Oxidative stress in adolescent passive smokers living in urban and rural environments

Elsevier Editorial System(tm) for International Journal of Hygiene and Environmental Health

Manuscript Draft

Manuscript Number: IJHEH-D-13-00022

Title: Title: Oxidative stress in adolescent passive smokers living in urban and rural environments.

Article Type: Original Article

Keywords: Oxidative stress, 15-F2t-isoprostane, passive tobacco smoke, urban pollution, adolescent health

Corresponding Author: Prof. Roberto Bono, Ph.D.

Corresponding Author's Institution: University of Torino, ITALY

First Author: Roberto Bono, Ph.D.

Order of Authors: Roberto Bono, Ph.D.; Valeria Bellisario, dr.; Valeria Romanazzi, Ph.D.; Valentina Pirro, Ph.D.; Pavilio Piccioni, Ph.D. M.D.; Marco Pazzi, Ph.D.; Massimiliano Bugiani, Ph.D. M.D.; Marco Vincenti, Ph.D.

Abstract: Purpose of this study was to evaluate and compare the oxidative stress status - a pre condition often resulting from imbalance between the production of oxidants and antioxidants in the body - among a group of adolescents. This work was conducted through the measurement of a specific oxidative stress biomarker: the urinary 15-F2t-isoprostane (15-F2t-isoP) in 168 adolescents passively smokers: 110 urbanized and 58 living in the countryside. A general linear model (GLM) analysis was performed in order to evaluate the significance of two factors in the biosynthesis of 15-F2t-isoP measured with ELISA technique: residence and tobacco smoke exposure, measured with urinary cotinine. Formaldehyde (FA) concentration in air was also evaluated as a primary confounding factor in oxidative stress. No significant differences in FA contamination between the two sites were found. Conversely, direct relationship between oxidative stress status and residence of adolescents was found: oxidative stress level was 31% higher for adolescents living in urban site than for those living in the countryside area. Tobacco smoke exposure proved to play an important role in the distribution of 15-F2t-isoP levels ($p < 0.0001$). Lastly, an inversely proportional relationship was found between the age of adolescents and 15-F2t-isoP ($p < 0.0001$).
The biological responses evaluated in this study suggested that even a modest environmental level of pollutants may affect the exposure risk and should possibly be taken into account to plan primary prevention actions. Future investigations will explore the effect of other pollutants, including the particulate matter.

Suggested Reviewers: Franziska Ferk Ph.D.
Department of Medicine I, Institute of Cancer Research, Medical University of Vienna
franziska.ferk@meduniwien.ac.at

Stanley T. Omaye Ph.D.
Nutrition Department and Environmental Science, University of Nevada, Reno, U.S.A.
omaye@unr.edu

Alessandro Marcon Ph.D.
Epidemiology, University of Verona, ITALY
alessandro.marcon@univr.it

UNIVERSITY OF TORINO, ITALY
Department of Public Health and Pediatry
Via Santena 5 bis
10126 TORINO, Italy

January 11st, 2013

Dear Editor of ***International Journal of Hygiene and Environmental Health***:

Please find enclosed the manuscript "*Oxidative stress in adolescent passive smokers living in urban and rural environments*" by R. Bono *et al.* In my view, the relevance of the results summarized in the manuscript is two-fold. Firstly, the evidence that passive tobacco smoke exposure causes oxidative stress in adolescents, independently from the urbanization level. Secondly, oxidative stress increases according to the level of urbanization of the adolescents, independently from their passive tobacco smoke exposure.
Thus, data may hence represent a platform for designing a protective grid for prevention to tobacco smoke exposure and to urban pollution.

Some information about the results presented:

- The new version of the manuscript has been corrected according to author instructions.
- the manuscript is an original work, has not been previously published in whole or in part, and is not under consideration for publication elsewhere;
- the voluntary participation of all the human subjects did not occur until after informed consent was obtained;
- all authors have disclosed any potential competing interest regarding the submitted article and the nature of those interests;
- all the authors have read the manuscript, agree that the work is ready for submission to Journal of Adolescent Health, and accept responsibility for the manuscript's contents.

Hoping that the manuscript may fulfil the scientific standards of ***International Journal of Hygiene and Environmental Health***, my best regards.

Roberto Bono, Ph. D.

Contacts:
Phone: (+39) 011-670-5818
Fax: (+39) 011-236-5818
e-mail: roberto.bono@unito.it

Title: Oxidative stress in adolescent passive smokers living in urban and rural environments.

Authors: Roberto Bono Ph.D.[1*], Valeria Bellisario Ph.D.[1], Valeria Romanazzi Ph.D.[1], Valentina Pirro[2] Ph.D., Pavilio Piccioni[3] MD Ph.D., Marco Vincenti[2] Ph.D.

Affiliations:

[1]University of Torino, Department of Public Health and Pediatry, Torino, ITALY

[2]University of Torino, Department of Chemistry, Torino, ITALY

[3]Unit of Respiratory Medicine, National Health Service (ASL TO2), Turin, Italy

***Address correspondence to:** Prof. Roberto Bono. Department of Public Health and Pediatry. University of Torino, Via Santena 5 bis, 10126, Torino, ITALY. Phone: (+39) 011-670-5818, fax: (+39) 011-236-5818, e-mail: roberto.bono@unito.it

Abstract

Purpose of this study was to evaluate and compare the oxidative stress status - a pre condition often resulting from imbalance between the production of oxidants and antioxidants in the body - among a group of adolescents. This work was conducted through the measurement of a specific oxidative stress biomarker: the urinary 15-F_{2t}-isoprostane (15-F_{2t}-isoP) in 168 adolescents passively smokers: 110 urbanized and 58 living in the countryside. A general linear model (GLM) analysis was performed in order to evaluate the significance of two factors in the biosynthesis of 15-F_{2t}-isoP measured with ELISA technique: residence and tobacco smoke exposure, measured with urinary cotinine. Formaldehyde (FA) concentration in air was also evaluated as a primary confounding factor in oxidative stress. No significant differences in FA contamination between the two sites were found. Conversely, direct relationship between oxidative stress status and residence of adolescents was found: oxidative stress level was 31% higher for adolescents living in urban site than for those living in the countryside area. Tobacco smoke exposure proved to play an important role in the distribution of 15-F_{2t}-isoP levels ($p < 0.0001$). Lastly, an inversely proportional relationship was found between the age of adolescents and 15-F_{2t}-isoP ($p < 0.0001$).

The biological responses evaluated in this study suggested that even a modest environmental level of pollutants may affect the exposure risk and should possibly be taken into account to plan primary prevention actions. Future investigations will explore the effect of other pollutants, including the particulate matter.

Key words

Oxidative stress, 15-F_{2t}-isoprostane, passive tobacco smoke, urban pollution, adolescents

Introduction

Indoor and outdoor air pollution, hazardous chemicals, noise, food and water contaminants are factors possibly associated with environment-related health outcomes, including respiratory diseases, allergies and asthma, cardiovascular diseases, neurological effects, reproductive and developmental disorders, and cancer. Among the environmental factors, urban outdoor air pollution, partly generated by car exhaust, has become a problem of growing international interest (Bono et al., 2010a; Cohen et al., 2005; Tzivian, 2011).

Air pollution may present various physical, chemical, mutagenic and toxicological properties, according to geographical area and human socio-economic activities (Traversi et al., 2008). The resulting impact on human health may evolve with different characteristics and intensity levels (Gomes et al., 2012; Plummer et al., 2012). Epidemiological studies performed in metropolitan areas revealed that the exposure to urban air pollution is a significant factor in the increasing prevalence of many diseases and mortality, even if its mechanisms of action remains partially unclear (Brauner et al., 2007; Brunekreef, 2007). In urban areas, prevalent contribution to air pollution arises from motor vehicle emissions (Gomes et al., 2012). Thus, many researchers focused their studies on exposure assessment and measurement of primary biological and/or adverse health effects on citizens exposed to traffic-related pollutants (Bind et al., 2012; Gan et al., 2012).

Recently, many scientific evidences attributed to air pollution and vehicle exhaust emissions the effect to modulate the body's redox system through an increase of pro-oxidant species and a decrease of antioxidant molecules (Ghio et al., 2012; Yang andOmaye, 2009). This condition, defined as oxidative stress, is connected with several DNA lesions, including modifications of bases, which are considered potential causes of cancer (Ghio et al., 2012; Loeb, 2001).

Oxidative stress can be induced by outdoor and indoor environments (residential, public or occupational) (Bono et al., 2010b). The indoor environments, particularly the sites where people smoke or have smoked, are often characterized by the highest levels of pollutants that induce oxidative stress (Fuselli et al., 2010). Accordingly, oxidative stress plays a crucial role in the

inflammatory response to tobacco smoke (Doruk et al., 2011), frequently in co-exposure with airborne ultrafine particles (Mo et al., 2012). Tobacco smoke is a complex mixture of oxidizing compounds, capable to promote numerous biological damages, such as lipid peroxidation (Kalra et al., 1991; Morrow et al., 1995; Scherer, 2005), protein and thiol oxidation (Frei et al., 1991; Reznick et al., 1992), and oxidation of DNA (Park et al., 1998). The combustion-derived nanoparticles (CDNPs), present in tobacco smoke and all environmental contests, produce oxidative stress, inflammation and lung cancer. CDNPs can be redistributed to other organs, after pulmonary deposition (Donaldson et al., 2005). The knowledge of how exogenous and endogenous oxidants interact with molecules in the cells, tissues, and the epithelial lining fluid (ELF) of the lung is crucial for planning the most suitable prevention strategies.

F2-isoprostanes are specific products of lipid peroxidation and their metabolites were evaluated in vivo as potential biomarkers of oxidative stress status (Roberts andMorrow, 2000). F2-isoprostanes are a family of Prostaglandin (PG) F2α isomers, described as products of non-cycloxygenase after oxidative modifications of arachidonic acid, that resulted from free-radical attack of cell membrane phospholipids or circulating low density lipids (LDLs) (Lynch et al., 1994; Morrow et al., 1990). Thus, F2-isoprostanes, a chemically stable group of bioactive compounds, appear to play a role in acute and sub-clinical chronic inflammations (Basu et al., 2009) and are utilized as non-invasive markers of airways inflammation (Basu, 2008) and asthma (Wedes et al., 2009). They may describe the possible role of some exogenous factors in the expression of oxidative stress in selected populations. They can also be implicated in a large number of human diseases, even if a clear correlation between disease and oxidative stress is far from being proven for most pathological conditions (Giustarini et al., 2009).

Purpose of this study was to clarify the role of the exposure to tobacco smoke and air pollution in the biosynthesis of 15-F_{2t}-isoprostane (15-F_{2t}-IsoP) (Romanazzi et al.) within two population of adolescents, living in areas of the Piedmont Region (North-Western Italy) characterized by different geographical conditions and levels of urbanization.

105

106 **Methods**

107 Sampling Sites. Two sampling sites were selected taking into account different urbanization,
108 anthropization and vehicular traffic conditions. Chivasso is an urbanized city with about 26.000
109 inhabitants (514 inhabitants/km^2), located at 180 meters a.s.l. close to Torino, the capital of
110 Piedmont Region (900.000 inhabitants). Casalborgone (200 meters a.s.l.) is a rural site, 12 km away
111 from Chivasso, and populated by 1850 inhabitants (92 inhabitants/Km^2). (Sources by Piedmont
112 Region, 2011) (**Figure 1**).

113 Epidemiological sample**.** All adolescents (N=168) involved in the present study were volunteers
114 who attended two schools located in Chivasso (N=110) and Casalborgone (N=58), respectively.
115 Since the subjects were underage, during a public meeting, parents and teachers were informed on
116 the objective of this study and to parents were asked to sign a written informed consent. Thus, the
117 participation of all the human subjects did not occur until after informed consent was obtained.
118 Sampling was carried out from March to April 2011, involving one class per day, according to a
119 pre-established timetable. From each student, the following items were collected: (1) a
120 questionnaire, gathering information about general features, (2) a urine sample for the
121 quantification of urinary cotinine, 15-F_{2t} IsoP and creatinine (CREA), (3) spirometry data to
122 evaluate respiratory health and vital capacity.

123 Questionnaire. A questionnaire was administered to each subject during the school time, to collect
124 information concerning individual and clinical features, such as age, gender, residence, hobbies,
125 therapies, and parent' smoking habits.

126 Urinary cotinine. Urinary cotinine was measured in order to consider the possible role played by
127 passive tobacco smoke in the onset of an oxidative stress status. An aliquot of fresh urine was
128 collected in the early morning and approximately at the same time from each volunteers, and stored
129 at -80 °C prior to analysis, performed within 20 working days. 10 ml of urine was transferred into a
130 glass tube and 4 g of NaCl, 500 µl of NaOH (5M) and 10 µl of cotinine-d_3 (internal standard) were

added. Subsequently, for two times, 2 ml of trichloromethane ($CHCl_3$) were added to the sample to perform liquid-liquid extraction which was carried out in a shaking wheel for 15 minutes. Sample was then centrifuged for 10 min at 1000 g and the resulting organic phase was collected in a new glass tube and evaporated to dryness in a rotary evaporator at room temperature. The dry residue was reconstituted in 200 µl of $CHCl_3$ and transferred into a conical vial for GC-MS determination. All the details of this last instrumental procedure were reported in a previous paper (Bono et al., 2012).

The cotinine calibration curve was built by fortifying a blank urine pool of non-smoking subjects, to obtain a concentration range from 0.02 µg/ml to 2 µg/ml. the fortified urine was extracted as for the samples. The limit of detection (LOD) was calculated as the concentration of the analyte that gives a signal equal to the average background of the blank (S_{blank}) plus three times its standard deviation ($LOD = S_{blank} + 3S_{blank}$), while the limit of quantification (LOQ) was estimated as twice of the LOD value. LOD and LOQ were respectively 0.01 µg/ml and 0.02 µg/ml. Coefficients of variation (CV%) calculated to test repeatability were below 5% for both cotinine and the internal standard.

Urinary Isoprostane. 15-F_{2t}-IsoP in urine was measured by ELISA technique performed with a specific microplate kit (Oxford, MI, USA), according to manufacturer's instructions. All the details of this procedure were reported in a previous paper (Romanazzi et al.). Dilution 1:4 was adopted to achieve better accuracy in the competitive ELISA method.

Creatinine quantification. An aliquot of fresh urine was used to measure the concentration of urinary creatinine (crea) by the kinetic Jaffé procedure (Bartels andCikes, 1969) so as to normalize the excretion rate of urinary cotinine and 15-F_{2t}-IsoP.

Spirometry. For each subject, maximal expiratory flow-volume curves were obtained in standing position, wearing a noseclip and breathing into a Stead-Wills spirometer. The instrument was calibrated with a 3L syringe daily. Spirometry curves were collected three times until they were repeatable within a 5% experimental error. Values were corrected for BTPS (Body Temperature Pressure Standard). Measured spirometric parameters included best forced vital capacity (FVC),

157 forced expiratory volume in one second (FEV_1) and maximal expiratory flows at peack, 50%, 25%
158 (PEF, MEF_{50}, MEF_{25}) (Miller et al., 2005).

159 Formaldehyde sampling and analysis

160 Formaldehyde determinations were carried out daily during three months of sampling (March,
161 April, May 2011), in two sites close to the adolescent' schools. For each sampling site, a sampling
162 line working at 1.0 l/min sampling speed during 24 hours/day and equipped with a adsorbent vial
163 containing silica gel coated with 2,4-dinitrophenylhydrazine (DNPH) was used. The reaction of
164 DNPH with FA yielded 2,4-dinitrophenylhydrazone which was subsequently quantified by a GC-
165 MS method, as described elsewhere [33].

166 Statistical analysis. The analysis was performed by means of Stata 12 Statistical Package
167 (StataCorp LP, Lakeway Drive, TX, USA). Appropriate linear transformation was applied on data
168 whenever suggested by distributional diagnostic plots (symmetry plot, quantile plot) and descriptive
169 statistic inspection (looking at variance stability among categories). To alleviate multivariate
170 heteroscedasticity, a Box-Cox power transformation on the dependent variable was applied to the
171 data by means of maximum likelihood estimates of the parameters. To compare the values among
172 groups, a median test (a nonparametric K-sample test on the equality of medians) was performed,
173 checking the null hypothesis that the K samples were drawn from populations with the same
174 median.

175 The relationship of $15F_{2t}$-IsoP with the other relevant variables was studied by means of the general
176 linear model (GLM) analysis, that considers 15-F_{2t}-IsoP as the dependent variable, while respiratory
177 symptoms recorded by the questionnaire (cough, colds, catarrh, whistles, shortness of breath, lung
178 disease, bronchitis, rhinitis and allergies), sampling sites, gender, Box-Cox transformed level of
179 cotinine, age (expressed as days since the birth date divided by 365.25), weight (Kg), height
180 (meters) and B.M.I. (weight/height(m)^2), were the independent variables. To assess the
181 relationship between lung function parameters (FEV1, FEF 25-75 and FEF50) and 15-F_{2t}-IsoP, a
182 different linear model (GLM) analysis was performed that considers 15-F_{2t}-IsoP as the dependent

variable, and gender, Box-Cox transformed single lung function parameter and level of cotinine, age (years), weight (Kg), height (meters) and B.M.I. (weight/height(m)^2), as independent variables.

The link function for GLM was selected following the Box-Cox transformation. A p value of ≤ 0.05 (two-tailed) was considered significant for all tests. All the variables that proved non-significant at 5% were excluded with a step-wise backward removal procedure.

Results

The main characteristics of the population examined are presented in **Table 1**. Subjects were grouped according to their residence municipality, which in turn coincides with the school location: Chivasso (urban site) and Casalborgone (rural site), respectively. For each group of students, numerousness, gender, averaged age, weight, height, and information about passive smoke exposure are reported.

Normalized 15-F_{2t} IsoP values for the investigated populations are given in **Table 2**. On average, 15-F_{2t} IsoP values recorded in Chivasso are significantly higher than those found in Casalborgone ($p = 0.03$). This outcome highlights an unspecific "urban factor" that promotes a higher level of oxidative stress in the adolescent subjects living and attending the school in the urban site.

The Box-Cox estimate and variables inspection suggested to apply a natural log-transformation on urinary cotinine concentrations and to use log-function as a link in the GLM: the other covariates and factors were not transformed. No significant relationship was detected between 15-F_{2t}-IsoP and respiratory symptoms, gender, weight, and height ($p > 0.10$). **Table 3** shows GLM outcomes considering log-urinary cotinine levels, sampling site and age.

A positive relationship was found between urinary 15-F_{2t}-IsoP and cotinine levels and urbanization of the sampling sites; while a negative relationship was found with respect to the adolescents' age. Multivariate analysis shows a positive effect of log-cotinine concentration on $15F_{2t}$-IsoP level ($p < 0.0001$). In particular, a 27% increment of $15F_{2t}$-IsoP is observed for each one-unit-increment of

log-cotinine (2,71828 in natural scale). Thus, it can be hypothesized that passive tobacco smoke exposure causes oxidative stress in the adolescent subjects, independently from the urbanization level (urban or rural site) and the subject's age (**Figure 2A**). Similarly, GLM analysis shows significant higher 15-F_{2t}-IsoP levels for the Chivasso population ($p < 0.0001$), i.e., the mean level of 15-F_{2t}-IsoP 31% is higher than for the Casalborgone sample population, independently from passive smoke exposure (**Figure 2B**). Finally, the GLM analysis shows a 15F_{2t}-IsoP decrease as a function of the increasing subjects' age ($p < 0,001$) with a 19% decreases every 12 months of age (**Figure 2C**). Lung function parameters are not significantly related (5% level) to 15-F_{2t}-IsoP, after controlling for age, sampling site and cotinine level (data not shown). Finally, mean concentrations of airborne FA (expressed as $\mu g/m^3$) were simultaneously measured outside the urban and rural schools. No evident differences between the two sites (t-test) were found. The recorded values are also comparable with those reported in the literature (NTP, 2011).

Discussion and conclusion

The specific purpose of this study was to assess the level of oxidative stress, as estimated from the urinary 15-F_{2t}-IsoP specific biomarker, as a function of various individual parameters, using two populations of adolescents respectively living in an urbanized site and a countryside area. Among these parameters, the passive exposure to tobacco smoke was also quantified for each subject by means of its urinary cotinine.

Adolescents were used as a target population for investigating the effects of different environmental conditions on the onset of an oxidative stress status, since they have on average lower mobility and a simpler life-style than adults, so that most of their time is spent within resident domestic and scholar indoor ambients.

The principal result of this study is the observation of positive and direct relationship between urbanization level and oxidative stress status, which appears to be independent from passive smoke exposure. In the urban site of Chivasso, detected urinary 15-F_{2t}-IsoP mean levels were 31% higher

than those found for the population living in the rural site of Casalborgone. This result could be partially explained by a generic "urban air pollution" factor which is definitely higher in Chivasso. However, air-FA, identified *a priori* as a possible marker of air pollution, failed to show significantly higher concentrations in the urbanized area than in the rural site. The similar of air-FA concentration in the two sites could be attributed to the complex and multiple generation mechanisms of this pollutant, ranging from primary origins, chiefly occurring in the urban environments, to secondary (photochemical) origin in the case of rural sites. Probably, determinations of individual exposure to air-FA and other airborne pollutants by using personal samplers may provide more accurate and highly sensitive observations and, therefore, could be performed in future investigations.

The second important result gained in this study is the evidence of the role played in the 15-F_{2t}-IsoP formation by tobacco smoke exposure, independently from the urbanization level. This means that an exposure, although passive, to about 4000 chemicals present in tobacco smoke acts as an inductor of significant oxidative stress. Thus, both independent factors investigated in this study, namely urbanization level and passive tobacco smoke, were found to have a powerful role in the induction of oxidative stress status detected by 15-F_{2t}-IsoP.

The students' age also appears to play a role in the formation of 15-F_{2t}-IsoP, although a very limited age range is deliberately represented in the investigated population (12, 13 and 14 years old). Surprisingly, age and 15-F_{2t}-IsoP appear to be inversely correlated, whereas, in previous studies, an increase of plasma free and total (free plus esterified) 15-F_{2t}-IsoP levels was found with the patients' age (Cruz et al., 2009; Ward et al., 2005). On the other hand, previous studies did not consider homogeneous populations of healthy adolescents, as in the present case. Thus, further investigations expanding the studied age range appear to be necessary.

Finally, others factors taken into account, including the non specific respiratory symptoms (as collected by questionnaire) and spirometric measurements, proved not to be associated with 15-F_{2t}-IsoP in urine, at least in the age range considered in this study. This could be related to the

relatively small concentration of the biomarker. However, although a cross-sectional effect cannot be demonstrated in our study, a possible long-term effect on lung function should not be excluded, as demonstrated for environmental pollutants in other sites (Arossa et al., 1987)

Remarkably, no 15-F_{2t}-IsoP outliers were detected in the studied healthy population Therefore, the detection of such a sensitive biological response as a consequence of limited differences of environmental pollution could provide new and useful knowledge for the appraisal of preventive strategies, particularly for young subjects, which are known to be more sensitive, since they spend most of their time in indoor environments (i.e. school and home), and they have a respiratory system not immunologically fully mature yet (Neri et al., 2006). Thus, we intend to extend this study to a larger number of subjects and sites, presenting different environmental characteristics. To better explain the biological effects, we plan to consider a higher number of air pollutants and, in particular, the ultrafine particulate as another important "urban air factor", possibly involved in the onset of oxidative stress of environmental origin.

Acknowledgments

The authors kindly thank to prof. Isabella Di Leo and all the students who have generously collaborated on this study and doctors Massimiliano Bugiani and Marco Pazzi for their collaboration.

Founding sources: no external founding was secured for this study

Financial disclosure statement: The authors have no financial relationships relevant to this article to disclose

Conflict of interest: The authors have no conflicts of interest to disclose

References

Arossa, W., Spinaci, S., Bugiani, M., Natale, P., Bucca, C. and de Candussio, G., 1987. Changes in lung function of children after an air pollution decrease. Arch Environ Health, 42, 170-4.

Bartels, H. andCikes, M., 1969. Chromogens in the creatinine determination of Jaffè. Clin Chim Acta, 26, 1-10.

Basu, S., 2008. F-2-isoprostanes in human health and diseases: From molecular mechanisms to clinical implications. Antioxidants & Redox Signaling, 10, 1405-1434.

Basu, S., Helmersson, J., Jarosinska, D., Sallsten, G., Mazzolai, B. and Barregard, L., 2009. Regulatory factors of basal F2-isoprostane formation: Population, age, gender and smoking habits in humans. Free Rad Res, 43, 85-91.

Bind, M.A., Baccarelli, A., Zanobetti, A., Tarantini, L., Suh, H., Vokonas, P. and Schwartz, J., 2012. Air pollution and markers of coagulation, inflammation, and endothelial function: associations and epigene-environment interactions in an elderly cohort. Epidemiology, 23, 332-40.

Bono, R., Degan, R., Pazzi, M., Romanazzi, V. and Rovere, R., 2010a. Benzene and formaldehyde in air of two Winter Olympic venues of "Torino 2006". Environ Int, 36, 269-75.

Bono, R., Romanazzi, V., Munnia, A., Piro, S., Allione, A., Ricceri, F., Guarrera, S., Pignata, C., Matullo, G., Wang, P., Giese, R.W. and Peluso, M., 2010b. Malondialdehyde-deoxyguanosine adduct formation in workers of pathology wards: the role of air formaldehyde exposure. Chem Res Toxicol, 23, 1342-8.

Bono, R., Romanazzi, V., Pirro, V., Degan, R., Pignata, C., Suppo, E., Pazzi, M. and Vincenti, M., 2012. Formaldehyde and tobacco smoke as alkylating agents: the formation of N-methylenvaline in pathologists and in plastic laminate workers. Sci Total Environ, 414, 701-7.

Brauner, E.V., Forchhammer, L., Moller, P., Simonsen, J., Glasius, M., Wahlin, P., Raaschou-Nielsen, O. and Loft, S., 2007. Exposure to ultrafine particles from ambient air and oxidative stress-induced DNA damage. Environ Health Perspect, 115, 1177-82.

Brunekreef, B., 2007. Health effects of air pollution observed in cohort studies in Europe. J Expo Sci Environ Epidemiol, 17 Suppl 2, S61-5.

Cohen, A.J., Ross Anderson, H., Ostro, B., Pandey, K.D., Krzyzanowski, M., Kunzli, N., Gutschmidt, K., Pope, A., Romieu, I., Samet, J.M. and Smith, K., 2005. The global burden of disease due to outdoor air pollution. J Toxicol Environ Health A, 68, 1301-7.

Cruz, M.J., Sanchez-Vidaurre, S., Romero, P.V., Morell, F. and Munoz, X., 2009. Impact of age on pH, 8-isoprostane, and nitrogen oxides in exhaled breath condensate. Chest, 135, 462-7.

Donaldson, K., Tran, L., Jimenez, L.A., Duffin, R., Newby, D.E., Mills, N., MacNee, W. and Stone, V., 2005. Combustion-derived nanoparticles: a review of their toxicology following inhalation exposure. Part Fibre Toxicol, 2, 10.

Doruk, S., Ozyurt, H., Inonu, H., Erkorkmaz, U., Saylan, O. and Seyfikli, Z., 2011. Oxidative status in the lungs associated with tobacco smoke exposure. Clin Chem Lab Med, 49, 2007-12.

Frei, B., Forte, T.M., Ames, B.N. and Cross, C.E., 1991. Gas phase oxidants of cigarette smoke induce lipid peroxidation and changes in lipoprotein properties in human blood plasma. Protective effects of ascorbic acid. Biochem J, 277 (Pt 1), 133-8.

Fuselli, S., De Felice, M., Morlino, R. and Turrio-Baldassarri, L., 2010. A three year study on 14 VOCs at one site in Rome: levels, seasonal variations, indoor/outdoor ratio and temporal trends. Int J Environ Res Public Health, 7, 3792-803.

Gan, W.Q., Davies, H.W., Koehoorn, M. and Brauer, M., 2012. Association of long-term exposure to community noise and traffic-related air pollution with coronary heart disease mortality. Am J Epidemiol, 175, 898-906.

Ghio, A.J., Carraway, M.S. and Madden, M.C., 2012. Composition of air pollution particles and oxidative stress in cells, tissues, and living systems. J Toxicol Environ Health B Crit Rev, 15, 1-21.

Giustarini, D., Dalle-Donne, I., Tsikas, D. and Rossi, R., 2009. Oxidative stress and human diseases: Origin, link, measurement, mechanisms, and biomarkers. Crit Rev Clin Lab Sci, 46, 241-81.

Gomes, J.F., Bordado, J.C. and Albuquerque, P.C., 2012. Monitoring exposure to airborne ultrafine particles in Lisbon, Portugal. Inhal Toxicol, 24, 425-33.

Kalra, J., Chaudhary, A.K. and Prasad, K., 1991. Increased production of oxygen free radicals in cigarette smokers. Int J Exp Pathol, 72, 1-7.

Loeb, L.A., 2001. A mutator phenotype in cancer. Cancer Res, 61, 3230-9.

Lynch, S.M., Morrow, J.D., Roberts, L.J. and Frei, B., 1994. Formation of Non-Cyclooxygenase-Derived Prostanoids (F-2-Isoprostanes) in Plasma and Low-Density-Lipoprotein Exposed to Oxidative Stress in-Vitro. Journal of Clinical Investigation, 93, 998-1004.

Miller, M.R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., Crapo, R., Enright, P., van der Grinten, C.P., Gustafsson, P., Jensen, R., Johnson, D.C., MacIntyre, N., McKay, R., Navajas, D., Pedersen, O.F., Pellegrino, R., Viegi, G. and Wanger, J., 2005. Standardisation of spirometry. Eur Respir J, 26, 319-38.

Mo, Y., Wan, R., Feng, L., Chien, S., Tollerud, D.J. and Zhang, Q., 2012. Combination effects of cigarette smoke extract and ambient ultrafine particles on endothelial cells. Toxicol In Vitro, 26, 295-303.

Morrow, J.D., Frei, B., Longmire, A.W., Gaziano, J.M., Lynch, S.M., Shyr, Y., Strauss, W.E., Oates, J.A. and Roberts, L.J., 2nd, 1995. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. N Engl J Med, 332, 1198-203.

Morrow, J.D., Hill, K.E., Burk, R.F., Nammour, T.M., Badr, K.F. and Roberts, L.J., 2nd, 1990. A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proc Natl Acad Sci U S A, 87, 9383-7.

Neri, M., Ugolini, D., Bonassi, S., Fucic, A., Holland, N., Knudsen, L.E., Sram, R.J., Ceppi, M., Bocchini, V. and Merlo, D.F., 2006. Children's exposure to environmental pollutants and biomarkers of genetic damage. II. Results of a comprehensive literature search and meta-analysis. Mutat Res, 612, 14-39.

NTP (2011).Formaldehyde in *National Toxycology Program - Twelfth Edition* Department of Health and Human Services, NC: U.S.

Park, E.M., Park, Y.M. and Gwak, Y.S., 1998. Oxidative damage in tissues of rats exposed to cigarette smoke. Free Radic Biol Med, 25, 79-86.

Plummer, L.E., Ham, W., Kleeman, M.J., Wexler, A. and Pinkerton, K.E., 2012. Influence of season and location on pulmonary response to California's San Joaquin Valley airborne particulate matter. J Toxicol Environ Health A, 75, 253-71.

Reznick, A.Z., Cross, C.E., Hu, M.L., Suzuki, Y.J., Khwaja, S., Safadi, A., Motchnik, P.A., Packer, L. and Halliwell, B., 1992. Modification of plasma proteins by cigarette smoke as measured by protein carbonyl formation. Biochem J, 286 (Pt 2), 607-11.

Roberts, L.J. andMorrow, J.D., 2000. Measurement of F-2-isoprostanes as an index of oxidative stress in vivo. Free Radical Biology and Medicine, 28, 505-513.

Romanazzi, V., Pirro, V., Bellisario, V., Mengozzi, G., Peluso, M., Pazzi, M., Bugiani, M., Verlato, G. and Bono, R., 15-F_{2t} Isoprostane as biomarker of oxidative stress induced by tobacco smoke and occupational exposition to formaldehyde in workers of plastic laminates. Sci Total Environ, *In press.*

Scherer, G., 2005. Biomonitoring of inhaled complex mixtures--ambient air, diesel exhaust and cigarette smoke. Exp Toxicol Pathol, 57 Suppl 1, 75-110.

Traversi, D., Degan, R., De Marco, R., Gilli, G., Pignata, C., Ponzio, M., Rava, M., Sessarego, F., Villani, S. and Bono, R., 2008. Mutagenic properties of PM2.5 air pollution in the Padana Plain (Italy) before and in the course of XX Winter Olympic Games of "Torino 2006". Environ Int, 34, 966-70.

Tzivian, L., 2011. Outdoor air pollution and asthma in children. J Asthma, 48, 470-81.

Ward, W.F., Qi, W., Van Remmen, H., Zackert, W.E., Roberts, L.J., 2nd and Richardson, A., 2005. Effects of age and caloric restriction on lipid peroxidation: measurement of oxidative stress by F2-isoprostane levels. J Gerontol A Biol Sci Med Sci, 60, 847-51.

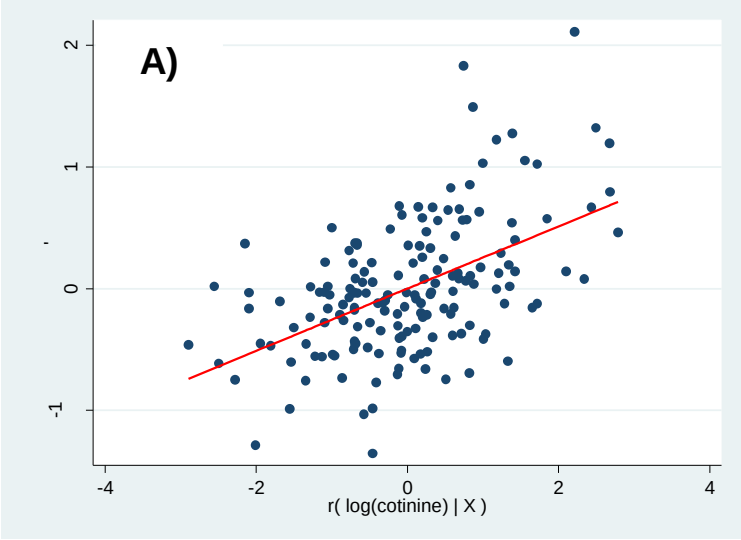
Wedes, S.H., Khatri, S.B., Zhang, R., Wu, W., Comhair, S.A., Wenzel, S., Teague, W.G., Israel, E., Erzurum, S.C. and Hazen, S.L., 2009. Noninvasive markers of airway inflammation in asthma. Clin Transl Sci, 2, 112-7.

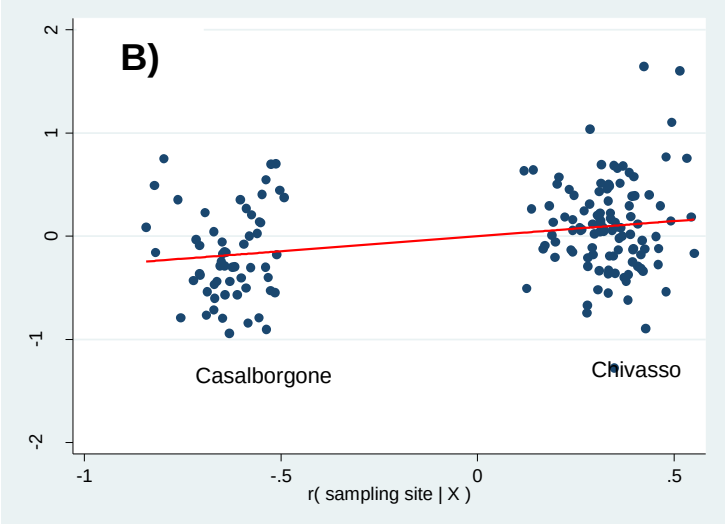
Yang, W. andOmaye, S.T., 2009. Air pollutants, oxidative stress and human health. Mutat Res, 674, 45-54.

Figure 1


TORINO
CHIVASSO
CASALBORGONE
Volpiano
Chivasso
Leini
Brandizzo
Mappano
Settimo Torinese
Castagneto Po
Parco Fluviale del Po
San Raffaele Cimena
Gassino Torinese
Castiglione Torinese
Rivalba
Casalborgone
San Mauro Torinese
Sciolze
Cinzano
Berzano di San Pietro
Rivodora
Tetti San Defendente
Baldissero Torinese
Pavarolo
Montaldo Torinese
Autostrada Torino-Milano
Tangenziale Nord
Via Torino
Via Casale
Via Asti
Stradale Casale
Stradale Torino
Strada Statale 11 Padana Superiore
Strada Statale Torino-Aosta
Corso Novara
Lungo Stura Lazio
SS460
SS460
SP267
SP11
SP267
SP39
SP500
SP94
SP122
SS590
SS590
SS458
SS458
SS11
SS11
SS11
SR11
SR11
E612
E64
E64
E64
A4
A4
A4
A5

Figure 2


A)
2
1
0
-1
-4
-2
0
2
4
r(log(cotinine) | X)


B)
2
1
0
-1
-2
-1
-.5
0
.5
Casalborgone
Chivasso
r(sampling site | X)

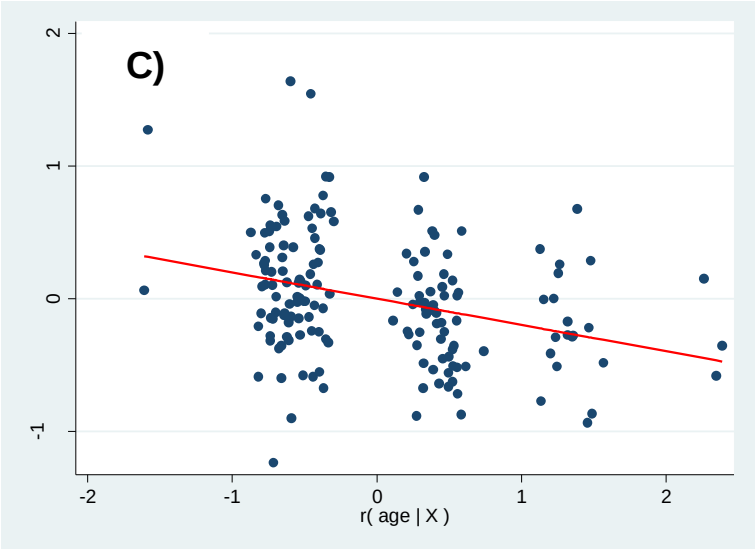

C)
2
1
0
-1
-2
-1
0
1
2
r(age | X)

Figure captions

Figure 1: The two sampling sites: Chivasso (urban site) and Casalborgone (rural site) compared to Torino.

Figure 2: **A)** Added-variable plot of log (15F2t-IsoP) levels by log (cotinine), adjusted by age and "sampling site". r = multivariate residual given age and sampling site. **B)** Added-variable plot of log (15F2t-IsoP) levels by the two sampling sites, adjusted by age and log(cotinine). r = multivariate residual given age and log (cotinine). **C)** Added-variable plot of log($15F_{2t}$-IsoP) by age, adjusted by "sampling site" and "log (cotinine)".

		CHIVASSO	CASALBORGONE	TOTAL
N.		110	58	168
GENDER	Male n (%)	59 (53%)	26 (45%)	85 (50.5%)
	Female N (%)	51 (47%)	32 (55%)	83 (49.5%)
AGE	years ± s.d.	12.7 ± 0.8	12.5 ± 0.6	12.6 ± 0.8
WEIGHT	kg ± s.d.	47.3 ± 12.3	47.7 ± 12.2	47.5 ± 12.2
HEIGHT	cm ± s.d.	154.7 ± 8.9	153.0 ± 9.8	154.2 ± 9.2
SMOKE	Active smokers n. (%)	1 (0.9 %)	0	1 (0.5 %)
	Passively exposed n. (%)	50 (45.5 %)	23 (60.3%)	73 (43.5 %)
	not exposed n. (%)	59 (53.6 %)	35 (39.7%)	94 (56.0 %)

Table 1. Epidemiological characteristics and exposure to tobacco smoke of the whole populations grouped according to their residence and school.

	15-F_{2t} Isoprostane (ng/mg crea)			
SAMPLING SITE	MEAN ± S.D.	MIN	MAX	*p*
Chivasso (urban)	5,8 ± 5,1	1,2	39,8	0,03
Casalborgone (rural)	4,8 ± 2,9	1,5	14,7	
Total	5,5 ± 4,5	1,2	39,8	

Table 2. 15-F_{2t} IsoP values in the two group of students attended the two schools.

Variables	B (C.I. 95%)	*p* <	Exp(B)
Log(Cotinine)	+0.238 (+0.177 +0.299)	0.000	1.27
Sampling site (Chivasso)	+0.304 (+0.161 +0.447)	0.000	1.35
Age	-0.212 (-0.300 -0.124)	0.000	0.80
Constant	3.598 (2.474 - 4.722)		

Table 3. GLM analysis results using $15F_{2t}$-IsoP as dependent variable with link log and normal distribution assuntion (non significant effects at 5% level are not reported).