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Exposure to air pollution interacts with obesogenic nutrition to induce tissue-specific response patterns☆

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ABSTRACT

Obesity and exposure to particular matter (PM) have become two leading global threats to public health. However, the exact mechanisms and tissue-specificity of their health effects are largely unknown. Here we investigate whether a metabolic challenge (early nutritional obesity) synergistically interacts with an environmental challenge (PM exposure) to alter genes representing key response pathways, in a tissue-specific manner. Mice subjected to 7 weeks obesogenic nutrition were exposed every other day during the final week and a half to aqueous extracts of PM collected in the city of London (UK). The expression of 61 selected genes representing key response pathways were investigated in lung, liver, white and brown adipose tissues. Principal component analysis (PCA) revealed distinct patterns of expression changes between the 4 tissues, particularly in the lungs and the liver. Surprisingly, the lung responded to the nutrition challenge. The response of these organs to the PM challenge displayed opposite patterns for some key genes, in particular, those related to the Nrf2 pathway. While the contribution to the variance in gene expression changes in mice exposed to the combined challenge were largely similar among the tissues in PCA1, PCA2 exhibited predominant contribution of inflammatory and oxidative stress responses to the variance in the lungs, and a greater contribution of autophagy genes and MAP kinases in adipose tissues. Possible involvement of alterations in DNA methylation was demonstrated by cell-type-specific responses to a methylation inhibitor. Correspondingly, the DNA methyltransferase Dnmt3a2 increased in the lungs but decreased in the liver, demonstrating potential tissue-differential synergism between nutritional and PM exposure. The results suggest that urban PM, containing dissolved metals, interacts with obesogenic nutrition to regulate diverse response pathways including inflammation and oxidative stress, in a tissue-specific manner. Tissue-differential effects on DNA methylation may underlie tissue-specific responses to key stress-response genes such as catalase and Nrf2.

1. Introduction

Exposure to particulate matter (PM) air pollution and obesity are among the most prevailing global health risks (Chen et al., 2006; Collaborators, 2016; Zanobetti et al., 2014; Yang et al., 2018.). Epidemiological studies in the last decade uncover multi-directional associations between these risk factors. Obese persons (compared to the general population), are more susceptible to air pollution-associated cardiovascular disease (CVD) (Dubowsky et al.,

Abbreviation: PM, particulate matter; HFD, high fat diet; CVD, cardiovascular disease; ROS, reactive oxygen species; GTT, glucose tolerance test; ITT, insulin tolerance test; HPRT, Hypoxanthine Phosphoribosyltransferase 1; TNF-α, tumor necrosis factor α; IL-6, interleukine 6; WAT, white adipose tissue; BAT, brown adipose tissue; IT, intra-tracheal; PC, principal component; MAPK, mitogen-activated protein kinase.

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2006), with impaired vascular reactivity, and CVD-associated hospitalizations (Pearson et al., 2010; Zanobetti et al., 2014), as well as higher vulnerability to PM-related respiratory diseases (Dong et al., 2013; McCormack et al., 2015). Indeed, lung exposure to a given PM concentration was higher with increasing weight in children (Bennett and Zeman, 2004). Complementarily, exposure to PM2.5 (2.5 μm diameter particles or smaller, fine particles) was associated with enhanced risk of diabetes incidence and/or to diabetes-associated mortality (Chen and Schwartz, 2008; Collaborators, 2016; Meo et al., 2015; Pearson et al., 2010, Yang et al., 2018). Thus, exposure to air pollution mainly adversely affects the lungs and cardiovascular system, while obesogenic nutrition (high fat diet, HFD) affects classical "metabolic tissues" such as the liver and adipose tissues. However, emerging data suggests that exposure of the lungs to PM, secondarily affects remote tissues' metabolism (Brook et al., 2013; Liu et al., 2013). It was recently shown that PM2.5 exposure induced pulmonary oxidative stress, which induced vascular insulin resistance and inflammation (Haberzettl et al., 2016). Yet, the mechanisms mediating the observed epidemiological evidence for interactions between obesity and lung exposure to air pollution on human health are still poorly understood.

Reactive oxygen species (ROS) regulate different cell processes such as response to stress (Ray et al., 2012), inflammation (Fernandez-Sanchez et al., 2011), cell division (Cui et al., 2015), autophagy (Liu et al., 2015) and more (Giacco and Brownlee, 2010; Lodovici and Bigagli, 2011; Ray et al., 2012; Savini et al., 2013). Evidently, both environmental and metabolic challenges can increase ROS production and lead to oxidative stress, which can affect health. Increased ROS levels can alter metabolic signaling and induce insulin resistance in obese mice (Houstis et al., 2006), and the development of diabetes-related complications (Giacco and Brownlee, 2010). Air pollution can induce adverse health effects by increasing ROS production that contribute to prevailing oxidative stress (Haberzettl et al., 2016; Shuster-Meiseles et al., 2016) and to inflammation (Lodovici and Bigagli, 2011). To maintain redox homeostasis, antioxidant defense genes can be induced by a master transcription factor regulator, Nrf2, and its related genes (Kensler et al., 2007), which are involved in PM-induced health effects (Lin et al., 2016; Lodovici and Bigagli, 2011; Pardo et al., 2015, 2016; Shuster-Meiseles et al., 2016), and in diabetes/obesity development (Giacco and Brownlee, 2010; Haberzettl et al., 2016; Kensler et al., 2007; Kowluru and Mishra, 2017). However, synergism between exposure of the lungs to PM and obesogenic stress through oxidative stress mechanisms and tissue-specific outcomes are not well-understood.

It was hypothesized that obesity is associated with systemic inflammation and increased ROS-induced oxidative stress (Fernandez-Sanchez et al., 2011; McMurray et al., 2016; Savini et al., 2013). In addition, it is evident that deposition of pollution particles in the lungs can provoke not only local, but also systemic effects by releasing signaling agents and soluble components from the respiratory system through the blood system (Kampfrath et al., 2011; Pardo et al., 2016). As we have previously shown that metals from the water soluble PM extracts increased the inflammatory response in mice's lung (Pardo et al., 2016) and the systemic inflammatory response in the blood (Pardo et al., 2016). This, along with the epidemiology-level evidence, suggest possible interactions that may synergize metabolic challenge (such as obesogenic nutrition) with PM exposure (Hooper et al., 2014; Mendez et al., 2013). To address this question, we hypothesized that such interactions may be evident in a tissue-specific manner by investigating the expression levels of genes representing key players and pathways, including Nrf2, antioxidant defense, inflammation and autophagy and apoptosis. Here we challenged this hypothesis by exposure of mice to combined environmental challenges: exposure to extracts from roadside urbans, and an obesogenic diet (total of seven weeks high fat diet, HFD), and studied the expression of selected gene sets in the lungs, liver, white and brown adipose tissues.

2. Materials and methods

2.1. Particulate matter (PM) collection and extracts characterization

Detailed description of the PM sample collection and characterization has been reported previously (Pardo et al., 2015, 2016; Shuster-Meiseles et al., 2016). Briefly, PM3 samples were collected over a period of a week in a roadside monitoring site in central London (Marylebone Road near Baker Street) in spring 2012 using a Hi-Vol sampler (Tisch (TE-230) Hi-Volume Environmental Impactor Sampler). The samplers operated continuously at a nominal flow rate of 1.2 $m^3\ min^{-1}$, and collected about 50–300 mg of PM on mixed-cellulose ester (MCE) filters.

Sections of the MCE filter-collected with PM3 impactor according to the manufacturer's instructions were extracted with high-purity Milli-Q (18 mΩ) water. We consider PM3 to be comparable to PM2.5 (Shuster-Meiseles et al., 2016) (particles of 2.5 μm and smaller, fine particles) rather than the coarse fraction (PM10, particles between 10 and 2.5 μm). Extraction started with 15 min of sonication, followed by 16 h of continuous agitation at room temperature in the dark and then another 15 min sonication. At the end of the extraction period, the suspension was divided to aliquots and distributed for various assays and analyses, (named "PM extract"). Blank samples replicated the processing procedure (named "Control"). The extracts were subjected to a broad range of characterization tools, including: total and water-soluble elements [ICPMS (SF-ICPMS)]; soluble ions (K^+, Na^+, NH_4^+, SO_4^{2-}, NO_3^-, Cl^-) by IC; and soluble organic carbon, as further detailed in (Pardo et al., 2015, 2016).

2.2. Animal studies and exposures

Five weeks old male C57BL/6 mice were purchased from Harlan laboratories (Rehovot, Israel) and maintained in a temperature-controlled room (22 °C) on a reverse 12-h light-dark cycle. The study was approved by The Institutional Animal Care and Use Committee (IACUC) at the Weizmann institute of science. One week after arrival, 40 mice were randomly divided into four groups, two groups were fed *ad libitum* either a high-fat diet (HFD, Research Diets, D12492) and two groups were fed normal-chow diet (Normal chow, NC) until the end of the experiment. On a caloric basis, the HFD consisted of 60% kcal fat, 20% carbohydrate, and 20% protein, whereas the normal diet contained 13.5% kcal fat, 56% carbohydrate, and 30% protein. After 5 weeks on either diet, mice were exposed to water extracts of resuspended PM collected in urban London or to a blank extract using our previously-published protocol (Pardo et al., 2016). A detailed description of the study design appears in Fig. 1. Briefly, mice were exposed every other day for a total of 5 times, using intra-tracheal (IT) administration model (Pardo et al., 2016). The intra-tracheal instillation technique is non-invasive and was proven to be an adequate method to deliver low dose of particles/extracts into the lungs. However, the technique cannot be used to determine the particle deposition patterns in the lungs as would occur following inhalation. In addition, the procedure requires mice to be fully anesthetized, therefore it is difficult to anesthetize the mice everyday as the anesthesia may kill the mice. Each dose of 50 μL of PM water extract corresponded to 10 μg PM. Therefore, a total amount of 50 μg PM was administered during the exposures. The final groups (n = 10 mice/group) were; mice on NC diet that received the blank extract (NC C), mice on NC diet that