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 PM2.5 and PM10 using three a-cellular assays; dithiotreitol (OPDTT), electron spin resonance (OPESR) and ascorbic acid depletion (OPAA).

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.8–10 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.11 Also, issues such as high variability in time and space and high costs of the different assays currently hamper wide-scale use.10

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.\(^\text{11-12}\) 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,\(^\text{13}\) nasal pro-inflammatory\(^\text{14}\) and vascular inflammatory and coagulation parameters\(^\text{15}\) 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\(_{0,10}\), 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).\(^\text{16}\) These methods will be referred to as OP\(_{\text{DTT}}\), OP\(_{\text{ESR}}\) and OP\(_{\text{AA}}\), 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.\(^\text{16}\)

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\(_{\text{DTT}}\), OP\(_{\text{ESR}}\) and OP\(_{\text{AA}}\). 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.\(^\text{16,17}\) 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.\(^\text{13-15}\) 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.\(^\text{16}\)

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\(_{\text{SCO}}\), 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,\(^\text{13-15}\) we measured OP of PM\(_{2.5}\) and PM\(_{10}\) using three a-cellular assays; OP\(_{\text{DTT}}\), OP\(_{\text{ESR}}\) and OP\(_{\text{AA}}\).\(^\text{16}\)

**Exposure assessment**

PM mass, PM composition and gaseous air pollution Details about the air pollution measurements are described elsewhere.\(^\text{13,16}\) 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\(_2\): 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.\(^\text{13,16}\) 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 \(\mu\)g/mL.

For OP\(_{\text{DTT}}\), 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\(_{\text{ESR}}\), PM suspensions are diluted to 125 \(\mu\)g/mL and mixed with H\(_2\)O\(_2\) and 5,5-dimethylpyrroline-N-oxide. After incubation, the suspension is vortexed and transferred into a 50 \(\mu\)L glass capillary without any filtration. The DMPO-OH quartette signal is measured with a MiniScope MS-400 spectrometer. OP\(_{\text{ESR}}\) is calculated as the average of the total amplitudes of the DMPO-OH quartette in arbitrary units per \(\mu\)g PM. For OP\(_{\text{AA}}\), PM suspensions are diluted to 12.5 \(\mu\)g/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/\(\mu\)g. Field blank corrected OP values in OP/\(\mu\)g were multiplied with the PM mass concentration (\(\mu\)g/m\(^3\)) to calculate OP/m\(^3\). 88% (OP\(_{\text{DTT}}\)) to 97% (OP\(_{\text{AA}}\)) of the samples were above the detection limit. Coefficients of variation of field duplicates ranged from 8% for OP\(_{\text{AA}}\) to 18% for OP\(_{\text{DTT}}\).\(^\text{16}\) Extreme outlying OP\(_{\text{PM10}}\) values from one measurement day at the farm were excluded.\(^\text{16}\)

**Health assessment**

Details about the health measurements are given elsewhere.\(^\text{13-15}\) In brief, we measured FE\(_{\text{SCO}}\), lung function\(^\text{18}\); interleukin (IL)-6, total protein and lactoferin in NAL\(^\text{14}\); IL-6 and high-sensitivity C reactive protein (CRP) in serum\(^\text{14,15}\); Fibrinogen, von Willebrand Factor (vWF) antigen and the complex between tissue plasminogen activator and plasminogen activator inhibitor-1 (tPA/PAI-1) in citrate plasma\(^\text{15}\) and platelets as part of complete blood cell counts.\(^\text{15}\) 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.\(^\text{14}\)

**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...
health outcomes within the RAPTES project. In brief, the
difference in health parameters between postexposure and pre-
exposure 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,\textsuperscript{13};
- Markers in NAL: IL-6, protein and lactoferrin\textsuperscript{14};
- Blood markers: CRP, fibrinogen platelets, vWF, TPA/PAI1 complex,\textsuperscript{15} IL-6.\textsuperscript{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 stron-
gest 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\textsuperscript{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,\textsuperscript{14}
we adjusted for endotoxin in all models investigating the asso-
ciations 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 under-
ground’ 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, rela-
tive humidity and season for all parameters; pollen and respira-
tory infections for FE_{NO} and lung function; use of oral contraceptives
for all blood parameters except IL-6\textsuperscript{16–18}), 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.\textsuperscript{12,15,16,18} A comparison between the previously published
results and results using the modified data analysis strategy for the
previously reported OP concentrations (ie, OP\textsuperscript{RTV}, 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\textsubscript{2.5} and PM\textsubscript{10} separately, that is, we adjusted assos-
ciations for OP of PM\textsubscript{2.5} for PM\textsubscript{2.5} mass and PM\textsubscript{2.5} composition
and associations for OP of PM\textsubscript{10} for PM\textsubscript{10} mass and PM\textsubscript{10} composition.
Adjustment for PNC, NO\textsubscript{2} and O\textsubscript{3} was done for both
OP of PM\textsubscript{2.5} and OP of PM\textsubscript{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 vari-
ables 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.

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 para-
ters 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\textsubscript{2}
and O\textsubscript{3}, and in the online supplementary table S5 for PM com-
position. We found highly elevated OP at the underground site
for all three OP measures, PNC and NO\textsubscript{2} concentrations were not
(substantially) elevated at the underground site compared with
the outdoor sites, whereas O\textsubscript{3} was lower at the under-
ground. Correlations between air pollution concentrations are
shown in online supplementary table S6 for PM\textsubscript{2.5} and S7 for
PM\textsubscript{10}. More details about correlations between the three OP
measures and their correlation with PM composition are pre-
sent and discussed elsewhere.\textsuperscript{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\textsubscript{RTV} was moderately

correlated with OP\textsubscript{ESR} and OP\textsubscript{AA} (Spearman r 0.52–0.70),
whereas OP\textsubscript{ESR} and OP\textsubscript{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\textsubscript{ESR} of PM\textsubscript{2.5} (p 0.06–0.21) and OP\textsubscript{AA}
of PM\textsubscript{2.5} in the outdoor only data set (p=0.097) (table 2). Effect
estimates increased considerably after excluding the observa-
tions 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 para-
meters (FVC, FEV\textsubscript{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
Two-pollutant models

Results from two-pollutant models are presented in detail for FENO 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, FENO was consistently associated with PNC and NAL IL-6 with NO2, after adjustment for a range of co-pollutants including the OPRTLF 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 FENO and NAL IL-6 in single-pollutant models all disappeared after adjusting for PNC (FENO) or NO2 (NAL IL-6), whereas effects of PNC or NO2 were not affected by adjustment for OP (see online supplementary figure S1). Effects of PNC on FENO and of NO2 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 OPAA and OPESR (see online supplementary table S13).

Associations after excluding or adjusting for the underground

FENO and NAL IL-6

Results from two-pollutant models for combinations of OP and PM mass, PNC, NO2 and O3 for the outdoor sites are shown in figure 1 for FENO 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 FENO, the significant associations for OPDTT, OPESR and OPAA of PM2.5 all remained after adjustment for PM2.5 mass, PNC, NO2, O3 (figure 1) as well as after adjustment for PM2.5 composition (see online supplementary table S9). Effects of OPDTT remained significant after adjustment for OPESR or OPAA, and vice versa. OPESR and OPAA were too highly correlated to disentangle their independent effects. Results for OP of PM10 were similar to the results for PM2.5 albeit less consistent for OPESR and OPAA.

For NAL IL-6, no consistent associations were found for any of the OP-PM2.5 measures. PM2.5 mass was consistently associated with NAL IL-6 in both the outdoor data set and the underground adjusted model. For PM10, both OPDTT and PM10 mass were consistently associated with increases in NAL IL-6, whereas the effects of OPESR and OPAA lost significance after adjustment for (among others) PM10 mass and NO2. OPDTT and PM10 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 (FEV1 and FVC) all lost significance when adjusted for several co-pollutants, including PNC, NO2 and O3 (see online supplementary tables S16 and 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 FENO and all three OPRTLF 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 OPRTLF also lost significance when adjusted for the OP metrics used in the current analyses (ie, OPDTT, OPESR and OPAA), whereas effects of these OP metrics remained when adjusted for OPRTLF (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 (OPDTT, OPESR and OPAA) and markers of airway and nasal inflammation (FENO 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...
PNC and NO₂ remained significantly associated with FE\textsubscript{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. In those analyses, OP was calculated as the sum of OP from PM\textsubscript{0.18}, PM\textsubscript{0.18–2.5} and PM\textsubscript{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\textsubscript{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\textsubscript{10} and PM\textsubscript{2.5} filters, in the current study. We cannot
disentangle between the impact of the different sampling methods (HI vs MOI) and impact of the different OP assays (current assays vs OPRTLF), but speculate that sampling played a major role, based on the rather poor agreement between MOI mass and HI mass. 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 PM2.5 were consistently associated with FENO: effects of OPDTT remained after adjustment for OPESR or OPAA and vice versa, suggesting that (drivers of) OPDTT and (drivers of) OPESR or OPAA can have independent effects on FENO. OPESR and OPAA were too highly correlated to disentangle their independent effects. For NAL IL-6, consistent associations with OP were only observed for OPDTT of PM10, which could not be disentangled from effects of PM10 mass. Different PM components contribute to OPDTT compared with OPESR or OPAA, and OP is not easily predicted by single chemical. In our study, OPDTT showed the highest correlation with PM mass, OC (for OPDTT-PM2.5) and NO2 (for OPDTT-PM10), whereas OPESR and OPAA showed the highest correlation with the traffic-related PM component (eg, Fe, Cu, EC), especially for PM10. As none of the measured individual PM components was consistently positively associated with FENO 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.
When all sites were considered, OP was not associated with increases in FeNO 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, FeNO and NAL IL-6 were consistently associated with PNC and NO2, 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 OPDTT 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 FeNO in the outdoor data set are in line with two panel studies in California.11 12 Delfino et al11 studied the relationship between air pollution and weekly measurements of FeNO 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 PM2.5 samples and an IQR change in ROS was associated with a 4% increase in FeNO. In a study among 45 schoolchildren with persistent asthma, both the macrophage ROS assay and the DTT assay were used to characterise OP of PM2.5.12 FeNO was significantly positively associated with lag 1-day and 2-day averages of both macrophage ROS (3–5% increase per IQR) and OPDTT (9–10% increase per IQR).12

Apart from the observed associations with FeNO and NAL IL-6, none of the other health end points, including 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 OPLO and OPH. 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 PM2.5 and PM10 from a-cellular assays vs macrophage ROS of 5-day aggregated PM0.25 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.11–15 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, OPDTT and PM10 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** NAHI, MStr, FJK, RMH, BB, FRC, MSte and GH were involved in conception and design of the RAPTES study. NAHI, BB and GH contributed to the data analyses strategy of the current study. NAHI performed the statistical analyses and drafted the manuscript. MStr and MSte organised and carried out the fieldwork and assisted in the statistical analyses. AV, BH and TAIJ 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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