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Urban air and tobacco smoke as conditions that increase the risk of oxidative stress and respiratory response in youth

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ABSTRACT

Background: Air pollution and tobacco smoke can induce negative effects on the human health and often leads to the formation of oxidative stress.
Objective: The purpose of this study was to clarify the role of the urbanization degree and of passive exposure to tobacco smoke in the formation of oxidative stress. Thus, a group of non-smoking adolescents was recruited among those who live and attend school in areas with three different population densities. To each subject a spot of urine was collected to quantify 15-F_{2t} isoprostane as a marker of oxidative stress and cotinine as a marker of passive exposure to tobacco smoke. Furthermore, respiratory functionality was also measured.
Results: Multiple linear regression analysis results showed a direct correlation ($p < 0.0001$) of 15-F_{2t} isoprostane with both the urbanization and passive smoke. Lung function parameters proved significantly lower for the subjects living in the most populous city of Torino.
Conclusion: This remarks the negative effect that urbanization has on the respiratory conditions. Lastly, lung functionality presented a low inverse correlation with 15-F_{2t} isoprostane, suggesting an independent mechanism than that of the urban factor.

1. Introduction

The airborne particulate matter (PM) has several origins, is formed in different places where its precursors may be different; thus it possesses various physico-chemical and toxicological properties (Götschi et al., 2005; Hazenkamp-Von Arx et al., 2004; Traversi et al., 2008). Depending on type and quantity, the presence of airborne PM can determine deleterious effects on the global environment, cultural heritage, human activities and health (Fang et al., 2010; Henschel et al., 2012; Katsouyanni et al., 2009; Levy et al., 2012; Poschl, 2005; Raaschou-Nielsen et al., 2013; Strak et al., 2012). To contain the problem, the European Union established air quality guidelines for PM as well as for other risky air pollutants (European Union, 2008). At the same time, the research activities of the scientific community were focused on the urban air pollution and its potential risk for health (Bono et al., 2001, 2014; Cohen et al., 2005; Fraser et al., 2003; Tzivian, 2011), in search of the best preventive techniques against the onset of diseases related to air pollution.

Exposure to urban air pollutants, whose concentration is partly dependent on proximity and intensity of traffic, is connected with the onset of asthma, development of respiratory allergies (Badyda et al., 2013; Ghio et al., 2012; Laumbach and Kipen, 2012), lung dysfunction (Kelly and Fussell, 2011; Wright and Brunst, 2013), inflammation, and exacerbation of other respiratory and cardiovascular problems (Mills et al., 2009). Numerous among these pathological conditions can be preceded or highlighted by the presence of internal dose markers, by biosynthesis of biological effects markers or, in some cases, by the formation of oxidative stress (Castro-Giner et al., 2009; Patel et al., 2013). An imbalance of the oxidative status is often a condition that precedes the onset of these respiratory diseases, and it is due to the exposure to airborne

Abbreviations: PM, particulate matter; 15-F_{2t} IsoP, 15-F_{2t}-isoprostane; SIDRIA, Italian Studies on Respiratory Disorders of Childhood and the Environment; CREA, creatinine; E.L.I.S.A., Enzyme-Linked Immuno Sorbent Assay; FVC, forced vital capacity; FEV1, forced expiratory volume in one second; PEF, maximal expiratory flows at peak of FVC; FEF50, maximal expiratory flows at 50% of FVC; FEF25, maximal expiratory flows at 25% of FVC; FEF25-75, maximal expiratory flows among 25–75% of FVC; MLR, multiple linear regression; C.I., confidence interval

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oxidants (Sava and Carlsten, 2012) and a decreasing biosynthesis of endogenous antioxidant molecules (Yang and Omaye, 2009). To date, the mechanisms by which oxidants interact with molecules, cells, and tissue remain largely unclear. Remarkably, oxidative stress is also related to the inflammatory response due to tobacco smoke (Doruk et al., 2011; Howard et al., 1998), which contains a complex mixture of mutagenic chemicals (Granella et al., 1996) able to promote lipid peroxidation (Kalra et al., 1991), protein and DNA oxidation (Vadhanam et al., 2012; van Rijt et al., 2012).

F2-isoprostanes, specific and stable products of lipid peroxidation (Basu et al., 2009), are non-invasive biomarkers for *in vivo* investigations of oxidative stress status (Roberts and Morrow, 2000; Romanazzi et al., 2013), airways inflammation (Basu, 2008) and asthma (Wedes et al., 2009). They can also be implicated in a larger number of human diseases, even if a clear correlation between many of these pathological conditions and oxidative stress is far from being proven (Giustarini et al., 2009). The determination of F2-isoprostanes levels in selected populations may help understanding the role that some environmental factors play in the expression of oxidative stress. In particular, the 15-F_{2t}-isoprostane (15-F_{2t} IsoP) can be monitored, since it has been proven capable to highlight different biological responses to environmental stimuli, particularly those concerning airborne chemicals (Bono et al., 2014).

Quantification of oxidative stress by means of F2-IsoPs has several advantages if compared to other biomarkers, including the one that its levels are unaffected by diet (Gopaul et al., 2000; Jacob et al., 2013). At this concern, Roberts and Morrow reported that urinary F2-IsoPs, in subjects consuming a normal diet, does not decrease after a four days diet consisting only of glucose (Roberts and Morrow, 2000), and Richelle refers that the lipid content of the diet does not affect the level of urinary F2-IsoPs (Richelle et al., 1999). This aspect of F2-IsoPs is particularly useful when, as in this case, the role of the diet is not object of interest, although it is very important in the manifestation of oxidative stress.

Finally, the relationship between biosynthesis of 15-F_{2t} IsoP and levels of respiratory functionality, in relation to the environmental conditions of life, are still largely to deepen.

That is, the purpose of this study was to clarify the role that some independent environmental, individual, and physiological variables have on the oxidative stress status of a large population of healthy non-smoking adolescents, living in three different areas of the Piedmont region (northwestern Italy).

2. Materials and methods

15-F_{2t} IsoP levels were studied in relation to the urbanization degree of the selected areas where the adolescents live and attend school, in order to understand the role that urbanization might play on oxidative stress formation. Any additional information, essential for the study, was collected through a questionnaire filled out by all the adolescents, after their parents or legal tutors had signed an informed consent. In detail:

2.1. Sampling sites

As shown in Fig. 1, three geographic areas with different levels of urbanization and anthropization were chosen in the Piedmont region (northwestern Italy, 25.401,56 km^2): Torino, capital of Piedmont, a urbanized city with almost 900,000 inhabitants 6,700 inhabitants/km^2, 130.2 km^2, 240 m above sea level); Chivasso, a smaller and less urbanized city with about 26,000 inhabitants (507 inhabitants/km^2, 51.3 km^2 183 m above sea level); and Casalborgone, a rural site with 1880 inhabitants 93.3 inhabitants/km^2, 20.2 km^2, 205 m above sea level). Due to the relative proximity with one another, the three locations do not have significant differences in climate, geography, altitude or social habits.

2.2. Epidemiological sample

The epidemiological sample was prepared with the aim to represent the young population of the three locations of the Piedmont region. All subjects were volunteers recruited in lower secondary schools. In more detail, three schools were located in residential and commercial areas of the city of Torino and 214 subjects were recruited from there; one school was in Chivasso, where 119 subjects were recruited; one school was in the rural area of Casalborgone and 57 subjects were enrolled from there. Since all the students were minors, parents or legal tutors were asked to sign an informed consent. Sampling was carried out over the period from March to May 2012. Each adolescent was asked to fill out a questionnaire, perform a spirometry test to evaluate their respiratory functionality, and provide a urine sample for the determination of 15-F_{2t} IsoP and cotinine.

2.2.1. Questionnaire

For each student, a short version of the questionnaire "SIDRIA" was prepared to acquire information on age, sex, place of residence, hobbies, therapies, and parent's smoking habits (SIDRIA, 1997). An interviewer administered the questionnaire during school hours, the same day the urine sampling and the spirometry took place.

2.3. Biological samples and statistical analysis

2.3.1. Urinary cotinine

Cotinine measurement was carried out to quantify the passive exposure to tobacco smoke, which represents a possible factor of oxidative stress formation. A specimen of the first morning urine was collected from each volunteer and stored at −80 °C until analysis. Cotinine was measured by gas chromatography–mass spectrometry. The analytical procedure has been described in detail elsewhere (Bono et al., 2014). Cotinine concentrations were normalized to the urinary creatinine (CREA) levels, as usual for every urinary measurement.

2.3.2. Urinary Isoprostane

15-F_{2t} IsoP was measured in urine by ELISA, as previously described (Romanazzi et al., 2013). A microplate kit specific for urinary 15-F_{2t} IsoP (Oxford, MI, USA) was used following manufacturers' instructions. The declared limit of detection is 0.2 ng/ml and the possible cross-reactivity of this method is fixed below 3%. To achieve better accuracy by the ELISA method, a dilution rate of 1:4 (v/v) was adopted (Romanazzi et al., 2013). 15-F_{2t} IsoP concentrations were normalized to the CREA levels.

2.3.3. Spirometry

According to the current standards (ATS/ERS, 2005), maximal expiratory flow-volume curves were obtained while the subjects were in a standing position, wearing a nose clip and breathing into a pneumotachograph (Medicalgraphics). The instrument was calibrated with a 3-l syringe. The measurements were repeated until the volume variability did not exceed 150 ml for at least 2 times. Forced vital capacity (FVC), forced expiratory volume in one second (FEV1) and maximal expiratory flows at peak 50%, 25% and among 25–75% of FVC (PEF, FEF50, FEF25, FEF 25-75) were recorded (Bono et al., 1998; Miller et al., 2005).

2.3.4. Statistical analysis

Statistical analysis was carried out with the statistical package