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Particulate matter exposure is associated with inflammatory gene methylation in obese subjects

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ABSTRACT

Background: Overweight and obesity are becoming more widespread with alarming projections for the coming years. Obesity may increase susceptibility to the adverse effects of PM exposure, exacerbating the effects on cardiovascular diseases and altering the biomarkers of vascular inflammation. The associated biological mechanisms have not been fully understood yet; the common denominator in the pathogenesis of the co-morbidities of obesity is the presence of an active, low-grade inflammatory process. DNA methylation has been shown to regulate inflammatory pathways that are responsible for the development of cardiovascular diseases.
Objectives: The aim of the study was to investigate, in a population of overweight/obese subjects, the effects of PM on blood DNA methylation in genes associated to inflammatory response.
Methods: Using bisulfite pyrosequencing, we measured DNA methylation in peripheral blood mononuclear cells from 186 overweighted/obese subjects. In particular, we quantified DNA methylation in a set of 3 candidate genes, including CD14, TLR4 and TNF-α, because of the important roles that these genes play in the inflammatory pathway. Personal exposure to PM_{10} was estimated for each subject based on the local PM_{10} concentrations, measured by monitoring stations at residential address. Repeated measure models were used to evaluate the association of PM10 with each genes, accounting for possible correlations among the genes that regulate the same inflammatory pathway.
Results: We found an inverse association between the daily PM_{10} exposure and the DNA methylation of inflammatory genes, measured in peripheral blood of healthy overweight/obese subjects. Considering different exposure time-windows, the effect on CD14 and TLR4 methylation was observed, respectively, in days 4–5-6, and days 6–7-8. TNF-α methylation was not associated to PM_{10}.
Conclusions: Our findings support a picture in which PM_{10} exposure and transcriptional regulation of inflammatory gene pathway in obese subjects are associated.

1. Introduction

Previous studies have shown that the exposure to particulate matter (PM) is associated with increased morbidity and mortality, primarily from cardiovascular disease (CVD) (Dai et al., 2014; Nasser et al., 2015; Pope et al., 2015; Talbott et al., 2014; Weichenthal et al., 2014). To date, the underlying molecular mechanism responsible for this consistently observed association between air pollution and CVD risk is still poorly understood. It has been proposed that ambient particles might trigger pulmonary oxidative stress and inflammatory responses, leading to the release of molecular signals into the circulatory system (Bollati et al., 2015).

Several investigators have hypothesized that oxidative stress directly induced in the lungs after PM inhalation (Sofer et al., 2013), might cause a systemic inflammatory cascade, increasing cardiovascular risk among susceptible individuals (Bertazzi et al., 2014; Dubowsky et al., 2006; Weichenthal et al., 2013).

Obesity may increase susceptibility to PM_{10} exposure, exacerbating

Abbreviations: BMI, Body mass index; CD14, cluster of differentiation 14; LPS, lipopolysaccharide; NF-κB, nuclear factor κB; PM, particulate matter; PM10, particulate matter with aerodynamic diameters < 10 μm; TLR4, toll-like receptor 4; TNF, tumor necrosis factor

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its effects on cardiovascular disease (Qin et al., 2015) and on biomarkers of vascular inflammation (Matsuda and Shimomura, 2014); in fact, it has been suggested that obese subjects inhale more air-per-day than normal-weight individuals (Brochu et al., 2014; WHO, 2013), thus potentially increasing their overall dose (Weichenthal et al., 2014). Recent studies have further demonstrated that populations characterized by a high body mass index (BMI), when compared to normal weight populations, exhibit an increased risk of CVDs, due to a higher exposure to air pollutants (Chen et al., 2007; Huang et al., 2012; Jung CC and Liang, 2016)

The biological mechanisms behind these associations have not been fully elucidated yet, but many of the obesity-related diseases are thought to be linked to a state of chronic oxidative stress and inflammation. Obese subjects have indeed increased systemic oxidative stress and impaired oxidant defense (De Pergola and Silvestris, 2013; Keaney et al., 2003; Matsuda and Shimomura, 2014; Savini et al., 2013).

To try to improve our understanding on the role of obesity as a susceptibility factor, we designed an epidemiological study where we investigated the effects of PM_{10} on blood DNA methylation in a population of 186 overweight/obese subjects (Wang et al., 2010).

DNA methylation has been shown to regulate biological processes underlying CVDs, such as inflammation and immune response. (Baccarelli et al., 2010; Bayarsaihan, 2011) By regulating inflammatory pathways, that are key elements in the onset of CVDs, pro-inflammatory gene methylation might be related to cardiovascular health (Alexeeff et al., 2013).

In detail, we measured DNA methylation in a set of candidate genes, including of CD14, TLR4 and TNF-α (Bollati et al., 2014a), selected for the important roles that they play in the inflammatory pathway. CD14 is a pattern recognition receptor in the innate immune response against microorganisms and other exogenous and endogenous stress factors. The most important CD14 signaling co-receptor is TLR4, required to trigger the downstream signaling pathways that lead to the activation of the nuclear factor κB (NF-κB) inflammatory pathway that in turn promotes the increase in pro-inflammatory cytokine production including tumor necrosis factor (TNF) (Bes-Houtmann et al., 2007; De Loera-Rodriguez et al., 2014; Hoareau et al., 2010). In addition CD14/TLR4 are over-expressed in obese subjects, reflecting a state of low-grade chronic inflammation (Devevre et al., 2015; Hoareau et al., 2010).

2. Materials and methods

2.1. Study subjects

Overweight/obese subjects were recruited at the Center for Obesity and Work (Department of Preventive Medicine, IRCCS Fondazione Ca'Granda – Ospedale Maggiore Policlinico) in the period between September 2010 and April 2011. These subjects were the first 186 subjects consecutively enrolled in the context of the larger study SPHERE (ERC-2011-StG 282413), (Bollati et al., 2014b) which was aimed at examining the possible molecular mechanisms underlying the effects of PM exposure in relation to health. Each participant signed a written informed consent, approved by the Ethic Committee of the Fondazione Ca'Granda – Ospedale Maggiore Policlinico (Approval no. 1425), in accordance with the Helsinki Declaration principles.

All subjects willing to participate in the study had to unchanged body weight and constant dietary habits in the last year. In addition, they had constant physical activity for at least one month before entry into the study. Exclusion criteria was the presence of a major disease such as cancer, autoimmune disease, cardiovascular disease.

Subjects who agreed to participate were asked to complete lifestyle and dietary questionnaires and to donate a blood sample. Information about lifestyle factors, including current and past smoking habits, alcohol consumption and physical activity, were collected with a self-administered questionnaire.

Body mass index (BMI) was calculated as the ratio between the subject's weight (kg) and height squared (m^2). According to the current definition ((WHO), 2000) , subjects who had a BMI ranging from 25 to 29.9 were classified as overweight, and subjects who had a BMI of 30 or higher were classified as obese.

Waist circumference (cm) was also assessed as marker of central fat accumulation. Fasting level of total cholesterol was measured from serum samples by routine methods (Modular, Roche- Basel, Switzerland). Blood pressure was measured with the participant supine, after 5 min of rest.

2.2. Assessment of PM_{10} exposure

PM_{10} concentrations were assigned to each participant's residential address from September 1, 2010 to April 11, 2011. The PM_{10} concentrations were recorded by the Regional Environmental Protection Agency (ARPA Lombardia) through monitoring stations located throughout Lombardy and available online as daily means. To assign a PM_{10} exposure level to each subject, the addresses of the monitoring stations and the study subjects were geocoded and the PM_{10} measured from the nearest monitor to residential address was assigned. For each subject, we used the daily mean of PM_{10} concentrations measured 1 day before the date of recruitment and back to 14 days before enrollment,. In case of incomplete series, each missing value was imputed by using an algorithm that integrates the annual average of the incomplete series and the PM_{10} concentrations of the nearest and more correlated monitors (Cattani et al., 2010).

2.3. Sample collection, DNA extraction and bisulfite treatment

Seven milliliters of whole blood were collected into EDTA tubes from each participant by venous phlebotomy. Blood was centrifuged at 2500 rpm for 15 min. The buffy coat fraction was transferred to a cryovial and immediately frozen at −80 °C until use. DNA was extracted by the Wizard Genomic DNA purification kit (Promega, Madison, WI, USA), according to the manufacturer's instructions.

500 ng of genomic DNA was treated with the EZ DNA Methylation-Gold™ Kit (Zymo Research, Orange, CA, USA), in accordance with the manufacturer's protocol. Bisulfite-treated DNA was eluted in 30 μL of M-Elution Buffer and kept at −80 °C until use.

2.4. DNA Methylation

Analysis of DNA methylation was performed by previously published methods (Bollati et al., 2007; Tarantini et al., 2013), with minor modifications. Briefly, a 50-μL PCR reaction was carried out with 25 μL of Hot Start GoTaq Green Master mix (Promega), 1 pmol of forward primer, 1 pmol of biotinylated reverse primer and 25 ng of bisulfite-treated genomic DNA. Biotin-labeled primers were used to purify the final PCR product with Sepharose beads. The PCR product was bound to a Streptavidin Sepharose HP (Amersham Biosciences, Uppsala, Sweden). Sepharose beads containing the immobilized PCR product were purified, washed, denatured with 0.2 M NaOH and washed again with the Pyrosequencing Vacuum Prep Tool (Pyrosequencing, Inc., Westborough, MA, USA), according to the manufacturer's instructions. Pyrosequencing primer (0.3 μm) was annealed to the purified single-stranded PCR product, and pyrosequencing was performed with the PyroMark MD System (Pyrosequencing, Inc. Westborough, MA, USA). PCR cycling conditions and primer sequences are reported in Table 1. The methylation level at CpG positions within each gene's promoter region, was expressed as the percentage of cytosines that were methylated, determined as the number of methylated cytosines divided by the sum of methylated and unmethylated cytosines, multiplied by 100% (% 5-methyl-Cytosine). Every sample was tested two times for each marker to confirm reproducibility and to increase the precision of