ORIGINAL ARTICLE

Associations between three specific a-cellular measures of the oxidative potential of particulate matter and markers of acute airway and nasal inflammation in healthy volunteers

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ABSTRACT

Introduction We evaluated associations between three a-cellular measures of the oxidative potential (OP) of particulate matter (PM) and acute health effects.

Methods We exposed 31 volunteers for 5 h to ambient air pollution at five locations: an underground train station, two traffic sites, a farm and an urban background site. Each volunteer visited at least three sites. We conducted health measurements before exposure, 2 h after exposure and the next morning. We measured air pollution on site and characterised the OP of $PM_{2.5}$ and PM_{10} using three a-cellular assays; dithiotreitol (OP^{DTT}), electron spin resonance (OP^{ESR}) and ascorbic acid depletion (OP^{AA}).

Results In single-pollutant models, all measures of OP were significantly associated with increases in fractional exhaled nitric oxide and increases in interleukin-6 in nasal lavage 2 h after exposure. These OP associations remained significant after adjustment for co-pollutants when only the four outdoor sites were included, but lost significance when measurements at the underground site were included. Other health end points including lung function and vascular inflammatory and coagulation parameters in blood were not consistently associated with OP.

Conclusions We found significant associations between three a-cellular measures of OP of PM and markers of airway and nasal inflammation. However, consistency of these effects in two-pollutant models depended on how measurements at the underground site were considered. Lung function and vascular inflammatory and coagulation parameters in blood were not consistently associated with OP. Our study, therefore, provides limited support for a role of OP in predicting acute health effects of PM in healthy young adults.

What this paper adds

- The oxidative potential (OP) of particulate matter (PM) has been proposed as a more health relevant metric than PM mass.
- However, there is still limited evidence in epidemiological studies that the OP of PM is more closely associated with health effects than PM mass or individual PM characteristics.
- We found significant associations between three a-cellular measures of OP of PM and markers of airway and nasal inflammation in healthy young adults.
- These OP associations remained significant after adjustment for co-pollutants when only the four outdoor sites were included, but lost significance when measurements at the underground site were included.
- Other health end points, including lung function and vascular inflammatory and coagulation parameters in blood were not consistently associated with OP.

INTRODUCTION

Numerous studies have shown health effects related to exposure to ambient particulate matter (PM).[1 2] However, it is not well known which PM characteristics are responsible for the observed effects,[3–5] although various PM characteristics, such as particle number concentrations (PNC), transition metals, organic components and biological components have been proposed.

Oxidative stress has been suggested as an important underlying mechanism by which exposure to PM may lead to adverse health effects.[6 7] Oxidative stress results when the generation of reactive oxygen species (ROS), or free radicals, exceeds the available antioxidant defences. High levels of oxidative stress induce inflammatory responses via a cascade of events including activation of various transcription factors and stimulation of cytokine production.[6] The oxidative potential (OP), defined as a measure of the capacity of PM to oxidise target molecules, has been proposed as a metric that is more closely related to biological responses to PM exposures and thus could be more informative than PM mass alone.[8] Several methods for measuring OP have been developed, both a-cellular and cellular. No consensus has been reached yet as to which measures of OP are most appropriate to predict PM-related health effects.[9] Also, issues such as high variability in time and space and high costs of the different assays currently hamper wide-scale use.[10]

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Although OP is considered an attractive measure, there is still limited evidence from epidemiological studies that it predicts health effects better than PM mass or individual PM characteristics. Two panel studies in California, USA, found an association between measures of OP and biomarkers of airway or systemic inflammation.[11 12] In a series of papers investigating acute effects of being exposed for 5 h to air pollution at different locations on a range of respiratory,[13] nasal pro-inflammatory[14] and vascular inflammatory and coagulation parameters[15] in healthy volunteers, no consistent associations with OP for any of the evaluated health end points were reported. In these studies, OP of PM_{10} was calculated as the sum of OP from $PM_{0.18}$, $PM_{0.18–2.5}$ and $PM_{2.5–10}$ collected with a Micro-Orifice Impactor (MOI) and measured as the capacity of PM to deplete the antioxidants ascorbate and glutathione in a synthetic human respiratory tract lining fluid (RTLF). We recently conducted additional measurements of OP in that study, using both $PM_{2.5}$ and PM_{10} filters from Harvard Impactors (HIs) and three measures of OP: consumption of dithiotreitol (DTT), formation of hydroxyl radicals by electron spin resonance (ESR) and depletion of ascorbic acid (AA).[16] These methods will be referred to as OP^{DTT}, OP^{ESR} and OP^{AA}, respectively. Contrasts in OP among sites, differences in size fractions and correlations with PM composition depended on the specific OP assay, suggesting that the different assays can provide different information regarding the oxidative properties of PM.[16]

Here, we investigated associations between OP of $PM_{2.5}$ and PM_{10} and acute changes in respiratory, nasal pro-inflammatory, vascular inflammatory and coagulation parameters, using three different measurement methods for OP: OP^{DTT}, OP^{ESR} and OP^{AA}. We studied these associations in healthy volunteers, exposed for 5 h to ambient air pollution at selected real-world locations with substantial differences in OP and other PM characteristics.[16 17] We hypothesised that these OP measures will have attributable value to predict PM-related health effects.

METHODS

Study design

The study was conducted within the framework of the 'Risk of Airborne Particles: a Toxicological-Epidemiological hybrid Study' (RAPTES). The RAPTES study design has been described previously.[13–15] In brief, we exposed 31 healthy volunteers to ambient air pollution at five different sites in the Netherlands: an underground train station, an animal farm, a continuous traffic site, a stop and go traffic site and an urban background site. The rationale for selecting different sites was to create high contrast and low correlations among different air pollutants.[17] Site visits were performed on 30 week days from March to November 2009. Each sampling day, we visited one site and each site was visited at least five times. Volunteers were healthy, non-smoking students living at the campus of Utrecht University. Participants participated in 3–7 visits scheduled at least 14 days apart for each individual. Exposure started around 09:00 and lasted for 5 h. Participants performed moderate exercise (minute ventilation 20 L/min/m^2) on a bicycle ergometer for 20 min every hour. We chose a 5 h exposure period with intermittent exercise in order to increase the contrast with exposure outside of the study. We conducted measurements of lung function and FE_{NO}, as well as collected blood and nasal lavage (NAL) samples before exposure, 2 h after exposure and the next morning.

During each 5 h exposure, we performed a detailed characterisation of air pollution on-site. In addition to the characterisation previously,[13–15] we measured OP of $PM_{2.5}$ and PM_{10} using three a-cellular assays; OP^{DTT}, OP^{ESR} and OP^{AA}.[16]

Exposure assessment

PM mass, PM composition and gaseous air pollution

Details about the air pollution measurements are described elsewhere.[13 16] In brief, we collected $PM_{2.5}$ and PM_{10} samples using HIs and measured endotoxin content of the PM_{10} samples. We analysed $PM_{2.5–10}$ and $PM_{2.5}$ samples collected with a high volume sampler for EC, OC, metals (eg, Fe, Cu), PAHs, nitrate and sulfate. We measured PNC and gaseous pollutants (O_3, NO_2) using real-time monitors (PNC: CPC model 3022A; O_3: UV Photometric O_3 Analyzer model 49, Thermo Environmental Instruments; NO_x: Chemiluminescence NO/NO_2/NO_x analyser model 200E, Teledyne API).

Oxidative potential

Measurement methods for the characterisation of OP are described in detail elsewhere.[16 18] In brief, we extracted PM_{10} and $PM_{2.5}$ Teflon filters with methanol and resuspended with traceselect ultrapure water to a fixed concentration of 500 μg/mL.

For OP^{DTT}, PM suspensions are incubated with DTT and the reaction is stopped at designated time points (0, 10, 20 and 30 min). The absorbance at 412 nm is recorded on a spectrophotometer and the rate of DTT consumption is calculated using linear regression of absorbance against time. For OP^{ESR}, PM suspensions are diluted to 125 μg/mL and mixed with H_2O_2 and 5,5-dimethylpyrroline-*N*-oxide. After incubation, the suspension is vortexed and transferred into a 50 μL glass capillary without any filtration. The DMPO-OH quartette signal is measured with a MiniScope MS-400 spectrometer. OP^{ESR} is calculated as the average of the total amplitudes of the DMPO-OH quartette in arbitrary units per μg PM. For OP^{AA}, PM suspensions are diluted to12.5 μg/mL and incubated in a spectrophotometer. After adding AA, the absorption at 265 nm is measured every 2 min for 2 h. The maximum depletion rate of AA is determined by performing a linear regression of the linear section of absorbance against time.

For all assays, the results were initially expressed as OP/μg. Field blank corrected OP values in OP/μg were multiplied with the PM mass concentration (μg/m^3) to calculate OP/m^3. 88% (OP^{DTT}) to 97% (OP^{AA}) of the samples were above the detection limit. Coefficients of variation of field duplicates ranged from 8% for OP^{AA} to 18% for OP^{DTT}.[16] Extreme outlying OP^{PM10} values from one measurement day at the farm were excluded.[16]

Health assessment

Details about the health measurements are given elsewhere.[13–15] In brief, we measured FE_{NO}, lung function[13]; interleukin (IL)-6, total protein and lactoferrin in NAL[14]; IL-6 and high-sensitivity C reactive protein (CRP) in serum[14 15]; Fibrinogen, von Willebrand Factor (vWF) antigen and the complex between tissue plasminogen activator and plasminogen activator inhibitor-1 (tPA/PAI-1) in citrate plasma[15] and platelets as part of complete blood cell counts.[15] Health parameters were expected to increase in relation to air pollution, with the exception of lung function (expected decrease), although decreases in blood IL-6 have also been reported.[14]

Data analysis

We analysed the associations between OP of PM during exposure and health end points following the same data analysis strategy as used in previous papers on respiratory and vascular

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health outcomes within the RAPTES project.[13–15] In brief, the difference in health parameters between postexposure and preexposure was used as the dependent variable in mixed linear regression to account for the influence of repeated observations per subject (using compound symmetry of the residuals). The 5 h average concentrations of air pollutants measured on-site were used as independent variables.

First, we analysed all health parameters in single-pollutant models:

- Respiratory parameters: FE_{NO}, FVC and FEV_1[13];
- Markers in NAL: IL-6, protein and lactoferrin[14];
- Blood markers: CRP, fibrinogen platelets, vWF, TPA/PAI1 complex,[15] IL-6.[14]

For the respiratory and NAL markers, we analysed effects 2 h after exposure, whereas for the blood markers we analysed effects the next morning, as these time points showed the strongest associations in our previous analyses.

We made the following modifications and additions to the previously described analysis strategy:

1. Log-transformation of exposure variables. The distributions of the different measures of OP as well as several other PM characteristics (eg, Fe, Cu) were highly skewed. We evaluated whether log-transformation of exposure improved the fit of the models by comparing the Akaike information criterion (AIC) (see online supplementary table S1 for FE_{NO} and table S2 for NAL IL-6). Log-transformation of exposure resulted in a lower AIC for all measures of OP and most other exposure variables in the all sites as well as in the outdoor only models.
2. Additional adjustment for endotoxin for NAL parameters and blood IL-6 (ie, the parameters previously reported by Steenhof *et al*[14]). Highly elevated levels of endotoxin were observed at the farm site, which were significantly positively associated with NAL IL-6 and significantly negatively associated with serum IL-6. Rather than excluding the observations from the farm,[14] we adjusted for endotoxin in all models investigating the associations with NAL and serum IL-6. Results after excluding the farm were similar (see online supplementary table S3).
3. Additional adjustment for exposure at the underground. As the underground site, compared to each outdoor site, had substantially higher concentrations of nearly all exposure parameters, we analysed the data separately after excluding the underground location (outdoor data set), as was done in our previous papers. In the current paper we added a third model, where we included 'measurement at the underground' as a dummy variable in the model. Inclusion of this variable resulted in a lower AIC for all measures of OP and most other exposure variables (see online supplementary table S1 for FE_{NO} and table S2 for NAL IL-6).

We included the same confounding factors as in our previous analyses of the respective health parameters (ie, temperature, relative humidity and season for all parameters; pollen and respiratory infections for FE_{NO} and lung function; use of oral contraceptives for all blood parameters except IL-6[13–15]), with the addition of endotoxin in the models for NAL and serum IL-6, as described above. Post- and pre-exposure values of NAL IL-6, lactoferrin and all blood parameters were log-transformed to reduce the effect of outliers.[13–15] A comparison between the previously published results and results using the modified data analysis strategy for the previously reported OP concentrations (ie, OP^{RTLF}, measured on MOI filters) was made to assess potential differences.

Two-pollutant models

We further evaluated associations in two-pollutant models for those health parameters that were significantly associated with at least one of the measures of OP. We specified two-pollutant models for $PM_{2.5}$ and PM_{10} separately, that is, we adjusted associations for OP of $PM_{2.5}$ for $PM_{2.5}$ mass and $PM_{2.5}$ composition and associations for OP of PM_{10} for PM_{10} mass and PM_{10} composition. Adjustment for PNC, NO_2 and O_3 was done for both OP of $PM_{2.5}$ and OP of PM_{10}. We considered an association consistent if the p value in the one-pollutant model was smaller than 0.1 and remained so after adjusting for all other co-pollutants in two-pollutant models. Models in which two pollutants had a Spearman's rank correlation coefficient >0.7 were not interpreted, because including highly correlated variables may result in unstable effect estimates (co-linearity).

We present effect estimates and their 95% CI as percentage increases over our study population mean of the baseline (t=0) values. We express these values as percentage increases per changes in IQRs in the log-transformed concentrations. We express results from all analyses using the IQRs of the outdoor data set to allow direct comparison of effect estimates between the outdoor data set and the data set including all sites. Statistical significance was defined as $p<0.05$ and borderline significance as $p<0.10$. We performed all analyses using SAS 9.3 (SAS Institute, Cary, North Carolina, USA).

RESULTS

We obtained 170 observations from 31 volunteers (21 female; 10 male). Each participant participated 3–7 times. Mean age was 22 (range 19–26) years. Baseline levels of the different health parameters are given in the online supplementary table S4.

Geometric means and ranges of air pollutants during the 5 h exposures are presented in table 1 for OP, PM mass, PNC, NO_2 and O_3, and in the online supplementary table S5 for PM composition. We found highly elevated OP at the underground site for all three OP measures. PNC and NO_2 concentrations were not (substantially) elevated at the underground site compared with the outdoor sites, whereas O_3 was lower at the underground. Correlations between air pollution concentrations are shown in online supplementary table S6 for $PM_{2.5}$ and S7 for PM_{10}. More details about correlations between the three OP measures and their correlation with PM composition are presented and discussed elsewhere.[16] In brief, when data from all sites were considered, we observed high correlations among all OP measures (Spearman R 0.80–0.97), which were partly driven by the high OP values at the underground site. When only the outdoor sites were considered, OP^{DTT} was moderately correlated with OP^{ESR} and OP^{AA} (Spearman r 0.52–0.70), whereas OP^{ESR} and OP^{AA} were highly correlated (Spearman r 0.88–0.94).

SINGLE-POLLUTANT MODELS

Measures of OP were significantly ($p<0.05$) associated with increases in FE_{NO} or NAL IL-6 2 h after exposure, with for NAL IL-6 the exception of OP^{ESR} of $PM_{2.5}$ (p 0.06–0.21) and OP^{AA} of $PM_{2.5}$ in the outdoor only data set ($p=0.097$) (table 2). Effect estimates increased considerably after excluding the observations from the underground. When associations in the all sites data set were additionally adjusted for measurement at the underground (yes/no), effect estimates were generally similar to effects observed in the outdoor data set.

Significant associations between OP and lung function parameters (FVC, FEV_1) were observed in the outdoor data set and/or underground adjusted models, whereas NAL-lactoferrin was significantly associated with OP in the all sites data set.

None of the blood markers showed significant associations with OP in the outdoor data set and/or underground adjusted

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Table 1 Geometric mean and range (minimum–maximum) of 5 h average OP, PM mass, PNC, NO_2 and O_3 concentrations during the exposure

	All sites (n=170)	Outdoor sites (n=125)	Underground (n=45)
$OP^{DTT\text{-}PM2.5}$(nmol DTT/min/m^3)	3.7 (0.4–25.7)	2.0 (0.4–5.8)	19.3 (12.7–25.7)
$OP^{DTT\text{-}PM10}$(nmol DTT/min/m^3)	5.7 (0.8–68.4)	2.3 (0.8–6.7)	49.9 (38.9–68.4)
$OP^{ESR\text{-}PM2.5}$(AU/1000/m^3)	12.8 (0.5–916.4)	2.9 (0.5–19.7)	773.2 (569.7–916.4)
$OP^{ESR\text{-}PM10}$(AU/1000/m^3)	30.9 (0.7–2612.4)	5.7 (0.7–41.4)	2.152.7 (1617.6–2612.4)
$OP^{AA\text{-}PM2.5}$(nmol AA/s/m^3)	95.6 (9.2–2122.2)	32.9 (9.2–264.5)	1853.2 (1482.4–2122.2)
$OP^{AA\text{-}PM10}$(nmol AA/s/m^3)	177.5 (4.5–5221.8)	48.4 (4.5–415.4)	4037.4 (3138.2–5221.8)
$PM_{2.5}$ (μg/m^3)	37.8 (8.3–167.1)	23.4 (8.3–95.0)	142.0 (123.0–167.1)
PM_{10} (μg/m^3)	70.6 (18.1–449.6)	38.0 (18.1–130.2)	395.1 (353.7–449.6)
PNC (10^3/cm^3)	23.0 (7.0–74.7)	21.0 (7.0–74.7)	29.6 (14.6–39.8)
NO_2 (ppb)	20.1 (9.0–33.8)	20.2 (9.0–33.8)	19.7 (14.1–26.0)
O_3 (ppb)	8.2 (0.3–31.8)	18.3 (5.8–31.8)	0.9 (0.3–0.6)

OP, oxidative potential; PM, particulate matter; PNC, particle number concentrations; AU, arbitrary units.

models (see online supplementary table S8). In the all sites data set, all measures of OP of $PM_{2.5}$ were significantly associated with increases in vWF the next morning, and OP^{DTT} was significantly associated with increases in TPA/PAI-1 complex.

TWO-POLLUTANT MODELS

Results from two-pollutant models are presented in detail for FE_{NO} and NAL IL-6, as these health parameters were significantly associated with OP in both the all sites and the outdoor only or underground adjusted models. In our previous analyses, 2 h after exposure, FE_{NO} was consistently associated with PNC and NAL IL-6 with NO_2, after adjustment for a range of co-pollutants including the OP^{RTLF} used in those analyses.[13–14]

Associations including all sites

Results from two pollutant models for health parameters that showed significant association with OP are given in the online supplementary tables S9–S15. The significant associations of OP with FE_{NO} and NAL-IL6 in single-pollutant models all disappeared after adjusting for PNC (FE_{NO}) or NO_2 (NAL IL-6), whereas effects of PNC or NO_2 were not affected by adjustment for OP (see online supplementary figure S1). Effects of PNC on FE_{NO} and of NO_2 on NAL IL-6 also remained after adjustment for all other pollutants and cancelled out the effect of all other pollutants (see online supplementary tables S9–S12).

Associations with lactoferrin remained significant after adjustment for co-pollutants, especially for OP^{AA} and OP^{ESR} (see online supplementary table S13).

Associations after excluding or adjusting for the underground

FE_{NO} and NAL IL-6

Results from two-pollutant models for combinations of OP and PM mass, PNC, NO_2 and O_3 for the outdoor sites are shown in figure 1 for FE_{NO} and figure 2 for NAL IL-6. Results from two-pollutant models with PM composition and results for the underground adjusted models are included in the online supplementary tables S9–S12.

For FE_{NO}, the significant associations for OP^{DTT}, OP^{ESR} and OP^{AA} of $PM_{2.5}$ all remained after adjustment for $PM_{2.5}$ mass, PNC, NO_2, O_3 (figure 1) as well as after adjustment for $PM_{2.5}$ composition (see online supplementary table S9). Effects of OP^{DTT} remained significant after adjustment for OP^{ESR} or OP^{AA}, and vice versa. OP^{ESR} and OP^{AA} were too highly correlated to disentangle their independent effects. Results for OP of PM_{10} were similar to the results for $PM_{2.5}$ albeit less consistent for OP^{ESR} and OP^{AA}.

For NAL IL-6, no consistent associations were found for any of the OP-$PM_{2.5}$ measures. $PM_{2.5}$ mass was consistently associated with NAL IL-6 in both the outdoor data set and the underground adjusted model. For PM_{10}, both OP^{DTT} and PM_{10} mass were consistently associated with increases in NAL IL-6, whereas the effects of OP^{ESR} and OP^{AA} lost significance after adjustment for (among others) PM_{10} mass and NO_2. OP^{DTT} and PM_{10} mass were too highly correlated to disentangle their independent effects.

Lung function

The significant associations in the outdoor data set between OP and lung function (FEV_1 and FVC) all lost significance when adjusted for several co-pollutants, including PNC, NO_2 and O_3 (see online supplementary tables S16and S17).

Associations with previously reported OP metrics

Associations between the previously reported OP metrics using the current data analysis strategy involving log-transformation did not differ materially from the previously published results with non-transformed OP values (see online supplementary table S18).

Although significant associations were observed between FE_{NO} and all three OP^{RTLF} metrics in the outdoor only and underground adjusted models, these associations all decreased and lost significance when adjusted for (among others) PNC. In addition, associations with OP^{RTLF} also lost significance when adjusted for the OP metrics used in the current analyses (ie, OP^{DTT}, OP^{ESR} and OP^{AA}), whereas effects of these OP metrics remained when adjusted for OP^{RTLF} (see online supplementary figure S2 and S3).

DISCUSSION

In single-pollutant models, we found significant associations between three different measures of the OP of PM (OP^{DTT}, OP^{ESR} and OP^{AA}) and markers of airway and nasal inflammation (FE_{NO} and NAL IL-6) 2 h after exposure. Effect estimates increased considerably after excluding measurements at the underground train station. Adjusting for, rather than excluding, the underground data resulted in effect estimates similar to effects observed in the outdoor data set. Results from two-pollutant models differed substantially depending on how the underground data were considered: For all sites, not OP but

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Table 2 Adjusted associations† between different measures of the OP of PM_{10} and $PM_{2.5}$, and percentage changes in FE_{NO}, lung function and markers in NAL 2 h after exposure

	All sites		Outdoor only		All sites, adjusted underground	
	Estimate (%)	(95% CI)	Estimate (%)	(95% CI)	Estimate (%)	(95% CI)
FE_{NO}‡						
OP^{AA}_PM_{10}	3.8*	(0.8 to 6.8)	10.0**	(3.5 to 16.4)	9.0**	(2.9 to 15.0)
OP^{AA}_$PM_{2.5}$	3.6**	(1.1 to 6.2)	13.6**	(7.4 to 19.8)	13.1**	(7.1 to 19.2)
OP^{ESR}_PM_{10}	2.4*	(0.5 to 4.4)	9.2**	(3.9 to 14.5)	9.2**	(4.1 to 14.2)
OP^{ESR}_$PM_{2.5}$	2.5*	(0.6 to 4.4)	10.4**	(5.2 to 15.7)	10.2**	(5.2 to 15.3)
OP^{DTT}_PM_{10}	2.2*	(0.2 to 4.2)	14.8**	(6.5 to 23.0)	13.9**	(6.8 to 21.0)
OP^{DTT}_$PM_{2.5}$	3.6**	(1.1 to 6.2)	10.8**	(4.7 to 17.0)	10.9**	(5.4 to 16.5)
FVC‡						
OP^{AA}_PM_{10}	−0.22	(−0.79 to 0.34)	−1.17*	(−2.35 to 0.00)	−0.94	(−2.08 to 0.20)
OP^{AA}_$PM_{2.5}$	−0.12	(−0.60 to 0.35)	−0.98	(−2.17 to 0.21)	−0.85	(−2.02 to 0.32)
OP^{ESR}_PM_{10}	−0.13	(−0.50 to 0.23)	−1.05*	(−2.02 to −0.08)	−0.96*	(−1.91 to 0.00)
OP^{ESR}_$PM_{2.5}$	−0.07	(−0.43 to 0.29)	−0.67	(−1.67 to 0.33)	−0.61	(−1.58 to 0.36)
OP^{DTT}_PM_{10}	−0.05	(−0.42 to 0.32)	−1.50#	(−3.03 to 0.02)	−0.76	(−2.12 to 0.60)
OP^{DTT}_$PM_{2.5}$	0.03	(−0.46 to 0.51)	−0.09	(−1.26 to 1.07)	0.05	(−1.04 to 1.15)
FEV_1‡						
OP^{AA}_PM_{10}	−0.33	(−0.86 to 0.20)	−1.29*	(−2.37 to −0.21)	−1.00	(−2.08 to 0.07)
OP^{AA}_$PM_{2.5}$	−0.25	(−0.70 to 0.21)	−1.17*	(−2.31 to −0.04)	−1.03	(−2.16 to 0.10)
OP^{ESR}_PM_{10}	−0.20	(−0.54 to 0.14)	−1.09*	(−1.99 to −0.18)	−1.01*	(−1.91 to −0.11)
OP^{ESR}_$PM_{2.5}$	−0.19	(−0.53 to 0.16)	−0.95*	(−1.90 to 0.00)	−0.96*	(−1.89 to −0.02)
OP^{DTT}_PM_{10}	−0.11	(−0.46 to 0.24)	−1.08	(−2.52 to 0.36)	−0.63	(−1.91 to 0.66)
OP^{DTT}_$PM_{2.5}$	−0.07	(−0.54 to 0.40)	−0.15	(−1.27 to 0.98)	0.01	(−1.05 to 1.07)
NAL IL-6§						
OP^{AA}_PM_{10}	14.7*	(2.3 to 28.7)	30.3*	(3.6 to 64.0)	36.9**	(8.3 to 72.9)
OP^{AA}_$PM_{2.5}$	10.7*	(1.2 to 21.1)	17.8	(−2.9 to 42.8)	23.3*	(0.9 to 50.7)
OP^{ESR}_PM_{10}	7.9*	(0.3 to 16.0)	23.5*	(2.1 to 48.9)	27.8*	(4.9 to 55.0)
OP^{ESR}_$PM_{2.5}$	6.8	(−0.2 to 14.2)	10.9	(−5.8 to 30.5)	13.0	(−4.7 to 34.1)
OP^{DTT}_PM_{10}	8.8*	(1.5 to 16.5)	39.2**	(14.6 to 69.0)	41.5**	(17.2 to 70.9)
OP^{DTT}_$PM_{2.5}$	12.4**	(3.3 to 22.2)	20.0*	(1.1 to 42.4)	29.0**	(9.1 to 52.7)
NAL protein§						
OP^{AA}_PM_{10}	2.9	(−5.6 to 11.4)	16.5*	(0.7 to 32.4)	11.8	(−5.7 to 29.2)
OP^{AA}_$PM_{2.5}$	2.2	(−4.3 to 8.7)	11.8	(−1.3 to 24.9)	10.4	(−4.3 to 25.0)
OP^{ESR}_PM_{10}	1.2	(−4.1 to 6.6)	8.9	(−4.3 to 22.1)	9.0	(−5.6 to 23.5)
OP^{ESR}_$PM_{2.5}$	1.0	(−3.8 to 5.9)	6.4	(−4.8 to 17.5)	5.9	(−6.5 to 18.2)
OP^{DTT}_PM_{10}	1.2	(−3.8 to 6.2)	11.4	(−2.7 to 25.4)	9.6	(−4.8 to 24.0)
OP^{DTT}_$PM_{2.5}$	1.5	(−4.7 to 7.6)	6.1	(−5.7 to 17.9)	5.4	(−7.1 to 17.8)
NAL lactoferrin§						
OP^{AA}_PM_{10}	20.9*	(3.6 to 41.0)	24.7	(−6.5 to 66.5)	25.3	(−8.6 to 71.7)
OP^{AA}_$PM_{2.5}$	14.0*	(1.2 to 28.5)	13.1	(−11.6 to 44.6)	13.8	(−13.1 to 48.9)
OP^{ESR}_PM_{10}	11.2*	(0.9 to 22.6)	14.9	(−9.4 to 45.8)	12.0	(−14.0 to 45.8)
OP^{ESR}_$PM_{2.5}$	10.5*	(1.1 to 20.7)	15.2	(−6.2 to 41.4)	12.4	(−10.3 to 40.8)
OP^{DTT}_PM_{10}	9.6*	(0.0 to 20.0)	8.0	(−16.4 to 39.6)	4.8	(−19.4 to 36.3)
OP^{DTT}_$PM_{2.5}$	10.1	(−1.6 to 23.3)	−0.2	(−19.8 to 24.1)	0.6	(−19.9 to 26.4)

#p<0.10; *p<0.05; **p<0.01.
†Expressed as percentage increase per change in IQR in the log-transformed concentrations at the outdoor sites: 0.72 and 0.71 for OP^{DTT} of $PM_{2.5}$ and PM_{10}; 1.14 and 1.27 for OP^{ESR} of $PM_{2.5}$ and PM_{10}; 1.15 and 1.51 for OP^{AA} of $PM_{2.5}$ and PM_{10}, implying a p75/p25 ratio ranging from 2.0 ($OP^{DTT\text{-}PM10}$) to 4.5 ($OP^{AA\text{-}PM10}$).
‡Adjusted for temperature, relative humidity, season, pollen counts and respiratory infections (as in ref 13).
§Adjusted for temperature, relative humidity, season (as in ref 14) and endotoxin.
NAL, nasal lavage; OP, oxidative potential; PM, particulate matter; NAL, nasal lavage.

PNC and NO_2 remained significantly associated with FE_{NO} and NAL IL-6, respectively, whereas after excluding the underground we found consistent associations with OP. Other health end points, including lung function and vascular inflammatory and coagulation parameters in blood were not consistently associated with OP.

In previous publications from the RAPTES project, no consistent associations with OP were found in either the all sites or the outdoor data set.[13–15] In those analyses, OP was calculated as the sum of OP from $PM_{0.18}$, $PM_{0.18–2.5}$ and $PM_{2.5–10}$ collected with a MOI and measured as the capacity of PM to deplete the antioxidants AA and glutathione (GSH) in a synthetic human RTLF. Our results suggest that the health relevance of OP^{RTLF}, as measured with an MOI sampler in the previous study, is less than the health relevance of the three OP metrics, as measured on PM_{10} and $PM_{2.5}$ filters, in the current study. We cannot

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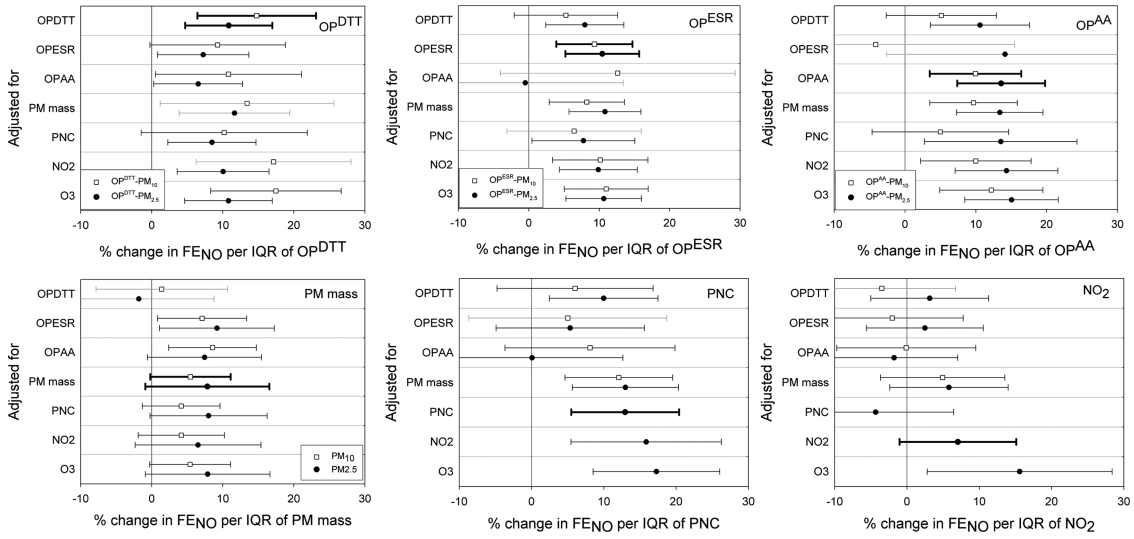

Figure 1 Associations between oxidative potential (OP) of particulate matter (PM), PM mass, particle number concentration (PNC), NO_2 and FE_{NO} in single-pollutant and two-pollutant models after excluding the underground. Single-pollutant effect estimates in bold; grey indicates high (>0.7) correlation between the two pollutants (see online supplementary table S6–S7).

disentangle between the impact of the different sampling methods (HI vs MOI) and impact of the different OP assays (current assays vs OP^{RTLF}), but speculate that sampling played a major role, based on the rather poor agreement between MOI mass and HI mass.[16] We documented that the log-transformation of exposure did not explain the difference between the current OP findings and our previous publications (see online supplementary table S18).

When comparing the different assays used in the current study, after excluding the underground, all three measures of the OP of $PM_{2.5}$ were consistently associated with FE_{NO}: effects of OP^{DTT} remained after adjustment for OP^{ESR} or OP^{AA} and vice versa, suggesting that (drivers of) OP^{DTT} and (drivers of) OP^{ESR} or OP^{AA} can have independent effects on FE_{NO}. OP^{ESR} and OP^{AA} were too highly correlated to disentangle their independent effects. For NAL IL-6, consistent associations with OP were only observed for OP^{DTT} of PM_{10}, which could not be disentangled from effects of PM_{10} mass. Different PM components contribute to OP^{DTT} compared with OP^{ESR} or OP^{AA}, and OP is not easily predicted by single chemical.[16] In our study, OP^{DTT} showed the highest correlation with PM mass, OC (for $OP^{DTT\text{-}PM2.5}$) and NO_2 (for $OP^{DTT\text{-}PM10}$), whereas OP^{ESR} and OP^{AA} showed the highest correlation with the traffic-related PM component (eg, Fe, Cu, EC), especially for PM_{10}. As none of the measured individual PM components was consistently positively associated with FE_{NO} or NAL IL-6, this suggests that different assays could provide complementary information regarding the oxidative properties of PM and their associated health effects. The observed changes most likely do not reflect adverse clinical effects, but they do show that, at ambient levels, different air pollutants can trigger biological responses in healthy, young adults.

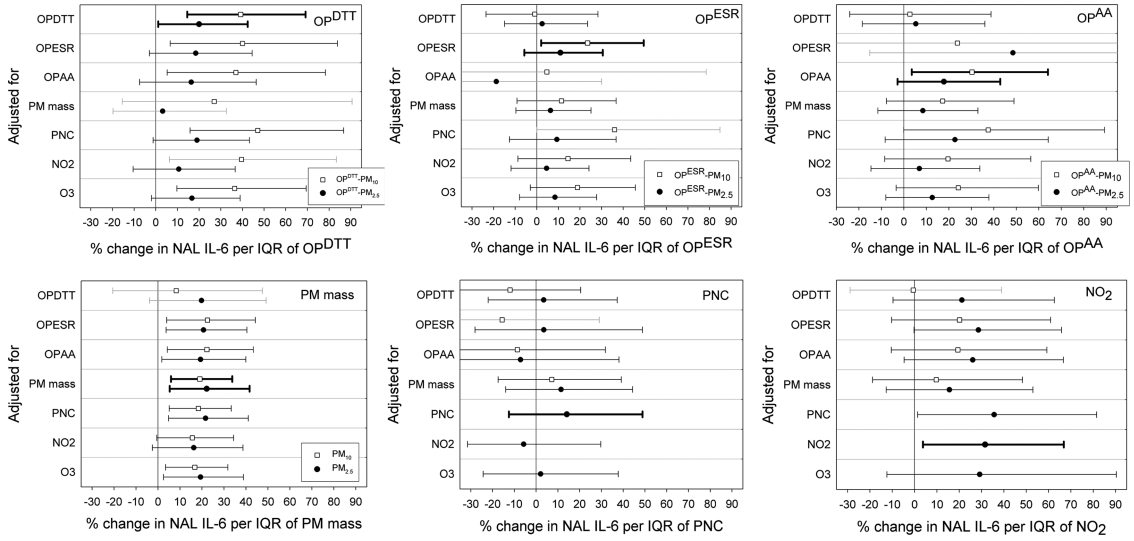

Figure 2 Associations between oxidative potential (OP) of particulate matter (PM), PM mass, particle number concentration (PNC), NO_2 and nasal lavage (NAL) interleukin (IL)-6 in single-pollutant and two-pollutant models after excluding the underground. Single-pollutant effect estimates in bold; grey indicates high (>0.7) correlation between the two pollutants (see online supplementary table S6–S7).

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When all sites were considered, OP was not associated with increases in FE_{NO} or NAL IL-6 after adjustment for co-pollutants. The differences in results, depending on how the underground is considered, are difficult to explain. In previous publications, we also observed differences in results for the all sites compared to the outdoor only models, especially for components that were highly elevated at the underground site. When the underground site was included in the analysis, FE_{NO} and NAL IL-6 were consistently associated with PNC and NO_2, respectively; two components that were not (substantially) elevated in the underground compared with the outdoor locations.[15] In an in vitro study, including samples from the five locations of the current study and three additional sites, a significant association between OP^{DTT} and pro-inflammatory activity was only observed after excluding the underground sample. However, the sample from the underground site was by far the most cytotoxic, which could have hampered the cellular responsiveness of that sample.[19] In another in vitro study, particles from a subway station in Stockholm were less potent to induce inflammatory cytokines compared with particles from an urban street.[20] Few studies have investigated the health effects of exposures in the underground settings.[21–24] Although these studies also measured high concentrations of air pollutants, they could not provide strong evidence of associations between exposure to air pollution and cardiorespiratory health effects. Overall, results from these in vitro and epidemiological studies suggest that the air pollution mixture and associated health effects in the underground are different from the outdoor environment. Alternatively, the lack of associations with OP when including the underground data suggests that the value of OP to predict health effects may be limited and cannot be easily extended to other exposure settings.

Few studies have investigated associations between OP of PM and acute health effects. The associations found for FE_{NO} in the outdoor data set are in line with two panel studies in California.[11 12] Delfino *et al*[11] studied the relationship between air pollution and weekly measurements of FE_{NO} in a panel of 60 elderly participants living in four retirement communities in the LA basin. A cellular macrophage ROS assay was used to characterise OP of 5-day aggregated $PM_{0.25}$ samples and an IQR change in ROS was associated with a 4% increase in FE_{NO}. In a study among 45 schoolchildren with persistent asthma, both the macrophage ROS assay and the DTT assay were used to characterise OP of $PM_{2.5}$.[12] FE_{NO} was significantly positively associated with lag 1-day and 2-day averages of both macrophage ROS (3–5% increase per IQR) and OP^{DTT} (9–10% increase per IQR).[12]

Apart from the observed associations with FE_{NO} and NAL IL-6, none of the other health end points, including lung function, total protein in NAL and vascular inflammatory and coagulation parameters in blood were consistently associated with OP. In contrast, in our previous studies we did report associations for these end points with pollutants such as NO_2, OC and sulfate/nitrate.[13–15] Although some significant associations with OP were observed in single-pollutant models in either the all sites or outdoor only models for lung function, vWF and TPA/PAI1 complex, these associations lost significance when adjusted for co-pollutants. The only exception was lactoferrin in the all sites data set, which remained significantly associated with especially OP^{ESR} and OP^{AA}. Given the lack of association between lactoferrin and OP in the outdoor data set, these associations were likely driven by the high exposures at the underground, as was also observed in our previous analyses.[14]

The lack of association for blood IL-6 contrasts with findings from the study among the elderly by Delfino *et al*,[11] in which an IQR change in macrophage ROS was associated with a significant 9% increase in blood IL-6. This inconsistency with our findings could be related to differences in design, study population and OP metric that was used (ie, 5 h average OP of $PM_{2.5}$ and PM_{10} from a-cellular assays vs macrophage ROS of 5-day aggregated $PM_{0.25}$ samples). In general, absence of associations with OP in our study may be related to the fact that the assays employed only examined the intrinsic potential of the particles to drive oxidation reactions in an a-cellular model, reflecting their content of redox active compounds rather than on interaction with a biological system. As PM can elicit oxidative stress through alternative pathways on interaction with the cellular/tissue matrix, an a-cellular assay does not necessarily reflect the total oxidative activity in vivo.[9]

Strengths and limitations of our design were discussed in detail previously.[13–15] Among others, since we performed air pollution characterisation on-site during exposure of volunteers, exposure measurement error was small compared with observational studies relying on data from central monitoring sites. In our design, we also reduced correlations between PM characteristics by performing repeated measurements at multiple locations with different source characteristics. Despite that, some correlations remained too high to interpret two-pollutant models and disentangle independent effects of OP from other PM characteristics (eg, OP^{DTT} and PM_{10} mass in relation to NAL IL-6). As we evaluated a large number of models, we potentially faced a problem of chance findings in our results. That is why, in our interpretation of the results, we focused on the consistency of (significant) associations rather than individual significant associations.

CONCLUSION

We found significant associations between three a-cellular measures of OP of PM and markers of airway and nasal inflammation in healthy young adults. These OP associations remained significant after adjustment for co-pollutants when the four outdoor sites were included, but lost significance when measurements at the underground site were included. Lung function and vascular inflammatory and coagulation parameters in blood were not consistently associated with OP. Our study, therefore, provides limited support for a role of OP in predicting acute health effects of PM in healthy adults. The difference in associations with different health end points in our study adds to the complexity of investigating which particle metric is more relevant in predicting health effects. Additional studies on the relation between OP and a range of health effects are needed to draw more firm conclusions on the added value of OP compared with more established metrics. Studies in susceptible populations and studies on effects of long-term exposure are needed to further evaluate the added value of OP in future air monitoring and assessments.

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Contributors NAHJ, MStr, FJK, RMH, BB, FRC, MSte and GH were involved in conception and design of the RAPTES study. NAHJ, BB and GH contributed to the data analyses strategy of the current study. NAHJ performed the statistical analyses and drafted the manuscript. MStr and MSte organised and carried out the fieldwork and assisted in the statistical analyses. AY, BH and TAJK contributed to the analyses and interpretation of the oxidative potential measurements used in the current study. FJK supervised the earlier oxidative potential analyses. RMH supervised the chemical analyses in the study. All authors reviewed and approved the manuscript.

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