Exposure to Ultrafine Particles from Ambient Air and Oxidative Stress-Induced DNA Damage

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Effects of Ambient Air Particulate Exposure on Blood–Gas Barrier Permeability and Lung Function

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Particulate air pollution is associated with increased risk of pulmonary diseases and detrimental outcomes related to the cardiovascular system, including altered vessel functions. This study's objective was too evaluate the effects of ambient particle exposure on the blood–gas permeability, lung function and Clara cell 16 (CC16) protein release in healthy young subjects. Twenty-nine nonsmokers participated in a randomized, two-factor crossover study with or without biking exercise for 180 min and with 24-h exposure to particle-rich (6169–15,362 particles/cm^3; 7.0–11.6 μg/m^3 $PM_{2.5}$; 7.5–15.8 μg/m^3 $PM_{10-2.5}$) or filtered (91–542 particles/cm^3) air collected above a busy street. The clearance rate of aerosolized ^{99m}Tc-labeled diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA) was measured as an index for the alveolar epithelial membrane integrity and permeability of the lung blood–gas barrier after rush-hour exposure. Lung function was assessed using body plethysmography, flow-volume curves, and measurements of the diffusion capacity of carbon monoxide. CC16 was measured in plasma and urine as another marker of alveolar integrity. Particulate matter exposure had no significant effect on the epithelial membrane integrity using the methods available in this study. Exercise increased the clearance rate of ^{99m}Tc-DTPA indicated by a 6.8% (95% CI: 0.4–12.8%) shorter half-life and this was more pronounced in men than women. Neither particulate matter exposure nor exercise had an effect on the concentration of CC16 in plasma and urine or on the static and dynamic volumes or ventilation distribution of the lungs. The study thus demonstrates increased permeability of the alveolar blood–gas barrier following moderate exercise, whereas exposure to ambient levels of urban air particles has no detectable effects on the alveolar blood–gas barrier or lung function.

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Acute and long-term impact of particulate matter (PM) in air pollution on the pulmonary and cardiovascular system are well documented, including the reversible decrement of pulmonary function (Brunekreef & Holgate, 2002). The lung blood–gas barrier (BGB) promotes efficient passage of gases between the alveolar space and circulation, whereas it restricts permeability to solutes and insoluble materials, including PM. If the barrier is injured, it may cause layers to become leaky, thereby increasing permeability. Consideration of changes in BGB permeability may therefore be important in relation to the possible effects of PM.

BGB permeability can be measured as the pulmonary clearance rate of ^{99m}Tc-labeled diethylenetriamine pentaacetic acid (DTPA) (Jones et al., 1983) that crosses the membrane by passive diffusion through the intercellular tight junctions of the alveolar epithelium and the capillary endothelium (Rinderknecht et al., 1980). Elevated rates of pulmonary clearance of ^{99m}Tc-DTPA are regarded as a marker of altered integrity of the alveolar epithelial membrane associated with increased permeability of the BGB. This is observed in a variety of lung diseases (Yeates et al., 2000), and as a part of the aging process (Groth et al., 1989).

In animal studies, exposure to PM in terminal-senescent mice (Tankersley et al., 2003) and ozone exposure in dogs (Foster & Freed, 1999) is associated with reduced BGB integrity. Relatively high ambient concentrations of ozone (400 ppb) have also previously been shown to increase epithelial permeability in humans (Kehrl et al., 1987). In keeping with tobacco-induced lung injury, it has been shown that smokers have increased BGB permeability (Nolop et al., 1987; Mason et al., 1983; Minty et al., 1981; Jones et al., 1980). A dose-dependent effect can also be inferred from observations that the BGB permeability is higher among smokers than nonsmokers, and passive smokers have BGB permeability in between these extremes (Beadsmoore et al., 2007). Cigarette smoke possibly elicits direct toxicity in alveoli cells with measurable alterations of lung function such as FEV_1. However, exercise is also associated with altered BGB integrity and increased permeability (Hanel et al., 2003).

The measurement of Clara cell 16 protein (CC16) in plasma or urine is considered to be a biomarker of lung epithelial cell damage or dysfunction (Bernard, 2008). CC16 is a 16- to 17-kD lung epithelium specific protein, produced by the Clara cells, and can diffuse passively across the bronchoalveolar-blood barrier into the plasma. It is believed that CC16 plays a role in lung immunosupression and anti-inflammation, after which it is eliminated by the kidneys. Baseline concentrations of CC16 show considerable variations in healthy subjects, which are due to different numbers of Clara cells. Concentrations increase slightly with aging, and reduced Clara-cell integrity in smokers is induced by tobacco smoke toxicity (Robin et al., 2002; Hermans & Bernard, 1999). Short-term increased CC16 baseline levels are regarded to reflect the integrity of the lung epithelial barrier (Hermans & Bernard, 1999). This has been observed in serum samples in firefighters after exposure to smoke (Burgess et al., 2001; Bernard et al., 1997) and cyclists in association with 2-h exercise in ozone rich air (Broeckaert et al., 2000).

We hypothesize that inhalation of ambient levels of PM may cause increased BGB permeability and that exercise may exacerbate this effect. To test this hypothesis we investigated alveolar membrane integrity in young healthy nonsmokers immediately after exposure to ambient particles from rush-hour morning traffic. This was assessed as the ^{99m}Tc-DTPA clearance rate and plasma and urine concentrations of CC16. In addition, lung function was assessed as an indicator of acute response, as brief exposures to higher levels of traffic have previously been associated with decreases in lung function in healthy adults (Kan et al., 2007).

MATERIALS AND METHODS

Study Design and Population

Study design and recruitment methods have previously been described in detail (Bräuner et al., 2007). Briefly, 30 healthy Caucasian nonsmokers participated and 29 completed the whole program. Baseline characteristics are shown in Table 1. The participants, 20 men, 9 women, were nonsmokers, had normal lung function ($FEV_1 > 100\%$ of predicted values), no history or findings of pulmonary or cardiovascular disease, and were free of respiratory infections at the time of the exposures.

The project design was a single blind, two-factor crossover study using four 24-h exposures with randomized exposure to particle-rich or filtered air with or without biking exercise. Exercise consisted of two 90-min periods on an ergometer bicycle after exposure times of 15 min and 7½ h at 60–75% maximal intensity (Table 2). The wash-out interval between individual exposures for each participant was 12 days and we observed no carryover effects of previous exposures. To avoid problems due to diurnal variation, participants entered the exposure chamber at the same time of the morning on each of their 24-h visits at either 7:00 or 7:30 a.m.

In the exposure chambers, the size distribution and number concentration (NC) of fine particles (6–700 nm) were continuously monitored using a custom-built differential-mobility particle sizer (Wåhlin et al., 2001), whereas concentrations of O_3, NO, NO_x, and CO were continuously measured using monitors from API, San Diego, CA. Nonfiltered air (NFA) cumulated 24-h particle samples were collected using dichotomous stacked filter units (Luhana et al., 2001) as fine ($<2.5\ \mu$m diameter) and coarse fractions (10–2.5 μm diameter). Sampling filters were polycarbonate membrane filters from Nucleopore. Particle mass in NFA was determined gravimetrically and elemental composition was determined using proton-induced x-ray emission as previously described (Wåhlin et al., 2006). Filter-based measurements were not performed on particle-filtered air (PFA) because of the low particle levels.

Outdoor levels of ultrafine particles (UFP) and gases were also measured at fixed monitoring stations. The first was located on the roof of the 20-m-high university H. C. Ørsteds campus

TABLE 1
Baseline characteristics, mean ± SD

Parameter	Men, $n = 20$	Percent of predicted value (95% CI)	Women, $n = 9$	Percent of predicted value (95% CI)
Age (yr)	27 ± 6	—	26 ± 3	—
Body mass index (kg/m^2)	23.4 ± 2.7	—	22.3 ± 2.5	—
Vital capacity (L)	5.9 ± 0.8	104 (97, 110)	4.4 ± 0.5	112 (102, 121)
Intrathoracic gas volume (L)	3.9 ± 0.8	111 (100, 123)	3.0 ± 0.4	107 (97, 117)
Residual volume (L)[a]	1.8 ± 0.4	102 (90, 114)	1.5 ± 0.4	104 (82, 126)
Total lung capacity (L)[b]	7.8 ± 1.0	105 (99, 111)	5.9 ± 0.6	112 (102, 122)
RV/TLC (%)	22.5 ± 4.4	—	25.2 ± 4.7	—
Forced expiratory volume 1s (L)[c]	4.9 ± 0.7	108 (101, 115)	3.7 ± 0.3	109 (101, 116)
Forced vital capacity (L)[d]	5.9 ± 0.8	108 (102, 115)	4.5 ± 0.5	114 (106, 121)
FEV_1/FVC (%)	80.3 ± 4.6	—	82.7 ± 6.9	—
FEV_1/VC_{max} (%)[e]	79.6 ± 4.6	—	82.0 ± 7.1	—
Peak expiratory flow (L/s)	10.3 ± 2.0	100 (91,108)	7.1 ± 1.0	97 (87, 107)
Maximal mid-expiratory flow rate (L/s)	4.4 ± 1.0	93 (84, 101)	3.8 ± 1.0	96 (76, 116)
Diffusion capacity of CO (mmol/min/kPa)	12.5 ± 1.4	101 (96, 102)	8.9 ± 0.9	91 (85, 98)

[a]RV.
[b]TLC.
[c]FEV_1.
[d]FVC.
[e]VC_{max}, maximum vital capacity.

building (background) in a park area in the center of Copenhagen, approximately 300 m from Tagensvej. The second was located on the kerbside of H. C. Andersen's Boulevard (busy street) with 60,000 vehicles per workday (Kemp et al., 1998).

Blood for CC16 determinations was sampled after 6 and 24 h of exposure and 24-h urine was collected. Each participant was his/her own control, which limited confounding.

The study was approved by the local ethics committee of Copenhagen (KF 01 255392) and in accordance with the Declaration of Helsinki. All participants gave written informed consent before inclusion.

Blood–Gas Barrier Permeability

Pulmonary ^{99m}Tc-DTPA Clearance

Measurements were performed directly after exposure to $2^1/_2$ h of either filtered or nonfiltered air from peak hour morning traffic during either rest or exercise at the Department of Clinical Physiology, Nuclear Medicine and PET, Rigshospitalet. In the supine position each subject inhaled ^{99m}Tc-DTPA aerosol (CIS Bio International Oris, Ind, GE-Amersham) with 3 min of tidal breathing and a 10-MBq dose was deposited. The total radiation dose to the bladder from 4 inhalations was 1.9 mGy (whole-body dose equivalent: 0.3 mSv), which is more than 10

TABLE 2
The project design, a single-blind two-factor crossover study with four randomized 24-h exposures to particle-rich air and/or exercise scenarios

Exposure scenario[a]	90-min cycling[b] or continued rest after 15-min exposure	90-min cycling[b] or continued rest after $7^1/_2$-h exposure	Remaining 19 h
Nonfiltered air	Bicycling	Bicycling	Rest
Nonfiltered air	Rest	Rest	Rest
Particle-filtered air	Bicycling	Bicycling	Rest
Particle-filtered air	Rest	Rest	Rest

[a]The four 24-h exposure scenarios were randomized.
[b]Participants bicycled at 60–75% of maximal intensity on an ergometer bicycle.

times lower than the yearly background exposure to the general population in Denmark. The aerosol was generated from a 400-MBq ^{99m}Tc-DTPA solution in 5 ml of 0.9% sodium chloride using a jet nebulizer (Swirler Nebulizer, AMICI) at a flow rate of 9 L/min. Radioactivity from the chest was detected in subjects positioned in the supine position above a gamma camera with a circular, low-energy, general-purpose collimator (General Electric, USA). Data were acquired as a 30-min dynamic acquisition (128 × 128 pixels) in 30-s frames. The time–activity curves obtained were fitted by a mono-exponential function, with the negative slope of the line being the rate constant of clearance as previously described (Hanel et al., 2003), and clearance half-life (ln 2)/k was calculated.

There are several technical issues to be considered and the distribution of inhaled ^{99m}Tc-DTPA in the lungs is an important factor for measurement of the pulmonary clearance. If primarily centrally deposited, ^{99m}Tc-DTPA could partly have been cleared by mucociliary action, but there were no signs of mucociliary transport of the inhaled ^{99m}Tc-DTPA as observed in our scintigrams herein (not shown) and in our previous study (Hanel et al., 2003). To avoid central distribution, the flow rate should be <0.6 L/min, the particles size <2 μm, and inspiratory volume slightly deeper than normal (Agnew et al., 1984), and all were met in our study. The radiochemical purity of ^{99m}Tc-DTPA is potentially the most important quality problem, because pulmonary clearance is five times faster for pertechnetate ($^{99m}TcO_4^-$) than for ^{99m}Tc-DTPA. Our in vitro binding of ^{99m}Tc-DTPA was on average 98% and always >95.5%—thus maximally 4.5% was free $^{99m}TcO_4^-$—and using the same equipment in an earlier study where scans were performed for 2 h after inhalation of ^{99m}Tc-DTPA, we observed no accumulation in the thyroid gland or stomach (Hanel et al., 2003), which would have been the case if a large amount of free $^{99m}TcO_4^-$ was present.

Pulmonary Function Tests

Baseline lung function was characterized by performing flow–volume curves (forced expiration and inspiration), body plethysmography (lung volumes), and measurements of the diffusion capacity of carbon monoxide ($D_{L,CO}$) using standardized methods with the subject in a seated position. These measurements were performed directly after clearance measurements.

Clara -Cell 16 Protein

The concentration of CC16 in plasma and urine was determined by an immunoassay relying on the agglutination of latex particles as previously described in detail (Bernard et al., 1991).

Statistics

All statistical analyses were performed using SAS software (version 9.1, SAS Institute, Inc., Cary, NC). Repeated-measure analysis of variance was used to investigate the effect of exposure to nonfiltered air and exercise on the outcome variables: BGB permeability, lung function (vital capacity [VC], total lung capacity [TLC], forced expiratory volume in 1 s [FEV_1],and peak expiratory flow [PEF]), and CC16 levels in plasma and urine. All statistical analyses were performed on the natural logarithm of these data. Participant was included as a random factor variable, and to assess differences in effect according to gender we stratified data according to gender. Particle exposure and exercise were included as fixed categorical explanatory variables, and possible carryover effects were tested by dummy variables for exposure during the preceding event. Relationships between BGB permeability and other lung function variables were assessed using analysis of covariance (Altman, 1999). The significance threshold was $p < .05$ in all analyses.

RESULTS

Exposure Characterization

Exposure chamber PM mass concentrations without filtration ranged from 7.5 to 15.8 μg/m^3 and from 7.0 to 11.6 μg/m^3 for $PM_{10-2.5}$ and $PM_{2.5}$, respectively. The filter effectively removed particles from chamber air, and the 24-h total number concentration ranged from 91 to 542 per cm^3 and from 6169 to 15,362 per cm^3 for particle-filtered and nonfiltered air, respectively (Table 3). NO_x and NO were unaffected by filtration, whereas O_3 was significantly reduced and CO significantly increased. During the nonfiltered air chamber scenario the levels of PM and gases resembled the composition of a mix of urban background air with penetration and mixing with busy street air (Table 3). Particles with a median diameter of 57 nm were the most abundant and also represented the major part of the surface area in both chamber and outdoor (background and urban) air, as previously reported (Bräuner et al., 2007).

Means and SD of all functional tests and biomarkers according to gender, activity, and exposure scenario are presented in Table 4.

Blood–Gas Barrier Permeability

Exercise altered the integrity of the epithelial membrane by significantly decreasing the ^{99m}Tc-DTPA half-life by 6.8% (95% CI: 0.4–12.8%), and this effect was greatest in men (Table 5). The exposure to PM was not associated with alterations in the integrity of the epithelial membrane (Table 5). The ^{99m}Tc-DTPA clearance rates obtained after 0–30 min, 0–15 min, and 15–30 min were relatively constant, indicating a mono-exponential model function (Table 5).

Pulmonary Function

All baseline lung function parameters were within normal limits (Table 1) and no exclusion was necessary due to this criterion. TLC was significantly improved by exposure to PM, and this effect was predominant in men, but none of the lung function parameters studied were associated with exercise and no significant interaction relationships between exercise and exposure were observed (Table 5).

TABLE 3
Total number concentration (NC), of particles (aerodynamic diameter 6–700 nm), particle mass as well as gases in the exposure chamber and at outdoor monitoring stations

	Exposure chamber		Outdoor monitoring stations	
Parameter	NFA[a]	PFA[b]	Urban background	Busy urban street
NC_{total}(/cm^3)	10067 (6169–15,362)	235 (91–542)	6571 (4530–9645)	22809 (13,499–31,977)
$PM_{10-2.5}$	12.6 (7.5–15.8)	—	—	—
$PM_{2.5}$	9.7 (7.0–11.6)	—	—	—
NO_x (ppb)	25.83 (13.01–49.56)	28.03 (14.43–52.56)	11.56 (7.43–18.36)	59.52 (37.94–88.17)
NO (ppb)	3.24 (0.72–14.49)	3.21 (0.72–17.42)	1.22 (0.41–3.05)	—
CO (ppm)	0.35 (0.25–0.49)	0.41 (0.28–0.57)	0.21 (0.17–0.29)	0.55 (0.39–0.76)
O_3 (ppb)	12.08 (5.68–18.85)	4.29 (1.99–10.49)	30.05 (23.24–35.27)	19.52 (11.88–26.67)

Note. Values are median (interquartile range) of 24-h average exposure scenarios and outdoor monitoring data.
[a]Nonfiltered air.
[b]Particle-filtered air.

CC16 Protein

No significant association with exposure, exercise, and length of exposure was observed (Table 5).

BGB permeability correlated significantly with the FVC, PEF, and CC16 levels in plasma and urine (Table 6).

DISCUSSION

In this study we show that the pulmonary clearance of ^{99m}Tc-DTPA was significantly increased by 90 min of moderately intensive exercise, whereas there was no effect on the BGB, lung function, or CC16 levels after exposure to ambient air levels of air pollution particles, which were sufficient to induce oxidative damage to DNA in peripheral-blood mononuclear cells of this group of young, healthy volunteers (Bräuner et al., 2007). Accordingly, alveolar epithelial membrane integrity and lung function are not affected to an extent detectable by state-of-the-art methods at PM exposure levels sufficient to induce systemic oxidative stress. It has previously been reported that the permeability of the BGB is increased in nonsmoking humans after 3 days of cigarette smoking (Minty et al., 1984), whereas there was no additional effect of smoking one cigarette among smokers, who already had a high permeability of the BGB (Gil et al., 1995). Furthermore, smokers who stop smoking improve their abnormally high BGB permeability to a significant extent after only 24 h and the maximal effect appears to be reached 1 wk after cessation (Minty et al., 1981). The subjects in the present study were nonsmokers and had a normal BGB permeability in line with other published reference values (Nilsson et al., 1997). In this respect it should be emphasized that the reported effect of daily inhalation of cigarette smoke or environmental tobacco smoke (Beadsmoore et al., 2007; Mason et al., 1983; Jones et al., 1980) is a result of rather intense continuous exposures as compared to the relatively short term exposure to the levels of PM in ambient air in Copenhagen. Indeed, 2 h of ozone exposure at 400 ppb was sufficient to produce significant decrements in pulmonary function and also caused increased BGB permeability measured by ^{99m}Tc-DTPA clearance (Kehrl et al., 1987).

In line with the lack of measurable effect of the present PM exposure on pulmonary clearance of ^{99m}Tc-DTPA we found unaltered plasma concentration and urinary excretion of CC16 that indicate little damage to the Clara cells and membrane integrity. In contrast, 3 h of chamber exposure to high concentrations of wood smoke (fine PM: 240–280 μg/m^3) was associated with an approximately 20% increased serum CC16 concentration in samples obtained 20 h after the cessation of the exposure (Barregard et al., 2007). That concentration of wood smoke PM was markedly higher than the 9.7 μg/m^3 of fine PM in this study, suggesting a possible threshold for effect. Increased concentration of CC16 in plasma and urine has also been used as a marker of lung epithelial injury following ambient exposure to ozone and chemical airway irritants in children (Bernard et al., 2005). Although the filtering of air in the present study affected the ozone concentration from 12.1 to 4.3 ppb in the nonfiltered and filtered air, respectively, it has recently been shown that 70–80 ppb ozone is required to elicit an effect after a 2-h bicycle ride in an urban air polluted environment (Bergamaschi et al., 2001; Broeckaert et al., 1999). These data further support threshold for effects of air toxics on CC16 changes.

Recently, 2-h exposure to ambient levels of PM in a busy street in London was associated with small but significant decrements in lung function measures, including FVC and FEV_1 among 60 subjects with mild to moderate asthma (McCreanor et al., 2007). This study indicates the need for large number of susceptible subjects to show significant effects, which were mainly associated with elemental carbon and ultrafine particles at levels substantially higher than ours. Similarly, we found the main effect on systemic oxidative stress with damage to DNA in our subjects to be related to ultrafine particles of 57 and

TABLE 4
Mean ± SD of all dependent variables according to sex, exposure, and activity

	Men, $n = 20$				Women, $n = 9$			
	Rest		Cycling		Rest		Cycling	
Parameter	Nonfiltered	Filtered	Nonfiltered	Filtered	Nonfiltered	Filtered	Nonfiltered	Filtered
Blood–gas barrier permeability $T_{1/2}$(min)[a]	89.9 ± 32.4	90.4 ± 24.7	83.8 ± 24.4	82.5 ± 25.0	102.6 ± 35.8	116.3 ± 69.0	112.3 ± 42.7	88.8 ± 31.6
Vital capacity (L)	5.8 ± 0.8	5.8 ± 0.8	5.9 ± 0.9	5.9 ± 0.8	4.5 ± 0.5	4.5 ± 0.5	4.5 ± 0.6	4.4 ± 0.5
Residual volume (L)[b]	1.7 ± 0.4	1.6 ± 0.5	1.9 ± 0.6	1.8 ± 0.5	1.4 ± 0.3	1.4 ± 0.3	1.3 ± 0.2	1.4 ± 0.3
Total lung capacity (L)[c]	7.7 ± 1.0	7.5 ± 1.1	7.9 ± 1.1	7.7 ± 1.0	6.0 ± 0.6	5.9 ± 0.7	5.8 ± 0.7	5.8 ± 0.7
Forced expiratory volume 1s (L)[d]	4.7 ± 0.6	4.7 ± 0.6	4.9 ± 0.8	4.7 ± 0.6	3.7 ± 0.5	3.7 ± 0.4	3.7 ± 0.4	3.7 ± 0.4
Forced vital capacity (L)[e]	5.9 ± 0.8	5.8 ± 0.8	6.0 ± 0.9	5.9 ± 0.8	4.6 ± 0.6	4.5 ± 0.5	4.5 ± 0.6	4.4 ± 0.5
FEV_1/FVC	79.8 ± 4.9	80.1 ± 4.8	81.2 ± 4.6	80.3 ± 4.4	82.0 ± 7.3	82.8 ± 6.9	82.3 ± 7.5	83.6 ± 7.3
Peak expiratory flow (L/s)	10.1 ± 1.8	10.2 ± 1.9	10.6 ± 2.4	10.2 ± 1.8	7.8 ± 1.1	7.8 ± 0.9	7.6 ± 0.7	7.9 ± 0.9
Urine Clara cell protein 16 (μg/g)[f]	8.3 ± 7.9	9.9 ± 13.3	10.4 ± 13.8	11.3 ± 11.9	6.1 ± 7.9	5.7 ± 5.3	4.2 ± 3.0	4.0 ± 2.7
Plasma Clara cell protein 16 (μg/L)								
Average all day	11.7 ± 4.6	11.9 ± 3.8	11.6 ± 3.6	11.7 ± 4.2	10.3 ± 4.5	9.9 ± 3.3	10.1 ± 3.1	9.8 ± 3.3
After 6-h exposure	11.2 ± 3.7	11.2 ± 4.4	11.4 ± 5.6	11.2 ± 3.3	10.2 ± 4.8	10.0 ± 3.6	9.7 ± 3.3	9.2 ± 4.0
After 24-h exposure	11.9 ± 3.4	12.1 ± 4.0	12.0 ± 3.4	12.6 ± 4.1	10.4 ± 4.3	9.7 ± 3.1	10.4 ± 3.1	10.3 ± 2.5

[a]Total lung clearance half-life determined using radiolabeled DTPA a detailed description is provided in the Methods section.
[b]RV.
[c]TLC.
[d]FEV_1.
[e]FVC.
[f]Creatinine-adjusted concentration.

TABLE 5
The predictive value of exposure to nonfiltered air and activity expressed as percent change

Outcome variable	Estimates (95% CI) n					
	Percent change in variable according to exposure with mutual adjustment for activity			Percent change in variable according to activity with mutual adjustment for exposure		
	All data, $n = 29$	Men, $n = 20$	Women, $n = 9$	All data, $n = 29$	Men, $n = 20$	Women, $n = 9$
BGB permeability $T_{1/2}$(min)[a]						
0–30 min	2.12 (–4.50, 9.53)	−0.80 (−7.78, 6.72)	8.87 (−6.20, 26.2)	**−6.76 (−12.8, −0.40)**	**−7.13 (−13.6, −0.10)**	−6.11 (−19.0, 8.98)
0–15 min	4.29 (−5.07, 14.7)	−0.90 (−8.33, 11.2)	12.3 (−10.2, 40.2)	−8.24 (−16.6, 0.80)	**−12.8 (−20.8, −3.92)**	2.63 (−17.8, 28.2)
15–30 min	3.15 (−4.78, 11.7)	−0.50 (−9.52, 11.3)	9.31 (−3.92, 24.5)	**−5.45 (−12.72, −2.43)**	**−6.85 (−9.24, −11.3)**	−2.18 (−14.02, 11.4)
Vital capacity (L)	0.60 (−0.80, 2.02)	0.40 (−1.29, 2.22)	0.95 (−1.39, 3.36)	0.50 (−0.90, 1.92)	1.31 (−0.40, 3.05)	−1.19 (−3.54, 1.11)
Total lung capacity (L)	2.22 (0.50, 4.08)	2.94 (0.90, 4.92)	0.96 (−2.37, 4.39)	1.31 (−0.40, 3.15)	3.05 (1.11, 5.02)	−2.47 (−5.64, 0.90)
Forced expiratory volume 1s (L)	1.41 (−0.40, 3.25)	1.92 (−0.40, 4.19)	0.30 (−2.47, 3.15)	1.21 (−0.50, 2.94)	2.02 (−0.30, 4.29)	−0.40 (−3.25, 2.43)
Forced vital capacity (L)	1.51 (j0.10, 3.15)	1.41 (−0.60, 3.67)	1.71 (−0.80, 4.29)	0.40 (−1.29, 2.02)	0.90 (−1.19, 3.05)	−0.80 (−3.15, 1.71)
Peak expiratory flow (L/s)	0.10 (−2.76, 2.94)	−0.70 (−3.05, 4.60)	−1.49 (−5.45, 2.63)	1.21 (−1.59, 4.19)	1.92 (−1.88, 5.76)	−0.10 (−4.11, 4.08)
Urine Clara cell protein 16 (μg/g)[b]	−6.67 (−24.3, 14.9)	−8.70 (−29.9, 0.06)	−2.27 (−30.3, 37.2)	−0.60 (−19.3, 22.5)	10.1 (−15.6, 43.5)	−20.6 (−43.5, 11.4)
Plasma Clara cell protein 16 (μg/L)	−0.90 (−6.29, 5.02)	−2.07 (−8.36, 4.66)	2.12 (−8.97, 14.5)	0.60 (−5.07, 6.50)	1.11 (−5.35, 8.00)	−0.60 (−11.5, 11.4)

Repeated-measures analysis of variance subject used as a random factor. The natural logarithm of the biomarker in question was included as a continuous outcome variable. Nonfiltered air and exercise were included in all the models as categorical (yes/no) fixed effects predictor variables. The predictive values (percent change) of activity and exposure are mutually adjusted for each other and all models are adjusted for age. Non-gender-stratified models are adjusted for gender. Blood samples were taken twice on each scenario; therefore, plasma Clara cell protein values are adjusted for length of exposure; in models stratified by length of exposure (not shown here) we found no significant effect of either exposure or activity. Numbers in bold depict significant estimates.

[a]Blood–gas barrier permeability was measured as total lung clearance half-life determined using radiolabeled DTPA; a detailed description is provided in the Methods section.

[b]Creatinine adjusted urinary concentration.

TABLE 6
The relationship between blood–gas barrier permeability (assessed as half-life of ^{99m}Tc-DTPA clearance) and each of the individual lung function parameters as well as urinary and plasma CC16 protein concentrations expressed as model estimates (95% confidence interval) from analysis of covariance

Parameter	^{99m}Tc-DTPA clearance
Vital capacity (L)	0.229 (–0.11, 0.56)[c,b]
Total lung capacity (L)	0.251 (–0.08, 0.58)[c,b]
Forced expiratory volume 1s (L)	0.303 (–0.04, 0.64)[c]
Forced vital capacity (L)	**0.339 (0.01, 0.67)**[c]
Peak expiratory flow (L/s)	**0.425 (0.16, 0.69)**[c]
Plasma Clara cell protein 16 (μg/L)	**0.240 (0.12, 0.36)**[c]
Urine Clara cell protein 16 (μg/g)[a]	**0.080 (0.03, 0.13)**[c]

Note. The analyses were made on log-transformed values and estimates are adjusted for activity, exposure, gender and age. Numbers in bold depict significant relationships.

[a]Creatinine adjusted urinary concentration.

[b]Gender was significant in this model.

[c]Age was significant in this model.

23 nm diameters associated with diesel vehicle exhaust, but at much lower levels, suggesting that this biomarker may more sensitive than lung-related measures (Bräuner et al., 2007).

Collectively, our data indicate that the BGB permeability and integrity are not altered to a detectable extent by the PM concentrations that humans are usually exposed to in the urban air of Europe and the United States. Although it might be necessary to use rather high PM exposures to exceed the threshold of effect, it should also be kept in mind that the particle size may be another important determinant because of minimum alveolar deposition of larger particles (Jaques & Kim, 2000). Indeed, there was no effect on pulmonary clearance of ^{99m}Tc-DTPA in healthy human subjects after 30 min of exposure to iron oxide particles with median aerodynamic diameter of 1.5 μm and an average mass concentration as high as 12.7 mg/m^3(Lay et al., 2001).

In accordance with findings from earlier studies with measurements during and immediately after exercise (Lorino et al., 1989), we detected a small increase of the pulmonary epithelial permeability indicated by a decrease in ^{99m}Tc-DTPA clearance half-life roughly 25 min after moderate exercise performed for 90 min. The effect of exercise appears to be transient and permeability normalizes 40–120 min after cessation of the exercise (Hanel et al., 2003; Edwards et al., 2000). The lack of effect of exercise on the CC16 concentrations in plasma and urine supports the notion that the pulmonary epithelial permeability was only briefly and reversibly altered. The precise mechanism behind the transient increase of the BGB permeability after exercise cannot be explained on the basis of the present study alone and we merely incorporated an exercise protocol in the study to increase the exposure to PM. Our data indicate that the exercise-induced increase in pulmonary epithelial permeability is not related to an increased burden of inhaled air pollutants, because there was no effect of air filtration, whereas the effect could be due to increased ventilation and elevated vascular pressure (Hanel et al., 2003). Both increased ventilation per se and increased lung volume without exercise are associated with increased pulmonary epithelial permeability, probably due to "stretching" the epithelial tight junctions (Suzuki et al., 1995; Evander et al., 1990). It can be speculated that the increased epithelial permeability, observed as a transient increase in ^{99m}Tc-DTPA clearance, is a physiological advantage to the organism because it is associated with efficient exchange of gases across the BGB. Indeed, it was not accompanied by increased CC16 levels, which would have indicated an adverse effect.

As shown in Table 6, there were positive associations between high ^{99m}Tc-DTPA clearance half-life indicating low BGB permeability and pulmonary functional endpoints with adjustment for exposures, age, and gender. More interesting, there were also significant positive associations between BGB permeability and CC16 concentrations in plasma and urine. It should be kept in mind that CC16 has a dual interpretation as biomarker, which may be one reason for recent critique (Lakind et al., 2007). Short-term increases within an individual can be considered as a marker of adverse effect with epithelial cell damage and leakage, whereas long-term decreased plasma concentrations can indicate malfunctioning or decreased number of Clara cells as seen in chronic lung diseases (Bernard, 2008). Thus, a well-functioning BGB indicated by a long ^{99m}Tc-DTPA clearance half-life would be expected to be positively associated with Clara cell number and function indicated by a high steady-state level of plasma and urine. This supports the validity of our biomarkers.

There are three limitations that need to be acknowledged regarding the present study. The first concerns the exposure concentrations. In order to demonstrate a clear significant change in any parameters in human subjects one would normally seek to show such an effect at a raised PM concentration and then track back to lower concentrations. In this study, however, we considered realistic concentrations that could reflect everyday exposures. Second, we could only measure the ^{99m}Tc-DTPA clearance after 2.5 h in order to catch the effect of rush-hour traffic, while prolonged exposure may have elicited detectable effects. Finally, the statistical power of the study could not detect very subtle biomarker changes, which may be relevant in a large population under exposure.

In conclusion, our results show that healthy, young nonsmokers display no detectable changes monitored by state-of-the-art methods in the integrity of BGB or lung function after exposure to PM at ambient air levels, despite these levels being sufficient to cause systemic oxidative stress among the same participants. It is possible that higher concentrations of PM might elicit detectable changes in terms of increased permeability of the BGB or that only susceptible individuals with preexisting conditions are affected.

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