Environmental Research 156 (2017) 341–348

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

The impact of ambient air pollution on the human blood metabolome

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ARTICLE INFO

Keywords:
Metabolite profiling
Air pollution
Blood
Cardio-respiratory health effects
Particulate matter components

ABSTRACT

Background: Biological perturbations caused by air pollution might be reflected in the compounds present in blood originating from air pollutants and endogenous metabolites influenced by air pollution (defined here as part of the blood metabolome). We aimed to assess the perturbation of the blood metabolome in response to short term exposure to air pollution.

Methods: We exposed 31 healthy volunteers to ambient air pollution for 5 h. We measured exposure to particulate matter, particle number concentrations, absorbance, elemental/organic carbon, trace metals, secondary inorganic components, endotoxin content, gaseous pollutants, and particulate matter oxidative potential. We collected blood from the participants 2 h before and 2 and 18 h after exposure. We employed untargeted metabolite profiling to monitor 3873 metabolic features in 493 blood samples from these volunteers. We assessed lung function using spirometry and six acute phase proteins in peripheral blood. We assessed the association of the metabolic features with the measured air pollutants and with health markers that we previously observed to be associated with air pollution in this study.

Results: We observed 89 robust associations between air pollutants and metabolic features two hours after exposure and 118 robust associations 18 h after exposure. Some of the metabolic features that were associated with air pollutants were also associated with acute health effects, especially changes in forced expiratory volume in 1 s. We successfully identified tyrosine, guanosine, and hypoxanthine among the associated features. Bioinformatics approach Mummichog predicted enriched pathway activity in eight pathways, among which tyrosine metabolism.

Conclusions: This study demonstrates for the first time the application of untargeted metabolite profiling to assess the impact of air pollution on the blood metabolome.

1. Introduction

The blood metabolome has been defined as the collection of biologically active chemicals in human blood derived from endogenous processes and exogenous exposure to foods, drugs, and pollutants (Rappaport et al., 2014). Untargeted metabolomic techniques such as mass spectrometry (MS) and nuclear magnetic resonance can measure exogenous and endogenous compounds in blood (among other media) and provide a powerful tool to study the potential direct and indirect impact of environmental risk factors on the composition of the blood metabolome (Holmes et al., 2008).

A metabolic signature in the blood of exposure to ambient air pollution is plausible as some ambient air pollutants (ultrafine particles and gaseous air pollutions) have been shown to enter the bloodstream directly from the lungs (Ewetz, 1993; Nemmar et al., 2002). In addition, larger particles (particulate matter $(PM)_{2.5}$, PM_{10}), incapable of crossing the lung epithelium have been shown to induce inflammation in the lungs, triggering a systemic response that can be observed in the peripheral blood (Hogg and van Eeden, 2009). Assessment of the blood metabolome in relation to air pollution is therefore of interest as it might simultaneously detect specific metabolites related to air pollution and their biological response.

Abbreviations: FENO, Concentration of NO in exhaled breath; CRP, C-reactive protein; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; IL-6, interleukin 6; ICC, intra class correlation coefficient; *m/z*, mass-to-charge ratio; PNC, particle number concentration; PM, particulate matter; QTOF, quadrupole time-of-flight mass spectrometer; tPA/PAI-1, tissue plasminogen activator/plasminogen activator inhibitor-1 complex; UHPLC, ultra-high performance liquid chromatography; VWF, Von Willebrand factor; MS, mass spectrometry

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http://dx.doi.org/10.1016/j.envres.2017.03.042
Received 18 October 2016; Received in revised form 1 March 2017; Accepted 27 March 2017

Two studies recently provided evidence for an association between (long- and short-term) exposure to air pollution (NO_2 and $PM_{2.5}$) and changes in the blood metabolome (Menni et al., 2015; Ward-Caviness et al., 2016). Both studies used a targeted approach in which a set of pre-specified metabolites (138 and 288) was assessed. Associations were observed with long-chain fatty acids, amino acids, a carbohydrate (glycerate), a salt (benzoate), and two cofactors and vitamins (a-tocopherol and threonate). Compared to targeted approaches, untargeted metabolomics has the potential to increase the coverage of the metabolome (increasing from several hundred markers to several thousands) and to reduce bias towards identifying well-studied metabolites. A recent study of cigarette smoking demonstrated the success of untargeted metabolomics by identifying 12 unexpected xenobiotic metabolites, in addition to a set of known nicotine metabolites, potentially opening up new avenues of studying the etiology of smoking-related diseases (Gu et al., 2016).

In this hypothesis generating study we used an untargeted high-resolution MS method to assess the profile of metabolic features in peripheral blood samples that were collected in the RAPTES study in which volunteers were exposed for five hours to different levels and mixtures of air pollution. The RAPTES study is highly standardized in terms of the exposure levels and potential confounding factors that were included. Furthermore, blood samples were taken before and after exposure allowing study participants to serve as their own control. Within RAPTES we previously observed associations between several air pollutants and changes in respiratory health parameters measured 2 and 18 h after exposure (Strak et al., 2013a). We also observed associations with markers of coagulation and vascular inflammation measured in peripheral blood that was collected at the same time points (Steenhof et al., 2014).

We assessed the association between changes in the serum concentrations of metabolic features, exposure to air pollutants, and markers of biological perturbations to acquire first insights into the perturbation of the blood metabolome in response to short term exposure to air pollution.

2. Methods

2.1. Study design

The study design has been described in detail before (Strak et al., 2012, 2013a, 2013b). Briefly, we exposed healthy human subjects to ambient air pollution for five hours at five locations in the Netherlands. On the day of exposure we standardized food intake and physical activity of the study participants. The study population consisted of healthy non-smoking volunteers (21 women and 10 men) with a median age of 22 years (range 19–26) and a median BMI of 22.3 (range 17–32). See Table A.1 for an overview of the characteristics of the study subjects. The locations were an underground train station, a continuous traffic location, a stop-and-go traffic location, a farm, and an urban background site (a large garden within a urban region). All locations were within 70 km of the Utrecht University campus where blood was collected in the morning (07:00–07:30), two hours before exposure (t_0) using standard venipuncture techniques. Blood was also collected two (t_9) and eighteen hours (t_{25}, the next morning) after exposure. In Table 1 we provide an overview of the total number of blood samples that were collected at t_0, t_9, and t_{25}, and indicate how many instances of repeated sampling (the same individual visiting a location twice) occurred at each location.

Serum samples were stored within 1 h of blood collection at −80 °C until analysis. We recruited healthy, young, nonsmoking Utrecht University students living on campus to minimize exposure to traffic-related air pollution when traveling to the data collection point. As the composition and the level of air pollution at the underground train station location has been shown to be very different from the other study locations in previous analyses (PM mass concentrations at the underground train station were up to 14 times higher than at the urban background, the sum of the concentrations of trace metals in fine and coarse PM was nearly 20 times above the outdoor levels, and elemental carbon was elevated in fine and coarse PM, in contrast to the outdoor sites where EC was predominantly found in fine PM) (Strak et al., 2011), we excluded this site from our main analysis, but assessed the impact of this decision in a sensitivity analysis.

Table 1
Number of blood samples[a] collected at the five study locations and included in the statistical analysis.

Timing of sampling	Urban background	Farm	Continuous traffic	Stop-and-go traffic	Underground
t_0[b,c]	29 (3)	28 (6)	31 (6)	37 (11)	45 (19)
t_9[b]	28 (3)	26 (6)	31 (6)	36 (10)	41 (17)
t_{25}[b]	28 (3)	24 (6)	30 (6)	36 (10)	43 (18)

[a] For some study participants blood was collected at the same location on multiple days. The number of repeated blood samples is indicated in brackets.
[b] t_0, blood was collected before exposure; t_9, blood was collected two hours after exposure; t_{25}, blood was collected eighteen hours after exposure.
[c] Blood samples collected at t_0 for which no matching blood sample at t_9 or t_{25} was available, were excluded from statistical analysis.

Participants were brought to one of the study locations once every 14 days in groups of eight and were exposed at the location for five hours, cycling 20 min each hour on a stationary bicycle (at a fixed minute ventilation rate of 20 L/min/m^2 body surface area) to increase and standardize inhalation. Each participant had to visit all five locations once, with the two remaining visits assigned randomly to a location. Visits were conducted from March until October 2009, and started at 09:00–09:30 in the morning. The participants completed an online screening questionnaire. Exclusion criteria included smoking or living in a household with a smoker; lifetime diagnosis of asthma or chronic obstructive pulmonary disease; or history of cardiovascular disease, diabetes mellitus, or pregnancy. Before the study, each participant was examined by a physician (blood pressure and pulmonary examination) and obtained medical clearance for participation. The study was approved by the ethics committee at University Medical Center Utrecht. Written informed consent was provided by all participants.

2.2. Exposure assessment

The methods used to characterize exposure to air pollutants have been described in detail before (Strak et al., 2011). Briefly, five hour air pollution measurements were collected at the five locations, each time study participants were present. We assessed the following markers of air pollution: particulate mass (PM) concentration for particles with a diameter < 2.5 μm ($PM_{2.5}$), < 10 μm (PM_{10}), and between 2.5 and 10 μm (PM_{coarse}), gaseous pollutants (nitrogen dioxide, NO_2; nitrogen oxides, NO_x; ozone, O_3), and particle number concentration (PNC, a proxy for ultrafine particles). Within the PM samples we assessed absorbance (a proxy for elemental carbon), trace metal composition, secondary organic and inorganic components, and endotoxin content. In Table A.1 we provide the mean and associated standard deviation of the measured levels of all air pollutants included in the current study. While the urban background location was generally the location where the lowest exposure levels were incurred, the ranking of the other locations based on exposure level varies from marker to marker (Strak et al., 2011). We conducted analyses using data from the 48 individual air pollutants.

2.3. Assessment of health markers

We assessed three respiratory health parameters (the concentration of NO in exhaled breath (FE_{NO}), Forced Vital Capacity (FVC), forced