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15-F2t Isoprostane as biomarker of oxidative stress induced by tobacco smoke and occupational exposition to formaldehyde in workers of plastic laminates

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1 **15-F_{2t} Isoprostane as biomarker of oxidative stress induced by tobacco**
2 **smoke and occupational exposition to formaldehyde**
3 **in workers of plastic laminates**

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36 **ABSTRACT**

37

38 **Background:** Formaldehyde (FA) is a suspected human carcinogen capable of inducing oxidative
39 stress through different metabolic ways. FA may origin from tobacco smoke, several environmental
40 sources, as well as occupational sources, like furnishing industries specialized in the production of
41 pressed-wood and laminate products.

42 **Object:** Our aim was to investigate the role of tobacco smoke and occupational **exposure** to air-FA
43 in the induction of oxidative stress status by comparing FA-exposed with non-exposed subjects who
44 smoked or did not.

45 **Methods:** Enrollment of 105 subjects was made in an industry of plastic laminates, including both
46 workers directly exposed to FA and non-exposed office personnel, as control group. 15-F_{2t}
47 Isoprostane (15-F_{2t} IsoP), detected by ELISA technique and urinary cotinine, detected by GC-MS,
48 were used for evaluating oxidative stress and tobacco smoke **exposure**, respectively. Air-FA levels
49 were detected by GC-MS.

50 **Results:** FA concentrations were significantly higher in subjects **occupationally** exposed than those
51 in controls. Smoking habits and air-FA **exposures** independently induce the formation of 15-F_{2t} IsoP
52 and increase the oxidative stress level.

53 **Conclusions:** Our findings show, for the first time, that 15-F_{2t} IsoP presents a dependency from
54 both the smoking habit and air-FA **exposures**, and consequently, that these breathable pollutants
55 could be considered as two important independent risk factors in increasing the oxidative stress in
56 human beings.

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61 **KEYWORDS**

62 15-F_{2t} Isoprostane, oxidative stress, formaldehyde, tobacco smoke

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64 **ABBREVIATION**

65 FA (formaldehyde), air-FA (professional exposure to formaldehyde), ELISA (enzyme-linked
66 immuno-sorbent assay), GC-MS (gas chromatography–mass spectrometry), IsoP (15-F_{2t}
67 Isoprostane), crea (creatinine).

68

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1. INTRODUCTION

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88 Oxidants, anti-oxidants and free radicals often play a useful role in cellular signalling, control of
89 vascular tones, cell generation, and defence against microorganisms. Their formation is the result of
90 an evolutive process. The oxidative balance can be disturbed by several adverse environmental
91 and/or occupational conditions, causing an uncompensated increase of pro-oxidants (Basu, 2010).
92 As a consequence, oxidative modification of cellular macromolecules, induction of cell death by
93 apoptosis or necrosis, damage of structural tissue may occur (Lykkesfeldt, 2007). Cells living under
94 aerobic conditions are continuously exposed to a large number of oxidizing compounds from
95 several endogenous and exogenous sources. Urban air is a typical exogenous mixture of chemical
96 compounds that can induce carcinogenic activity, oxidative stress and toxicity. For example, several
97 pollutants (PM, PAHs, benzene, etc.), emitted from cars (Rusconi et al., 2011) may: (i) inhibit cell-
98 mediated immunity toward infectious agents, (ii) exacerbate respiratory allergy, (iii) cause DNA
99 damage and (iv) induce lung cancer, after a long-term exposure (Rossner et al., 2008a, b). Many
100 epidemiologists related the presence of an oxidative stress status in human beings with traffic
101 emissions and air pollution (Rossner et al., 2011).

102 Formaldehyde (FA) is a breathable pollutant present in both living and working environments and
103 is considered the prevalent carbonyl specie in urban atmosphere. FA is emitted by several primary
104 sources (Bono et al., 2010a), it is formed in the troposphere by photochemical hydrocarbon-
105 oxidation processes (Correa et al., 2003; Flyvholm and Andersen, 1993; Salthammer et al., 2010),
106 and is also a component of tobacco smoke (Godish, 1989; Uchiyama et al., 2010). Production and
107 use of FA in the manufacture of resins, paints, disinfectants, preservatives, and a variety of other
108 chemicals or industrial products make this chemical compound potentially breathable in several
109 (indoor) working and living environments (Kelly et al., 1999; Zhang et al., 2010b). Thus, FA is

110 nowadays a relevant topic to be studied in the environmental and occupational health studies
111 (Nielsen and Wolkoff, 2010; Zhang et al., 2010b).

112 FA can induce local irritations, acute and chronic toxicity, and genotoxic and carcinogenic activity
113 (IARC, 2006; Schmid and Speit, 2007; Speit et al., 2007), as confirmed by an increased incidence
114 of nasopharyngeal cancer in some types of FA-exposed workers (Duhayon et al., 2008; Hauptmann
115 et al., 2004), the relationship reported between FA and leukemia (Zhang et al., 2010a; Zhang et al.,
116 2009), and a significant positive association between FA **exposure** and childhood asthma (McGwin
117 et al., 2010).

118 Rager et al. have recently suggested that FA alters the expression of 89 microRNA (miRNA)
119 profiles targeting mRNAs linked to numerous biological pathways, including those involved in the
120 inflammatory response (Rager et al., 2011). In some molecular epidemiology studies, oxidative
121 properties of FA have been reported in rats and humans (Bono et al., 2010b). Different metabolic
122 pathways, i.e., the production of FA detoxifying enzymes after FA **exposure**, appeared to be
123 involved. (Im et al., 2006; Kum et al., 2007). F₂-isoprostanes (F₂-IsoPs) are prostaglandin-like
124 bioactive compounds formed *in vivo* from the free radical-catalyzed peroxidation of essential fatty
125 acids. **F₂-IsoPs are stable, robust molecules and are detectable in all human tissues and biological**
126 **fluids analyzed, including plasma, urine, broncho-alveolar lavage fluid, cerebrospinal fluid, and**
127 **bile. Based on their mechanism of formation, four F₂-IsoP regioisomers are generated. Compounds**
128 **are denoted as 5-, 12-, 8-, or 15-series regioisomers depending on the carbon atom to which the side**
129 **chain hydroxyl is attached; thus the compound under investigation belongs to the last regioisomer.**
130 **Moreover, “2*t*” is for the – *trans* position of the oriented side chain to the prostane ring in the 15-**
131 **F_{2*t*}-IsoP (Roberts and Milne, 2009).**

132 **The metabolic fate of 15-F_{2*t*}-IsoP in humans has been assessed in previous studies (Mitsumoto et**
133 **al., 2008; Morrow et al., 1999; Roberts and Morrow, 2000). Findings of these studies show the**
134 **usefulness of 15-F_{2*t*}-IsoP to assess oxidant stress *in vivo* both in animal models and in humans.**

135 In fact, they appear to be related to quite a number of human diseases, although a clear correlation
136 between those pathological conditions and an oxidative stress status is far from being proven
137 (Giustarini et al., 2009). Moreover, F₂-IsoPs seem to play a role in acute and sub-clinical chronic
138 inflammations (Basu et al., 2009). Since F₂-IsoPs can be detected in urine samples, which sampling
139 is possible with a non-invasive procedure, they have been proposed as a suitable biomarker for
140 airways inflammation (Basu, 2008) and asthma diagnosis (Wedes et al., 2009), with the scientific
141 community agreement.

142 Considering that FA and tobacco smoke have toxic and cancerogenic activities in different
143 biological districts and may play a role in the onset of oxidative stress (Bono et al., 2010b; Campos
144 et al., 2011), the potentiality of urinary 15-F_{2t} IsoP as indicator of oxidative stress induced by FA
145 and tobacco smoke was investigated in this study, for the first time.

146 Healthy subjects, working in one of the world's leading manufacturers of decorative laminate
147 located in the north-western part of Italy, were enrolled as volunteers. Workers exposed to FA and
148 non-exposed office personnel, as control group, were statistically compared. To evaluate the FA
149 **exposure**, air-FA was quantified, while tobacco **exposure** was measured by urinary cotinine, a
150 metabolite of nicotine.

151

152 **2. MATERIAL AND METHODS**

153

154 *2.1 Epidemiological sample.*

155 51 healthy male workers of an industry of decorative laminates were recruited as subjects
156 potentially exposed to FA. The decorative laminate sheeting is made of melamine and phenolic
157 resins reacting with aldehydes during the thermosetting process. The resins are laminated onto
158 layers of kraft paper topped with a decorative sheet. Other 54 male subjects were enrolled as
159 controls from some offices and laboratories of the National Health System, where FA was not used.

160 All the subjects involved in this study live and work in Bra (town located in Piedmont region, Italy,
161 counting 28.000 inhabitants, 250 meters a.s.l.) or in its immediate surroundings. Only males were
162 selected for this epidemiological investigation, since gender is actually debated as confounding
163 factor in the 15-F_{2t} IsoP formation, moreover only male workers are usually employed in plastic
164 laminate industries. All subjects were informed about the objective of the study and gave a written
165 informed consent, voluntarily.

166 All samplings were executed during summer. For each subject, air-FA samples were passively
167 collected for an entire working shift (i.e., from 6 a.m. to 2 p.m.; about 8 h) in the middle of the
168 working week (Wednesday). A spot of the first urine in the morning was also collected for urinary
169 cotinine and 15-F_{2t} IsoP determinations.

170

171 2.2 Questionnaire.

172 At the end of the working shift, a questionnaire was administered to each subject to acquire
173 information about individual and clinical features (age, place of residence, hobbies, and therapies),
174 smoking habits, profession (qualifications, seniority, and job-specific work), the presence and use of
175 environmental and personal devices to prevent air exposure and health risks. More in detail, the
176 description of smoking habits for all subjects was established *a-priori*. Both subjects who never
177 smoked and smokers who had ceased smoking from at least 1 month were classified as “non-
178 smokers”, while subjects who smoked at least one cigarette per day were classified as “smokers”.

179

180 2.3 Air-FA sampling and analysis.

181 FA air sample was collected from each subject for the whole working shift (8 h) using a passive,
182 personal air sampler working with radial symmetry (Radiello®), clipped near the breathing zone of
183 the subject. Samplers were equipped with a specific sorbent tube containing 35–50 florisil mesh
184 coated with 2,4-dinitrophenylhydrazine (DNPH). DNPH reacts with FA yielding 2,4-

185 dinitrophenylhydrazone which was subsequently quantified by a GC-MS method, within the
186 following 10 days. Cartridges were stored at -80°C before GC-MS analysis. Each sample was
187 eluted with 3 ml of toluene and shaken at room temperature for 15 min. An aliquot was transferred
188 into a vial and then injected in a capillary Agilent Technologies 6890 gas chromatograph, interfaced
189 to a 5973 MSD Inert Agilent single quadrupole mass spectrometer. A Gerstel CIS4 PTV injection
190 system utilized an initial temperature of 65 °C followed by heating at 5 °C/s; with a final
191 temperature of 320 °C, held for 10 min. The injection volume was 2 µl in splitless mode. The
192 capillary column used was a HP-5MS 30m×0.25mm×0.25µm film thickness. Initial column
193 temperature was 70°C, and increased at 20°C/min up to 220°C and at 30°C/min up to 300°C. The
194 carrier gas was ultrapure He (1.0 ml/min). The transfer-line temperature was set at 280°C. The mass
195 spectrometer operated in electron impact and Selected Ion Monitoring (SIM) mode. The monitored
196 *m/z* values for FA were 63, 79, 180 and 210, while the ones for the internal standard
197 (isovaleraldehyde-DNPH) were 177, 206, 223 and 166. The FA calibration curve was built by
198 fortifying 3 ml of toluene so as to obtain a concentration range from 0.10 µg/ml to 10 µg/ml. The
199 fortified toluene was analyzed as for the samples.

200 The limit of detection (LOD) was calculated as the concentration of the analyte that gives a signal
201 equal to the average background of the blank (Sblank) plus three times its standard deviation (LOD
202 = Sblank + 3*SD Sblank), while the limit of quantification (LOQ) was estimated as twice of the
203 LOD value.

204 LOD and LOQ were respectively 0.05 µg/ml and 0.10 µg/ml. Coefficients of variation (CV%)
205 calculated to test repeatability were below 5%.

206

207 *2.4 Urine: collection and analyses.*

208 Two aliquots of fresh urine were collected in the early morning and approximately at the same time
209 from each volunteer, and stored at $-80\text{ }^{\circ}\text{C}$ prior to analysis, performed within 20 working days. One
210 aliquot of urine was used for cotinine quantification, the other one for 15-F_{2t} IsoP determination.

211 First, urinary creatinine (crea) was determined by the kinetic Jaffé procedure (Bartels and Cikes,
212 1969) so as to normalize the excretion rate of urinary cotinine and 15-F_{2t} IsoP.

213 Urinary cotinine was measured in order to consider the possible role played by tobacco smoke for
214 the subjects under study. 10 ml of urine was transferred into a glass tube and 4 g of NaCl, 500 μl of
215 NaOH (5M) and 10 μl of cotinine-d₃ (internal standard) were added. Subsequently, for two times, 2
216 ml of trichloromethane (CHCl_3) were added to the sample to perform liquid–liquid extraction which
217 was carried out in a shaking wheel for 15 min. Sample was then centrifuged for 10 min at 1000 g
218 and the resulting organic phase was collected in a new glass tube and evaporated to dryness in a
219 rotary evaporator at room temperature. The dry residue was reconstitute in 200 μl of CHCl_3 and
220 transferred into a conical vial for GC-MS determination (Bono et al., 2005). GC-MS analysis was
221 performed using an Agilent Technologies 6890 gas chromatograph, interfaced to a 5973 MSD Inert
222 Agilent mass spectrometer. A Gerstel CIS4 PTV injection system utilized an initial temperature of
223 $50\text{ }^{\circ}\text{C}$ followed by heating at $10\text{ }^{\circ}\text{C/s}$; with a final temperature of $300\text{ }^{\circ}\text{C}$, held for 10 min. The
224 injection volume was 1 μl in the split mode. The capillary column used was a HP-5MS
225 $30\text{m}\times 0.25\text{mm}\times 0.25\text{ }\mu\text{m}$ film thickness. The initial column temperature was $50\text{ }^{\circ}\text{C}$, increased at 15
226 $^{\circ}\text{C/min}$ up to $300\text{ }^{\circ}\text{C}$. The carrier gas was ultrapure Helium (1.0 ml/min). The transfer-line
227 temperature was set at $280\text{ }^{\circ}\text{C}$. The mass spectrometer operated in electron impact and SIM mode.

228 The monitored m/z values for cotinine were: 98, 118, 176; while the ones for the internal standard
229 were 101, 121, 179.

230 The cotinine calibration curve was built by fortifying a blank urine pool of non-smoking subjects, to
231 obtain a concentration range from 0.02 $\mu\text{g/ml}$ to 2 $\mu\text{g/ml}$. The fortified urine was extracted as for
232 the samples. LOD and LOQ (calculated as previously described) were respectively 0.01 $\mu\text{g/ml}$ and

233 0.02 µg/ml. Coefficients of variation calculated to test repeatability were below 5% for both
234 cotinine and the internal standard.

235 15-F_{2t} IsoP in urine was measured by ELISA technique performed with a specific microplate kit
236 (Oxford, MI, USA), according to manufacturer's instructions. The declared limit of detection is 0.2
237 ng/ml and cross-reactivity can likely occur for some other isoprostanes: prostaglandin E₂,
238 prostaglandin D₂, and arachidonic acid (< 0.01%), 9 α ,11 β prostaglandin F_{2 α} (4.1%), 13,14 dihydro-
239 15-keto-PGF_{2 α} (3.0%) and. Dilution 1:4 was adopted to achieve better accuracy in the competitive
240 ELISA method.

241 Because of the high percentage of 15-F_{2t} IsoP excreted in human urine conjugated to glucuronic
242 acid (over 50%), a preliminary incubation with β -glucuronidase for 2 h at 37°C was performed, in
243 order to detect the entire quantity of 15-F_{2t} IsoP present in each urine sample.

244

245 *2.5 Statistical analysis.*

246 The analysis was performed by means of Stata 12 Statistical Package (StataCorp LP, Lakeway
247 Drive, TX, USA). Table 1 shows some general epidemiological aspects of the resulting groups of
248 volunteers (according to their FA exposure and smoking habits).

249 Where suggested by distributional diagnostic plots (symmetry plot, quantile plot) and descriptive
250 statistic inspection (looking at variance stability among categories), appropriate linear
251 transformation was applied on data. Untransformed 15-F_{2t} IsoP , urinary cotinine and air-FA values
252 were compared among smoking and exposure categories by means of Nonparametric equality-of-
253 medians test.

254 Multiple regression analysis with robust standard error estimation was performed to assess the
255 relationship between urinary 15-F_{2t} IsoP values with urinary cotinine and air-FA concentrations. For
256 each category, means and 95% confidence intervals of transformed 15-F_{2t} IsoP values were

257 calculated as margins of responses from predictions of the complete fitted model integrating over
258 the covariates.

259

260 3. RESULTS

261

262 As reported in Table 1, subjects were classified according to their professional **exposure** to FA
263 (exposed and non-exposed), and their smoking habits (smokers and non-smokers). As reported
264 above, gender was not considered for describing the epidemiological sample population. Among the
265 non-exposed subjects, 14 out of 54 are smokers (26%), while the percentage of smokers into the
266 FA-exposed population grows up to 47%. The difference between the number of smokers into the
267 FA-exposed and non-exposed categories appears to be statistically significant.

268 Means and standard deviations for age, urinary cotinine, urinary 15-F_{2t} IsoP and air-FA levels are
269 reported in Table 2. Both the overall and single category values are reported.

270 From Table 2 outcomes, it is worth noting that the mean age of studied groups were comparable,
271 and any statistical differences were recorded excluding “age of the subjects” as possible
272 confounding factor. Considering the air-FA concentrations (ranged from 49 to 444 $\mu\text{g}/\text{m}^3$ for FA-
273 exposed subjects, and from 16 to 110 $\mu\text{g}/\text{m}^3$ for the control group), three FA-exposed workers
274 presented air-FA concentrations greater than 370 $\mu\text{g}/\text{m}^3$, namely the absolute **exposure** limit that
275 should not be exceeded at any time (TLV-ceiling), suggested by the American Conference
276 Governmental Industrial Hygienists (ACGIH). Moreover, the air-FA concentrations for the FA-
277 exposed workers appear to be significantly higher at 5% probability than those for the non-exposed
278 subjects (Fisher exact $p < 0,0001$). Conversely, urinary cotinine concentrations appear to be
279 significantly higher for smokers than those for non-smokers (Fisher exact $p < 0,0001$). Finally,
280 considered the four kind of **exposures**, 15-F_{2t} IsoP levels appear to be higher for FA-exposed
281 workers and smokers than those for non-exposed subjects and non-smokers, respectively; instead,

282 15-F_{2t} IsoP in the two groups having only one exposure shows intermediate mean levels (Fisher
283 exact $p < 0,0001$).

284 Due to non-normal distribution and heteroskedasticity (the variance increases linearly with the
285 mean), for further analysis appropriate log-transformation was applied to 15-F_{2t} IsoP levels, air-FA
286 and cotinine concentrations, in order to stabilize the variance and normalize the distributions.

287 Table 3 shows the outcomes for the linear regression analyses performed considering the
288 transformed 15-F_{2t} IsoP levels *versus* transformed air-FA and urinary cotinine concentrations, first
289 singly (Mod.1 and Mod.2, respectively), and then in association between them (Mod.3).

290 Despite the higher air-FA values for smokers than those for non-smokers, there are no significant
291 linear relationship between urinary cotinine and air-FA concentrations ($p = 0.24$; $R = 0.04$; $B = 0.018$;
292 C.I. 95% = -0.054, 0.09). The standardized regression coefficient (Beta) show the smoking
293 contribution to IsoP biosynthesis as equal in subjects exposed and not exposed to FA; in particular,
294 each increase of one S.D. unit both for Log-FA and Log-cotinine induces an Log-IsoP increase of
295 approximately 35%.

296 15-F_{2t} IsoP levels increase significantly with both air-FA and urinary cotinine concentrations. The
297 complete model confirms the multivariate relationship without substantial confounding effects. No
298 age main effect significant at 5% level and no significant at 5% level interactions were detected.

299 Table 4 reports the means and 95% confidence intervals of 15-F_{2t} IsoP levels for all the studied
300 categories, as estimated by the multiple regression analysis. The smoking effect is not significantly
301 different from the FA exposure effect. In addition, the two effects seem to be less than additive in
302 logarithmic scale (2.3 *versus* 3.3 expected, $p < 0.05$).

303 Figure 1 shows the added-variable plots - or partial regression plot - in which the effect of adding
304 one variable (FA or tobacco smoke exposure) to the model containing the other one can be
305 evaluated. Although both regression lines are significantly different from 0, the residuals are very
306 scattered around them, suggesting (as confirmed by the low R^2 value) that the model is able to

307 predict only part (20%) of the large 15-F_{2t} IsoP variability. Evident outliers (influential position or
308 evident lack of residual normality distribution) from the regression lines are not evident.

309

310 4. DISCUSSION

311

312 In this study we investigated the relationship occurring between human **exposure** to FA and
313 oxidative stress - measured by urinary 15-F_{2t} IsoP - in a population of male subjects. These subjects
314 were recruited in a laminate industry in Piedmont region (Italy) and information about FA and
315 tobacco smoke **exposures** was collected. They were classified as FA-exposed and non-exposed
316 (control group) subjects and as smokers or non-smokers too.

317 If compared with controls, FA-exposed workers reported significantly higher concentrations of air-
318 FA, although the mean concentration was lower than the ACGIH limit (370 µg/m³) (Table 2). Good
319 environmental work conditions and health status suggest that these high values of air-FA could
320 essentially depend on improper work behaviour by those workers, rather than improper working
321 conditions. On the other hand, non-exposed subjects presented an average air-FA concentration
322 equal to 40 µg/m³ (Table 2) and 10% of these subjects even exceed 70 µg/m³ concentration,
323 suggesting that other non-controlled domestic or environmental FA **exposures** may influence the
324 results.

325 As previously mentioned, urinary 15-F_{2t} IsoP were investigated as indicators of an oxidative stress
326 status due to FA and tobacco smoke **exposure**. The presence of aldehydes by lipid peroxydation
327 (LPO) processes is one of the factors related to the “physiological” oxidative stress status (Qujeq et
328 al., 2004; Sohal et al., 2002). FA **exposure** can increase the oxidative stress status with further
329 release of non-specific cellular membrane inflammation mediators, especially at the upper
330 respiratory tract (Bartsch and Nair, 2006). In the last decade, many experimental evidences
331 suggested that cellular damage, mediated by reactive species, may play a crucial role in the

332 pathogenesis of respiratory disorders (Wedes et al., 2009; Yang and Omaye, 2009) - that cover a
333 wide range of conditions: from simple acute effects due to tobacco smoke **exposure** in healthy
334 subjects to chronic damages characterized by interstitial lung destruction (emphysema) or
335 irreversible thickening.

336 The outcomes of this study globally show the effectiveness of urinary 15-F_{2t} IsoP in describing the
337 *in vivo* **exposure** to air-FA (Table 3 and Figure 1a). Age usually does not linearly correlate with 15-
338 F_{2t} IsoP levels (Basu et al., 2009) and, within the studied population, this tendency appeared to be
339 confirmed. The influence of tobacco smoke habits on 15-F_{2t} IsoP formation was also reported - as
340 previously described by Campos (Campos et al., 2011) – although it appeared to be independent
341 from the FA **exposure** effect (Table 3 and Figure 1b). Indeed, no statistically significant interaction
342 was detected between FA and tobacco smoke **exposures**, at least in the logarithmic scale. In
343 addition to FA, cigarette smoke contains quite a number of different carcinogenic compounds and
344 reactive oxygen species (ROS) - due to tobacco pyrolysis - that potentially can lead to an oxidative
345 stress status (Bhalla et al., 2009; Faux et al., 2009). Furthermore, FA and tobacco smoke **exposures**
346 seem not to have an additive effect in logarithmic scale (multiplicative in natural scale) on the 15-
347 F_{2t} IsoP formation and not to play a reciprocal confounding effect.

348 It is important to remark that urinary 15-F_{2t} IsoP is a non-specific oxidative stress indicator, and
349 therefore, other exogenous *stimuli*, other than FA and tobacco smoke, may influence their urinary
350 levels, as the wide dispersion of the residuals around the regression line (Figure 1 a and b) and by
351 the relatively low values of determination coefficient (<0,20 in the complete model) seem to
352 suggest.

353 The means of 15-F_{2t} IsoP, as predicted by the multiple regression model (as shown in Table 4), are
354 similar for FA-exposed/non-smokers and non-exposed/smokers, but the means of 15-F_{2t} IsoP for
355 subjects exposed both to FA and tobacco smoke (FA-exposed/smokers) are less than expected for
356 the additive model.

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5. CONCLUSION

In this study, urinary 15-F_{2t} IsoP levels were measured as indicator of oxidative stress status induced by FA and tobacco smoke **exposures**. Several endogenous/biological and exogenous/environmental or occupational factors, such as FA and tobacco smoke **exposures** - here investigated - affect the onset of oxidative stress and probably act synergistically in different ways. In this study a relationship between urinary 15-F_{2t} IsoP levels with FA and urinary cotinine concentrations has been reported, suggesting that primary prevention towards professional (and environmental) **exposure** to FA and tobacco smoke plays an important role for health care, as well as primary prevention towards several pathogenic conditions.

Finally, an enzyme-linked immunoassay procedure was adopted and proved to be appropriate for the purpose of this study. Comparison of the ELISA procedure with a GC-NCI-MS protocol for 15-F_{2t} IsoP determination can be considered as a perspective goal.

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551 **Caption Figure 1:** Partial regression plot: regression of Log (15-F2t IsoP) as dependent vs. (a) Log
552 (Air-Fa) and (b) Log (Cotinine) as predictive variables.

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