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

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## UNIVERSITÀ DEGLI STUDI DI TORINO

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**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.

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January 11<sup>st</sup>, 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.

A handwritten signature in black ink that reads 'Roberto Bono'.

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

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3

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27 **Abstract**

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29 resulting from imbalance between the production of oxidants and antioxidants in the body - among  
30 a group of adolescents. This work was conducted through the measurement of a specific oxidative  
31 stress biomarker: the urinary 15-F<sub>2t</sub>-isoprostane (15-F<sub>2t</sub>-isoP) in 168 adolescents passively smokers:  
32 110 urbanized and 58 living in the countryside. A general linear model (GLM) analysis was  
33 performed in order to evaluate the significance of two factors in the biosynthesis of 15-F<sub>2t</sub>-isoP  
34 measured with ELISA technique: residence and tobacco smoke exposure, measured with urinary  
35 cotinine. Formaldehyde (FA) concentration in air was also evaluated as a primary confounding  
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38 adolescents was found: oxidative stress level was 31% higher for adolescents living in urban site  
39 than for those living in the countryside area. Tobacco smoke exposure proved to play an important  
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41 relationship was found between the age of adolescents and 15-F<sub>2t</sub>-isoP ( $p < 0.0001$ ).

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44 prevention actions. Future investigations will explore the effect of other pollutants, including the  
45 particulate matter.

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49 **Key words**

50 Oxidative stress, 15-F<sub>2t</sub>-isoprostane, passive tobacco smoke, urban pollution, adolescents

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52

53 **Introduction**

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

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

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

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



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

89 F<sub>2</sub>-isoprostanes are specific products of lipid peroxidation and their metabolites were evaluated in  
90 vivo as potential biomarkers of oxidative stress status (Roberts andMorrow, 2000). F<sub>2</sub>-isoprostanes  
91 are a family of Prostaglandin (PG) F<sub>2</sub> $\alpha$  isomers, described as products of non-cyclooxygenase after  
92 oxidative modifications of arachidonic acid, that resulted from free-radical attack of cell membrane  
93 phospholipids or circulating low density lipids (LDLs) (Lynch et al., 1994; Morrow et al., 1990).  
94 Thus, F<sub>2</sub>-isoprostanes, a chemically stable group of bioactive compounds, appear to play a role in  
95 acute and sub-clinical chronic inflammations (Basu et al., 2009) and are utilized as non-invasive  
96 markers of airways inflammation (Basu, 2008) and asthma (Wedes et al., 2009). They may describe  
97 the possible role of some exogenous factors in the expression of oxidative stress in selected  
98 populations. They can also be implicated in a large number of human diseases, even if a clear  
99 correlation between disease and oxidative stress is far from being proven for most pathological  
100 conditions (Giustarini et al., 2009).

101 Purpose of this study was to clarify the role of the exposure to tobacco smoke and air pollution in  
102 the biosynthesis of 15-F<sub>2t</sub>-isoprostane (15-F<sub>2t</sub>-IsoP) (Romanazzi et al.) within two population of  
103 adolescents, living in areas of the Piedmont Region (North-Western Italy) characterized by different  
104 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<sup>2</sup>), 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<sup>2</sup>). (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<sub>2t</sub> 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<sub>3</sub> (internal standard) were

131 added. Subsequently, for two times, 2 ml of trichloromethane ( $\text{CHCl}_3$ ) were added to the sample to  
132 perform liquid-liquid extraction which was carried out in a shaking wheel for 15 minutes. Sample  
133 was then centrifuged for 10 min at 1000 g and the resulting organic phase was collected in a new  
134 glass tube and evaporated to dryness in a rotary evaporator at room temperature. The dry residue  
135 was reconstituted in 200  $\mu\text{l}$  of  $\text{CHCl}_3$  and transferred into a conical vial for GC-MS determination.  
136 All the details of this last instrumental procedure were reported in a previous paper (Bono et al.,  
137 2012).

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

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

149 Creatinine quantification. An aliquot of fresh urine was used to measure the concentration of  
150 urinary creatinine (crea) by the kinetic Jaffé procedure (Bartels and Cikes, 1969) so as to normalize  
151 the excretion rate of urinary cotinine and 15-F<sub>2t</sub>-IsoP.

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

157 forced expiratory volume in one second (FEV<sub>1</sub>) and maximal expiratory flows at peak, 50%, 25%  
158 (PEF, MEF<sub>50</sub>, MEF<sub>25</sub>) (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<sub>2t</sub>-IsoP with the other relevant variables was studied by means of the general  
176 linear model (GLM) analysis, that considers 15-F<sub>2t</sub>-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)<sup>2</sup>), were the independent variables. To assess the  
181 relationship between lung function parameters (FEV<sub>1</sub>, FEF 25-75 and FEF<sub>50</sub>) and 15-F<sub>2t</sub>-IsoP, a  
182 different linear model (GLM) analysis was performed that considers 15-F<sub>2t</sub>-IsoP as the dependent

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

186 The link function for GLM was selected following the Box-Cox transformation. A *p* value of  $\leq 0.05$   
187 (two-tailed) was considered significant for all tests. All the variables that proved non-significant at  
188 5% were excluded with a step-wise backward removal procedure.

189

## 190 **Results**

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

196 Normalized 15-F<sub>2t</sub> IsoP values for the investigated populations are given in **Table 2**. On average,  
197 15-F<sub>2t</sub> IsoP values recorded in Chivasso are significantly higher than those found in Casalborgone  
198 (*p* = 0.03). This outcome highlights an unspecific “urban factor” that promotes a higher level of  
199 oxidative stress in the adolescent subjects living and attending the school in the urban site.

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

205 A positive relationship was found between urinary 15-F<sub>2t</sub>-IsoP and cotinine levels and urbanization  
206 of the sampling sites; while a negative relationship was found with respect to the adolescents' age.

207 Multivariate analysis shows a positive effect of log-cotinine concentration on 15F<sub>2t</sub>-IsoP level (*p* <  
208 0.0001). In particular, a 27% increment of 15F<sub>2t</sub>-IsoP is observed for each one-unit-increment of

209 log-cotinine (2,71828 in natural scale). Thus, it can be hypothesized that passive tobacco smoke  
210 exposure causes oxidative stress in the adolescent subjects, independently from the urbanization  
211 level (urban or rural site) and the subject's age (**Figure 2A**). Similarly, GLM analysis shows  
212 significant higher 15-F<sub>2t</sub>-IsoP levels for the Chivasso population ( $p < 0.0001$ ), i.e., the mean level of  
213 15-F<sub>2t</sub>-IsoP 31% is higher than for the Casalborgone sample population, independently from passive  
214 smoke exposure (**Figure 2B**). Finally, the GLM analysis shows a 15F<sub>2t</sub>-IsoP decrease as a function  
215 of the increasing subjects' age ( $p < 0,001$ ) with a 19% decreases every 12 months of age (**Figure**  
216 **2C**). Lung function parameters are not significantly related (5% level) to 15-F<sub>2t</sub>-IsoP, after  
217 controlling for age, sampling site and cotinine level (data not shown). Finally, mean concentrations  
218 of airborne FA (expressed as  $\mu\text{g}/\text{m}^3$ ) were simultaneously measured outside the urban and rural  
219 schools. No evident differences between the two sites (t-test) were found. The recorded values are  
220 also comparable with those reported in the literature (NTP, 2011).

221

## 222 **Discussion and conclusion**

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

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

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

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

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

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

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

261 relatively small concentration of the biomarker. However, although a cross-sectional effect cannot  
262 be demonstrated in our study, a possible long-term effect on lung function should not be excluded,  
263 as demonstrated for environmental pollutants in other sites (Arossa et al., 1987)  
264 Remarkably, no 15-F<sub>2t</sub>-IsoP outliers were detected in the studied healthy population Therefore, the  
265 detection of such a sensitive biological response as a consequence of limited differences of  
266 environmental pollution could provide new and useful knowledge for the appraisal of preventive  
267 strategies, particularly for young subjects, which are known to be more sensitive, since they spend  
268 most of their time in indoor environments (i.e. school and home), and they have a respiratory  
269 system not immunologically fully mature yet (Neri et al., 2006). Thus, we intend to extend this  
270 study to a larger number of subjects and sites, presenting different environmental characteristics. To  
271 better explain the biological effects, we plan to consider a higher number of air pollutants and, in  
272 particular, the ultrafine particulate as another important “urban air factor”, possibly involved in the  
273 onset of oxidative stress of environmental origin.

274

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Figure 1

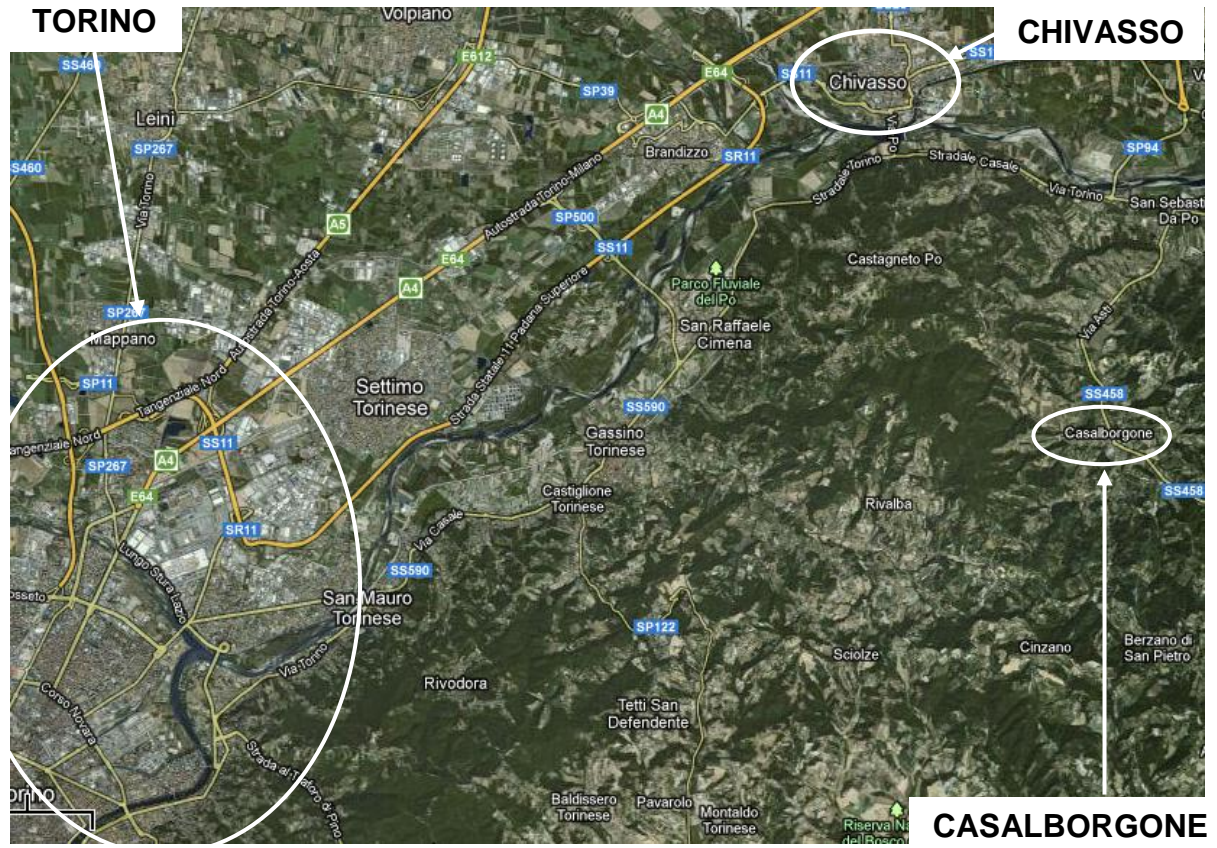
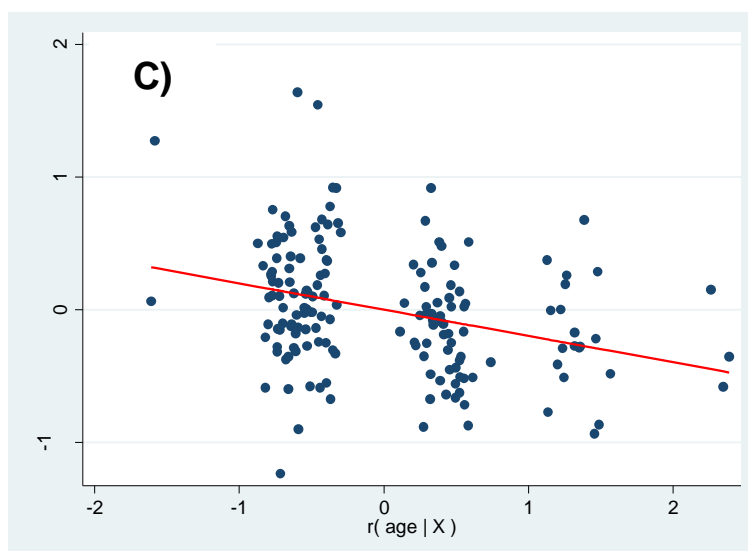
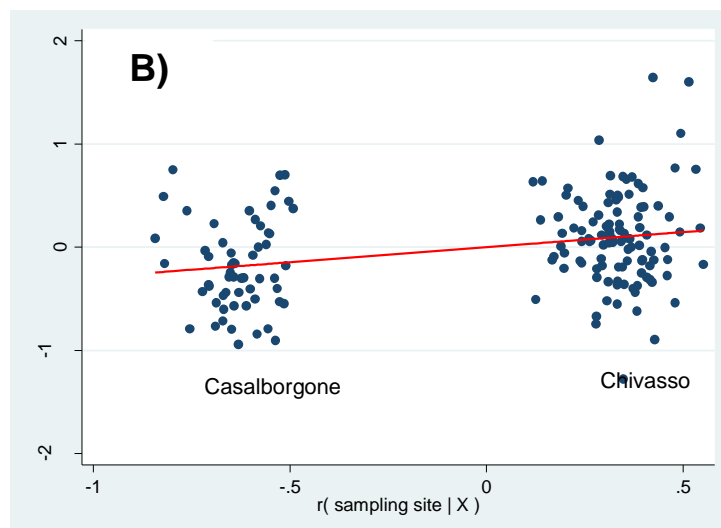
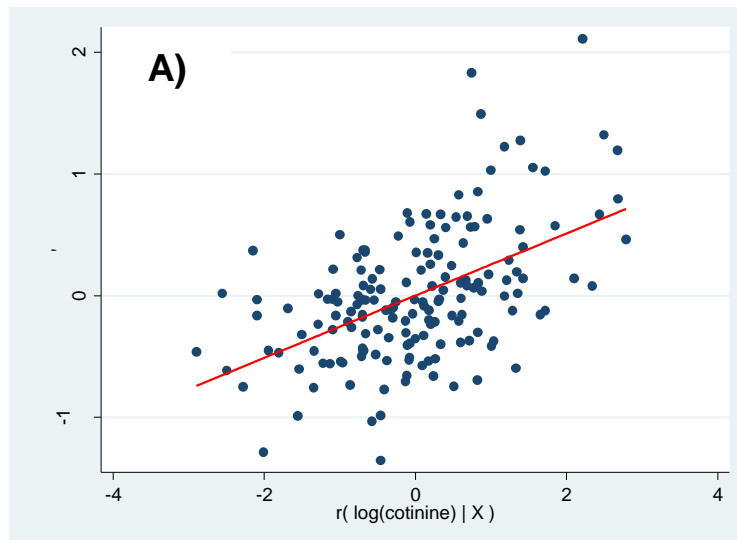


Figure 2



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

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

		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 $\pm$ s.d.	12.7 $\pm$ 0.8	12.5 $\pm$ 0.6	12.6 $\pm$ 0.8
WEIGHT	kg $\pm$ s.d.	47.3 $\pm$ 12.3	47.7 $\pm$ 12.2	47.5 $\pm$ 12.2
HEIGHT	cm $\pm$ s.d.	154.7 $\pm$ 8.9	153.0 $\pm$ 9.8	154.2 $\pm$ 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.

SAMPLING SITE	15-F <sub>2t</sub> Isoprostane (ng/mg crea)			<i>p</i>
	MEAN ± S.D.	MIN	MAX	
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<sub>2t</sub> 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<sub>2t</sub>-IsoP as dependent variable with link log and normal distribution assumption (non significant effects at 5% level are not reported).