$ were regarded as significant.

Results

Environmental exposure measurements

The exposure levels, for both control and subway environment, were similar to the previous study. Subway exposures were performed at totally eleven occasions. A comparison of exposure levels for $PM_{2.5}$, PM_{10}, UF particles, NO and NO_2 for both studies are presented in Table 2, as well as the content of metals in the PM_{10} fraction (mean level $\pm$ SD). All but three metals (iron, barium and manganese) were below the detection limits (ICP-MS).

In the control exposures the particle concentration of 0.1–10 μm (using DataRAM) was 18 $\pm$ 4 $\mu g/m^3$ (mean level $\pm$ SD), while the number concentration of particles with a diameter of 20–1000 nm (using P-Trak) was 910 $\pm$ 420 particles/mL. The levels with the same equipment in the subway environment were 150 $\pm$ 30 $\mu g/m^3$ and 9330 $\pm$ 1290 $\mu g/m^3$, respectively.

Symptoms

In comparison with the control exposures, self-reported symptoms of irritation from the eyes, nose and experience of disturbing smell were significantly higher during the exposure to the subway environment, see Table 4. No significant increase of irritations in the lower airways or experience of disturbing noise was reported.

Lung function tests

Fourteen hours after each exposure no significant changes between subway and control exposures were observed with regard to VC, FVC, FEV_1 or exhaled NO. None significant changes were seen in mean PEF performed 2–14 after the exposures or for PEF quotient (after exposure/before exposure). For details see Appendix (additional material).

Table 2 Mean $\pm$ SD values for environmental exposure measurements in the subway for the study with asthmatics in comparison with levels for a previously performed study with healthy volunteers.[23] Both studies were performed at the same subway station and during the same seasons.

Type of exposure	Healthy	Asthmatics
Ultrafine particles (number concentration)	8280 $\pm$ 1720/mL	8960 $\pm$ 660/mL
$PM_{2.5}$ (mass concentration)	77 $\pm$ 10 $\mu g/m^3$	71 $\pm$ 13 $\mu g/m^3$
PM_{10} (mass concentration)	242 $\pm$ 40 $\mu g/m^3$	232 $\pm$ 51 $\mu g/m^3$
NO	58 $\pm$ 12 $\mu g/m^3$	43 $\pm$ 14 $\mu g/m^3$
NO_2	24 $\pm$ 3 $\mu g/m^3$	20 $\pm$ 3 $\mu g/m^3$
Iron in PM_{10}	58.6 $\pm$ 21.0 %	49.3 $\pm$ 7.3%
Barium in PM_{10}	1.0 $\pm$ 0.4%	0.7 $\pm$ 0.1%
Manganese in PM_{10}	0.5 $\pm$ 0.2%	0.4 $\pm$ 0.1%
Copper in PM_{10}	0.8 $\pm$ 0.4%	Below detection limit

Abbreviations: $PM_{2.5}$ and PM_{10}: particulate mass of particles with diameter <2.5 and <10 μm, respectively; NO: nitrogen monoxide; NO_2: nitrogen dioxide.

Table 3 Overview of findings for T-cells expressing markers analyzed for healthy,[23] and asthmatic volunteers respectively. Analyses were done in erythrocyte-lysed human peripheral blood and bronchoalveolar lavage (BAL) fluid after volunteers being the exposed to control and subway environment. The data is presented as medians (interquartile ranges).

$CD4^{pos}$ cells markers	Healthy			Asthmatics		
	Control exposure	Subway exposure	*p*-value	Control exposure	Subway exposure	*p*-value
Regulatory T-cell marker (%)						
FOXP3 in blood	2.1 (1.5–5.4)	4.3 (2.5–5.9)	0.03	1.8 (1.4–2.6)	2.0 (1.1.-3.1)	0.4
FOXP3 in BAL fluid	8.1 (5.2–9.4)	8.8 (7.5–10.9)	0.1	6.8 (5.7–7.8)	7.3 (5.9–10.7)	0.4
$CD25^{bright}$/FOXP3 in blood	2.1 (0.8–2.7)	2.8 (1.7–4.5)	0.03	1.0 (0.6–1.2)	0.8 (0.5–1.4)	0.7
$CD25^{bright}$/FOXP3 in BAL fluid	4.9 (3.7–6.8)	5.4 (3.9–6.0)	0.8	4.9 (3.5–6.1)	4.5 (3.8–6.7)	0.2
T-cell activation (%)						
CD25 in BAL fluid	7.8 (6.6–9.2)	8.8 (7.2–10.9)	0.5	7.4 (6.0–9.6)	9.5 (6.4–12.2)	0.03

Peripheral blood

The total number of cells, cell concentrations and distribution of different cell populations in peripheral blood did not differ between control and subway exposures. Neither fibrinogen nor PAI-1 levels in the peripheral blood were affected. Levels of PAI-1 were often below the detection limit of <5 kilounits/L (10 volunteers after the subway exposure respectively 9 after the control). In order to perform statistical analysis, these cases were assigned a value of 3. For details see Appendix (additional material).

Bronchoalveolar lavage fluid and bronchial wash

No significant changes were seen in BALF or bronchial wash fluid between subway and control exposures with regard to recovery, cell viability, the total number of cells, cell concentration and distribution of different cell populations. Neither any significant change was seen in cytokines expression in the BALF. For details see Appendix (additional material).

Immunostaining and flow cytometric analysis

In BALF the percentage of $CD4^{pos}$ T-cell population expressing T-cell activation marker interleukin-2 (IL-2) receptor alpha chain (CD25) increased significantly after exposure in the subway ($p < 0.03$). In blood, no corresponding significant increase was seen. See Table 3 and Fig. 1.

No other significant change in TBNK-cells or lymphocyte subsets was seen after exposure in the subway. For comparative data with a previous study with healthy on regulatory T-cells and CD4 cells expressing the T-cell activation marker CD25, see Tables 3 and 4. No significant changes in the ratio between the T-helper/regulatory cells(CD4)/T-cytotoxic-suppressor cells (CD8) in BALF was found. It was 1.5 (control) *versus* 1.7 (subway). The ratio in blood was 2.5 (control) *versus* 2.3 (subway).

Discussion

Inhaled particles in air pollution may exacerbate the already on-going lung inflammation in asthmatics.[11] The mean

Table 4 (additional material). Changes in different parameters in healthy and asthmatic volunteers, respectively, after being exposed to control and subway environments.

Parameter	Healthy[16]	Asthmatics
Lymphocyte subsets in blood (CD3, CD4, CD8, CD25, CD19, CD45, $CD56^{pos}CD16^{pos}$, CD69, HLA-DR, FOXP3)	↗ $CD4^{pos}/CD69^{pos}$ ↗ $CD4^{pos}$/$HLA\text{-}DR^{pos}$ ↗ $CD8^{pos}/CD69^{pos}$ ↗ $CD4^{pos}$/FOXP3 ↗ $CD4^{pos}D25^{bright}$/ FOXP3	ns
Lymphocyte subsets in BAL fluid (as above)	ns	↗ $CD4^{pos}$/CD25
Self-reported symptoms during exposure (irritation from eyes, nose, lower airways; disturbing noise and smell)	↗ lower airways ↗ disturbing smell	↗ eyes ↗ nose ↗ disturbing smell
Fibrinogen in blood	↗	ns

Abbreviations: ns = non-significant; ↗ significance = values of $p < 0.05$ were regarded as significant with an increase after subway exposure in comparison to control exposure; BAL = bronchoalveolar lavage; BW = bronchial wash. For descriptive data, see Table 3 and Appendix.

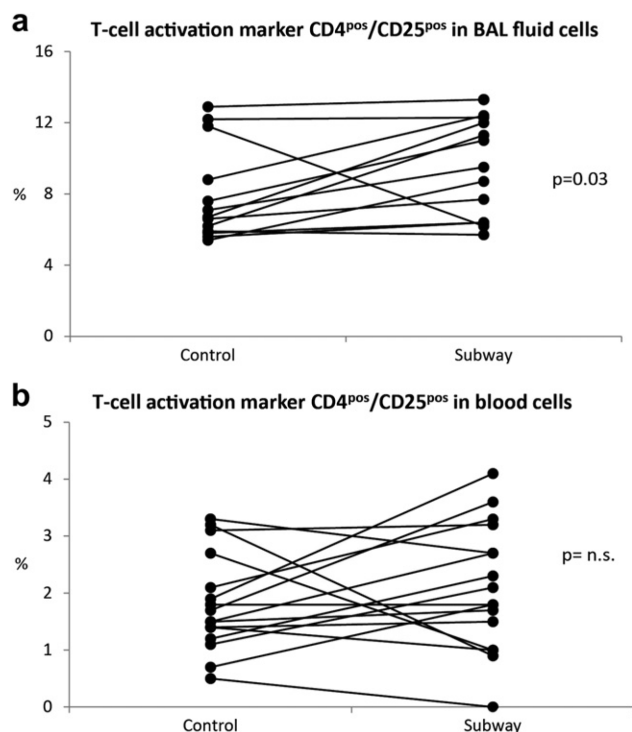

Figure 1 Percentage of CD4 cells expressing the T-cell activation marker CD25 ($CD4^{pos}/CD25^{pos}$) in bronchoalveolar lavage fluid (BALF) and peripheral blood after control and subway exposure. Data is presented as medians (interquartile ranges). (a) In BALF, the relative number increased from 7.4 (6.0–9.6) after control exposure to 9.5 (6.4–12.2) after subway exposure ($p = 0.03$). (b) In blood the relative number increased from 1.6 (1.3–2.6) after control exposure to 2.0 (1.1–3.1) after subway exposure.

average levels of particles in Stockholm subway are within levels reported world-wide in scientific literature.[17,18,20,21,30] We have found that mild asthmatics exposed to subway environment for 2 h, with particles mainly containing iron, showed statistically significant increase in percentage of lung recruited CD4 cells expressing the T-cell activation marker CD25 ($CD4^{pos}/CD25^{pos}$) in comparison to a control exposure. $CD25^{pos}$ is a general activation marker present on activated T-cells in inflammatory reactions, but in situations with no ongoing immune response it is also an appropriate marker for natural regulatory T-cell with a frequency of 5–10% in both mice and human blood.[31] In our study the $CD4^{pos}/CD25^{pos}$ changes were seen in BAL, but not in blood, which suggest a local effect on lungs.

There is to our knowledge no other study that has investigated acute health effects in asthmatics caused by exposure in the subway environment. Acute health effects in asthmatics to environmental pollutants may differ depending on the composition and concentration of pollutants, as well as on the severity of asthma. In our study we have chosen a group of "mild asthmatics", with no regular need of medication for asthma. Another *in vivo* study on asthmatics was undertaken where they were exposed for 2 h in a busy road in London.[32] Car exhausts causes exposure to much higher number concentration of ultrafine particles than subway environment does. The road traffic exposure had a negative effect on the lung function of the asthmatics measured as FEV_1 and FVC in comparison to exposure in a park. The effects were greater in moderate asthmatics compared to mild asthmatics. Associations were strongest with ultrafine particles and with elemental carbon. The association with ultrafine particles, rather than with $PM_{2.5}$ and PM10, and asthmatic symptoms and increased need of medication, has been shown in other studies.[12–15]

We have previously performed an identical *in vivo* study in the subway environment with healthy volunteers. The exposure levels of $PM_{2.5}$ and PM_{10}, number concentration of ultrafine and NO_x, were in the same order of magnitude in the two studies. No significant increase of CD4 cells expressing T-cell activation marker CD25 was found in healthy.[23] Healthy demonstrated significant increased expression of markers for regulatory T-cells ($CD4^{pos}CD25^{bright}$/FOXP3), as well as elevated levels of fibrinogen in peripheral blood, after being exposed to the subway environment. Even though asthmatics are known to be more sensitive to air pollution than healthy,[33] this was not seen in asthmatics.

Studies on T-regs in asthmatics is quite novel.[34] Generally T-regulatory cells are important in balancing or suppressing immune responses. At present there limited knowledge regarding the role of T-regs in asthma, but evidence suggests that asthma is characterized by a relative deficiency in T-regs.[31] Such tendency was also seen in our study. In an *in vivo* study evaluation was done on circulating T-reg cells in 14 control subjects and 29 patients with asthma.[35] In both groups numbers of peripheral blood $CD4^{pos}CD25^{high}$ and $CD4^{pos}CD25^{high}$/FOXP3 T-cells were similar, but asthmatic patients had decreased FOXP3 protein expression within their $CD4^{pos}CD25^{high}$ T-regs. Interestingly treatment of asthmatics with glucocorticosteroids had a tendency to increase FOXP3 expression within $CD4^{pos}CD25^{high}$ T-cells, when compared to steroid-naive asthmatic patients. In our study none of the asthmatic volunteers was treated with glucocorticosteroids.

In our study asthmatics reported significant increased irritation in the eyes and the nose during the exposure to the subway environment, as well as increased experience of disturbing smell. Similar to asthmatics, a self-reported experience of a disturbing smell was registered in healthy volunteers, but they had no significant changes in irritations in the eyes or in the nose. Instead healthy reported increased signs of irritation in the lower airways. For summary of comparative data, see Table 4 (additional material).

Overall the acute health effects after exposure to subway environment are few. The main result from our study shows that a 2 h long exposure of asthmatic individuals leads to a different T-cell response than for healthy volunteers in our previous study. There is a local activation of T-cells in asthmatics in BAL fluid, while healthy demonstrate a systemic increase of regulatory T-cells in blood that suppresses the inflammatory response. It is still too early to conclude if this difference has any influence on real health risk, but it would be of interest to expand the follow-up time frame with repeated measurements to monitor possible earlier or later effects on the inflammatory responses. BAL is not a suitable method for repetitive measurements, while repeated blood sampling could be used. In healthy volunteers, inflammatory effects were seen in blood, which makes them as interesting and feasible group to test.

Conflict of interest

None.

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Appendix

(additional material). Descriptive data for different outcome parameters for healthy[23] respectively asthmatic volunteers after being exposed to control and subway environment. Data for lung function are presented as mean ± SD, while data for cellular response in peripheral blood, as well as for PAI-1, and for bronchoalveolar lavage and bronchial wash findings are presented as medians (interquartile ranges)

Outcome parameter	Healthy Control exposure	Healthy Subway exposure	Asthmatics Control exposure	Asthmatics Subway exposure
Lung function				
VC (predicted %)	109 ± 14	111 ± 13	103 ± 15	101 ± 11
FVC (predicted %)	112 ± 13	112 ± 14	108 ± 13	107 ± 13
FEV_1 (predicted %)	105 ± 12	105 ± 14	102 ± 16	103 ± 17
Exhaled NO (ppb)	18 ± 8	17 ± 9	43 ± 42	40 ± 36
PEF, 2–14 h after exposure (L/min)	537 ± 138	522 ± 113	469 ± 119	460 ± 116
PEF quotient (after exposure/before exposure)	1.0	0.96	0.98	0.96
Blood cell concentration (10^9/L)				
Leucocytes	5.7 (5.1–6.7)	5.6 (5.2–6.5)	5.6 (5.2–7.1)	5.5 (5.1–6.2)
Neutrophils	2.8 (2.6–4.0)	2.9 (2.5–3.6)	2.6 (2.0–3.4)	2.5 (2.1–3.3)
Eosinophils	0.1 (0.1–0.2)	0.1 (0.1–0.2)	0.4 (0.1–0.5)	0.2 (0.1–0.4)
Basophils	0.1 (0.1–0.1)	0.1 (0.1–0.1)	0.1 (0.1–0.1)	0.1 (0.1–0.1)
Lymphocytes	2.4 (2.0–2.6)	2.4 (1.9–2.7)	2.5 (1.9–2.7)	2.2 (1.9–2.7)
Monocytes	0.4 (0.3–0.4)	0.4 (0.3–0.4)	0.6 (0.5–0.7)	0.5 (0.5–0.6)
Blood differential (%)				
Neutrophils	50.8 (46.5–54.8)	51.7 (45.8–54.9)	44.3 (39.1–53.1)	44.7 (38.5–52.8)
Eosinophils	2.0 (1.8–3.0)	2.0 (1.8–3.4)	6.5 (2.3–8.6)	3.9 (2.6–6.8)
Basophils	1.8 (1.5–2.0)	1.8 (1.6–1.9)	1.8 (1.4–1.9)	1.8 (1.6–2.0)
Lymphocytes	40.2 (35.1–43.9)	40.5 (35.9–45.2)	39.6 (32.9–44.4)	39.9 (35.1–43.1)
Monocytes	5.9 (5.3–7.0)	6.1 (5.0–7.1)	9.5 (8.6–12.2)	9.4 (7.3–12.7)
Bronchoalveolar lavage (BAL) findings				
Recovery (%)	74.0 (71.2–78.2)	70.7 (64.5–75.4)	70.5 (55.0–78.8)	69.0 (61.8–75.2)
Viability (%)	95.3 (91.3–98.1)	95.5 (94.3–97.7)	92.0 (90.0–94.8)	92.9 (90.3–95.0)
Total number of cells (*10^6)	14.3 (12.3–17.8)	12.7 (10.1–17.0)	13.7 (9.2–19.0)	12.7 (10.4–23.1)
Total cell concentration (*10^6/L)	79.5 (67.8–98.9)	75.1 (59.2–96.2)	81.2 (60.0–125.4)	98.6 (64.1–149.7)
Number of mast cells[a]	1.5 (0.0–5.0)	1.5 (1.0–4.0)	4.0 (1.0–8.8)	2.5 (1.0–4.8)
Cell concentrations in BAL fluid (10^6L)				
Macrophages	66.8 (56.7–84.9)	60.4 (50.1–86.1)	65.0 (52.6–103.5)	80.2 (54.6–137.4)
Lymphocytes	8.8 (5.6–17.2)	8.2 (6.0–13.5)	8.9 (5.1–18.0)	11.3 (8.8–15.2)
Neutrophils	1.4 (0.9–1.9)	1.7 (1.1–2.4)	1.4 (0.8–2.4)	1.6 (0.7–3.0)
Eosinophils	0.1 (0.0–0.4)	0.1 (0.0–0.5)	0.5 (0.3–1.1)	0.5 (0.1–2.5)

(*continued on next page*)

(continued)

Outcome parameter	Healthy Control exposure	Healthy Subway exposure	Asthmatics Control exposure	Asthmatics Subway exposure
Basophils	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.1)
Cell differential in BAL fluid (%)				
Macrophages	85.9 (80.4–89.4)	85.3 (81.9–88.3)	85.7 (80.0–89.7)	83.4 (79.2–90.1)
Lymphocytes	11.3 (8.3–17.9)	11.4 (8.2–15.9)	11.0 (7.6–14.8)	11.9 (7.7–15.5)
Neutrophils	1.9 (1.2–2.2)	2.2 (1.4–3.0)	1.5 (0.8–2.5)	1.8 (0.9–2.9)
Eosinophils	0.2 (0.0–0.4)	0.2 (0.0–0.6)	0.6 (0.2–1.6)	0.6 (0.3–2.4)
Basophils	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–1.2)
Bronchial wash (BW) findings				
Recovery (%)	40.0 (37.0–50.0)	40.0 (35.0–50.0)	60.0 (40.0–60.0)	55.0 (50.0–60.0)
Viability (%)	82.0 (67.0–94.0)	75.0 (58.0–86.0)	94.5 (84.3–99.5)	90.0 (86.8–97.3)
Total number of cells (*10^6)	0.4 (0.2–0.8)	0.7 (0.3–1.0)	0.4 (0.1–0.7)	0.4 (0.2–0.7)
Total cell concentration (*10^6/L)	52.5 (30.0–99.1)	85.0 (36.0–133.0)	31.5 (14.9–61.6)	33.8 (21.5–55.4)
Number of mast cells[a]	2.0 (0.0–4.5)	2.0 (0.0–3.0)	4.0 (1.0–15.0)	3.0 (2.0–16.5)
Cell concentrations in BW fluid (10^6/L)				
Macrophages	51.9 (51.7–52.2)	57.8 (57.8–57.8)	26.3 (14.1–36.0)	23.4 (12.9–40.8)
Lymphocytes	2.2 (2.1–2.3)	6.9 (6.9–6.9)	2.7 (0.9–4.3)	2.6 (1.0–4.7)
Neutrophils	14.7 (3.3–26.0)	5.2 (5.2–5.2)	3.8 (1.5–5.2)	2.7 (1.2–4.8)
Eosinophils	0.1 (0.1–1.2)	0.1 (0.1–0.1)	0.2 (0.0–0.4)	0.2 (0.1–0.8)
Basophils	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
Cell differential in BW fluid (%)				
Macrophages	64.6 (56.8–73.0)	55.8 (41.3–76.8)	80.9 (68.2–83.6)	76.5 (68.6–89.0)
Lymphocytes	5.6 (3.5–8.2)	5.7 (3.6–10.0)	8.3 (3.4–11.6)	8.0 (5.6–11.5)
Neutrophils	28.0 (18.2–34.5)	38.3 (11.4–54.0)	13.0 (6.2–19.2)	12.0 (2.0–18.0)
Eosinophils	0.2 (0.0–0.5)	0.3 (0.0–0.7)	0.8 (0.0–1.0)	0.7 (0.3–3.1)
Basophils	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
Cytokine concentrations in BAL fluid (pg/L)				
IL-1B	79.8 (57.8–165.9)	64.9 (53.1–139.8)	284.2 (154.4–439.0)	166.3 (92.1–296.4)
IL-6	394.9 (263.2–963.0)	391.9 (272.0–686.4)	711.6 (529.4–1287.4)	534.5 (398.6–1152.9)
IL-8	8569.5 (4646.8–19802.3)	4901.0 (3696.0–14260.0)	12 718.0 (6513.0–16723.0)	12 359.0 (5535.0–18459.0)
IL-10	15.2 (5.5–21.2)	9.6 (6.3–17.3)	26.4 (21.3–30.4)	20.1 (15.6–30.0)
IL-12p70	14.8 (10.6–24.0)	13.7 (8.0–22.1)	17.4 (0.0–28.5)	0.0 (0.0–20.0)
TNF-α	8.9 (1.0–58.6)	8.2 (0.0–49.0)	14.2 (8.4–46.3)	17.0 (0.0–54.3)
PAI-1 in blood (kilounits/L)				
PAI-1	3 (3–11)	3 (3–11)	3 (3–9)	3 (3–10)
Fibrinogen (g/L)				
Fibrinogen in blood	2.2 (2.00–2.38)	2.3 (2.21–2.58)	2.6 (2.2–2.9)	2.4 (2.2–2.9)

[a] Enlarged 16 times/10 visual field.

References

1. Brook RD, Rajagopalan S, Pope 3rd CA, Brook JR, Bhatnagar A, Diez-Roux AV, et al. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. *Circulation* 2010 Jun 1; **121**(21):2331–78.
2. Brunekreef B, Forsberg B. Epidemiological evidence of effects of coarse airborne particles on health. *Eur Respir J* 2005 Aug; **26**(2):309–18.
3. Brunekreef B, Holgate ST. Air pollution and health. *Lancet* 2002 Oct 19;**360**(9341):1233–42.
4. Dockery DW. Health effects of particulate air pollution. *Ann Epidemiol* 2009 Apr;**19**(4):257–63.
5. Dockery DW, Pope 3rd CA, Xu X, Spengler JD, Ware JH, Fay ME, et al. An association between air pollution and mortality in six U.S. cities. *N Engl J Med* 1993 Dec 9;**329**(24):1753–9.
6. Pope 3rd CA, Burnett RT, Thun MJ, Calle EE, Krewski D, Ito K, et al. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. *JAMA* 2002 Mar 6; **287**(9):1132–41.
7. Brook RD. Cardiovascular effects of air pollution. *Clin Sci (Lond)* 2008 Sep;**115**(6):175–87.
8. Pope 3rd CA, Burnett RT, Thurston GD, Thun MJ, Calle EE, Krewski D, et al. Cardiovascular mortality and long-term exposure to particulate air pollution: epidemiological evidence of general pathophysiological pathways of disease. *Circulation* 2004 Jan 6;**109**(1):71–7.

9. Franklin M, Koutrakis P, Schwartz P. The role of particle composition on the association between PM2.5 and mortality. *Epidemiology* 2008 Sep;**19**(5):680—9.
10. Perez L, Tobias A, Querol X, Kunzli N, Pey J, Alastuey A, et al. Coarse particles from Saharan dust and daily mortality. *Epidemiology* 2008 Nov;**19**(6):800—7.
11. Health effects of outdoor air pollution. Committee of the environmental and Occupational Health Assembly of the American Thoracic Society. *Am J Respir Crit Care Med* 1996 Jan;**153**(1):3—50.
12. Penttinen P, Timonen KL, Tiittanen P, Mirme A, Ruuskanen J, Pekkanen J. Ultrafine particles in urban air and respiratory health among adult asthmatics. *Eur Respir J* 2001 Mar;**17**(3): 428—35.
13. Ostro BD, Lipsett MJ, Wiener MB, Selner JC. Asthmatic responses to airborne acid aerosols. *Am J Public Health* 1991 Jun;**81**(6):694—702.
14. Peters A, Wichmann HE, Tuch T, Heinrich J, Heyder J. Respiratory effects are associated with the number of ultrafine particles. *Am J Respir Crit Care Med* 1997 Apr;**155**(4):1376—83.
15. Penttinen P, Vallius M, Tiittanen P, Ruuskanen J, Pekkanen J. Source-specific fine particles in urban air and respiratory function among adult asthmatics. *Inhal Toxicol* 2006 Mar; **18**(3):191—8.
16. Johansson C, Johansson P-Å. Particulate matter in the underground of Stockholm. *Atmos Environ* 2003;**37**(3—9).
17. Strak M, Steenhof M, Godri KJ, Gosens I, Mudway IS, Cassee FR, et al. Variation in characteristics of ambient particulate matter at eight locations in the Netherlands — the RAPTES project. *Atmos Environ* 2011 Aug;**45**(26):4442—53.
18. Aarnio P, Yli-Tuomi T, Kousa A, Makela T, Hirsikko A, Hameri K, et al. The concentrations and composition of and exposure to fine particles (PM2.5) in the Helsinki subway system. *Atmos Environ* 2005 Sep;**39**(28):5059—66.
19. Pfeifer GD, Harrison RM, Lynam DR. Personal exposures to airborne metals in London taxi drivers and office workers in 1995 and 1996. *Sci Total Environ* 1999 Sep;**235**(1—3):253—60.
20. Wang X, Gao HO. Exposure to fine particle mass and number concentrations in urban transportation environments of New York City. *Transportation Res Part D-Transport Environ* 2011 Jul;**16**(5):384—91.
21. Ripanucci G, Grana M, Vicentini L, Magrini A, Bergamaschi A. Dust in the underground railway tunnels of an Italian town. *J Occup Environ Hyg* 2006 Jan;**3**(1):16—25.
22. Kim Y, Kim M, Lim J, Kim JT, Yoo C. Predictive monitoring and diagnosis of periodic air pollution in a subway station. *J Hazard Mater* 2010 Nov;**183**(1—3):448—59.
23. Klepczynska Nyström A, Svartengren M, Grunewald J, Pousette C, Rodin I, Lundin A, et al. Health effects of a subway environment in healthy volunteers. *Eur Respir J* 2010 Aug; **36**(2):240—8.
24. Larsson BM, Grunewald J, Skold CM, Lundin A, Sandstrom T, Eklund A, et al. Limited airway effects in mild asthmatics after exposure to air pollution in a road tunnel. *Respir Med* 2010 Dec;**104**(12):1912—8.
25. Larsson BM, Sehlstedt M, Grunewald J, Skold CM, Lundin A, Blomberg A, et al. Road tunnel air pollution induces bronchoalveolar inflammation in healthy subjects. *Eur Respir J* 2007 Apr;**29**(4):699—705.
26. Svartengren M, Strand V, Bylin G, Jarup L, Pershagen G. Short-term exposure to air pollution in a road tunnel enhances the asthmatic response to allergen. *Eur Respir J* 2000 Apr;**15**(4): 716—24 [Article].
27. Price DD, McGrath PA, Rafii A, Buckingham B. The validation of visual analogue scales as ratio scale measures for chronic and experimental pain. *Pain* 1983 Sep;**17**(1):45—56.
28. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of lung function tests, European Community for Steel and Coal. Official statement of the European respiratory Society. *Eur Respir J Suppl* 1993 Mar;**16**: 5—40.
29. Eklund A, Blaschke E. Relationship between changed alveolar-capillary permeability and angiotensin converting enzyme activity in serum in sarcoidosis. *Thorax* 1986 Aug;**41**(8): 629—34.
30. Gomez-Perales JE, Colvile RN, Nieuwenhuijsen MJ, Fernandez-Bremauntz A, Gutierrez-Avedoy VJ, Paramo-Figueroa VH, et al. Commuters' exposure to PM2.5, CO, and benzene in public transport in the metropolitan area of Mexico City. *Atmos Environ* 2004 Mar;**38**(8):1219—29.
31. van Oosterhout AJ, Bloksma N. Regulatory T-lymphocytes in asthma. *Eur Respir J* 2005 Nov;**26**(5):918—32.
32. McCreanor J, Cullinan P, Nieuwenhuijsen MJ, Stewart-Evans J, Malliarou E, Jarup L, et al. Respiratory effects of exposure to diesel traffic in persons with asthma. *N Engl J Med* 2007 Dec 6; **357**(23):2348—58.
33. Boezen M, Schouten J, Rijcken B, Vonk J, Gerritsen J, van der Zee S, et al. Peak expiratory flow variability, bronchial responsiveness, and susceptibility to ambient air pollution in adults. *Am J Respir Crit Care Med* 1998 Dec;**158**(6): 1848—54.
34. Shi HZ, Qin XJ. CD4CD25 regulatory T lymphocytes in allergy and asthma. *Allergy* 2005 Aug;**60**(8):986—95.
35. Provoost S, Maes T, van Durme YM, Gevaert P, Bachert C, Schmidt-Weber CB, et al. Decreased FOXP3 protein expression in patients with asthma. *Allergy* 2009 Oct;**64**(10): 1539—46.