Urinary Excretion of 1-Hydroxypyrrene as a Marker for Exposure to Urban Air Levels of Polycyclic Aromatic Hydrocarbons

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Abstract

A cross-sectional study was conducted among 94 traffic police officers from the Municipality Police of Genoa, Italy, exposed to airborne pollutants and 52 referent subjects exposed to indoor air pollution levels to investigate the relationships between exposure to ambient air polycyclic aromatic hydrocarbons (PAHs) and urinary excretion of 1-hydroxypyrrene (1-OH-P). The effects of smoking, lifestyle factors such as exposure to ETS, and diet, along with the role played by the cytochrome P450IA1 (CYP1A1), and glutathione S-transferase M1 and δ metabolic susceptibility gene polymorphisms were examined. The geometric mean of benzo(a)pyrene air measurements (an index compound of PAH levels) was 70 times higher in traffic police officers (3.67 ng/m³) than in referents (0.05 ng/m³). The urinary concentration of 1-OH-P was clearly associated with cigarette smoking and, to a lesser extent, with exposure to ETS and particulate PAH pollution. No association was detected between 1-OH-P excretion and diet. Women exhibited a higher excretion level than did men, and an apparent effect of age was due to differences in cigarette smoking habits. Exposure to PAHs resulted in higher levels of 1-OH-P excretion in all groups except heavy smokers. Overall, no significant role of any metabolic polymorphism was detected. However, stratification of study subjects according to their smoking habits revealed higher levels of excretion of 1-OH-P in subjects smoking $\leq$15 cigarettes/day carrying the CYP1A1 polymorphism. No such effect was seen either with nonsmokers or with people smoking more than 15 cigarettes/day. These findings are suggestive of a gene-environment interaction, in which subjects with the CYP1A1 polymorphism, relative to subjects without it, have higher levels of 1-OH-P in their urine at low doses of exposure to PAHs.

Introduction

Human data suggesting that living in large urban settings may increase lung cancer risk were first reported during the 1950s by Stocks and Campbell (1), who detected a 2-fold increased mortality from lung cancer in nonsmoking individuals from Liverpool compared to residents of rural North Wales (2). Since then, what has been called the “urban effect” has been reported by cohort and case-control studies conducted among British, Chinese, Greek, Finnish, Japanese, Irish, Polish, Swedish, and USA residents (3, 4), by cancer registry-based incidence data (5, 6), and by human studies investigating the relationships between outdoor pollution monitoring data and delayed health effects (7–13). The available epidemiological data indicate urban/rural or high/low pollution areas lung cancer rate ratios on the order of 1.5 or lower (range, 1.0–2.3).

Incomplete combustion of fossil fuels in transportation, residential heating, power generation, and manufacturing contribute significantly to ambient air pollution, a complex mixture of compounds in the gas, vapor, and particulate phases. Fine particulate air pollution contains solid carbon cores that aggregate into particles with an aerodynamic diameter below 2.5 μm and absorb organic compounds, including carcinogenic agents, such as PAHs and nitro-PAHs (14, 15).

Urinary 1-OH-P, a metabolite of pyrene estimating the total uptake of PAHs, has been proposed as a biological marker of individual internal dose (16). Increased urinary levels of 1-OH-P have been reported in human biomonitoring studies, including subjects occupationally exposed to coal tar fumes, mineral oil and bitumen, and coal tar-based ointment (17–25). Urinary 1-OH-P correlates with high PAH levels in occupational settings (18, 23), and it is clearly influenced by cigarette smoking habits under conditions of low exposure (25–27). The relative contribution of different sources to daily pyrene intake has been estimated in human volunteers nonoccupationally exposed to PAHs (26). Mainstream smoke and foods containing PAHs accounted for some 99% of the total pyrene intake, while ETS and indoor/outdoor ambient air contribution was insignificant. The variation levels of urinary 1-OH-P explained by
smoking and foods were 66% and 2%, respectively, reflecting a poor estimation of pyrene intake or a relevant role of unaccounted-for lifestyle and genetic factors in 1-OH-P excretion. Interindividual variability in metabolic activation and detoxification may affect urinary 1-OH-P, the amount of PAH-diol epoxides that are delivered to target tissue, and possibly the cation may affect urinary I-OH-P, the amount of PAH-diol adducts in humans, and their allelic variants have been associated with increased lung and bladder cancer risks in cigarette-smoking subjects (27–31) and elevated PAHs-DNA adducts in humans (32, 33). GSTT1 conjugates potential carcinogens, and its polymorphic distribution in humans may contribute to the modification of cancer risk (34). Studies on its relationship with lung cancer risk failed to show a polymorphism-linked modification of the risk of lung cancer (35–37).

To evaluate the role of ambient air pollution in human exposure to PAHs, we conducted a cross-sectional study among traffic police officers and referent subjects working and living in Genoa, Italy. Individual exposure to selected PAHs [i.e., B(a)P, B(ghi)P, and B(k)F], experienced during a typical working shift and urinary excretion of 1-OH-P were determined in exposed and referent subjects to investigate the relationship between exposure to ambient air PAHs and urinary 1-OH-P while accounting for the effects of cigarette smoking and other lifestyle factors. All subjects were genotyped with respect to CYP1A1 (MspI RFLP), GSTM1, and GSTT1 to investigate the influence of genetic polymorphisms in the urinary excretion of 1-OH-pyrene.

Subjects and Methods

The present study was carried out among 94 traffic police officers from the Municipality Police of Genoa, Italy, exposed to ambient air pollutants from mobile sources (i.e., traffic) and 52 referent subjects employed at the National Institute for Research on Cancer of Genoa, exposed to indoor air pollution levels. All subjects were informed on the research aims and were asked to signed an informed consent form prior to their inclusion in the study. The characteristics of the study population are reported in Table 1. Exposed and referent subjects were frequency matched by age (±2 years), and a standardized questionnaire form was used to collect data on current smoking habits (including brand and number of cigarettes smoked per day); hours spent in ETS-polluted spaces; illnesses and medical treatments; consumption of broiled and grilled food, alcohol, coffee, fresh fruit, and vegetables; occupational history; and other demographic variables. Four to six subjects per week were enrolled in the study between July 1993 and June 1994. All subjects who worked from Monday through Wednesday were included in the study. They were asked to wear air samplers on Thursday morning during a typical 5-h working shift (between 7:00 am and 12:00 am) and to collect postshift morning urine in polyethylene containers. Questionnaire forms, air samplers, and urine tubes were transported to the National Institute for Cancer Research, where the urine samples were frozen and kept in the dark until analysis. Each environmental and biological sample was uniquely identified using a four-digit randomly generated code to mask the sample’s exposure-referent status. One measurement of 1-OH-P was made on each participant’s urine sample. The study was part of a larger coordinated research project involving 13 laboratories from eight European countries aimed at developing and evaluating biomonitoring procedures for human populations exposed to environmental pollutants (38).

Determination of Ambient Air Level of PAHs

Sampling. Suspended airborne particles, to which PAHs are adsorbed, were collected by personal samplers (Gilian Instrument Corp., Caldwell, NJ). Samplers were calibrated to a constant flow (3 ± 0.15 liters/min) and air velocity (1.25 m/s). Each sampler was fixed to a police officer’s belt and the sampling head was fixed to the uniform lapel, as close as possible to the mouth. Glass fiber filters (type A/E, diameter 37 mm; Gelman Science, Ann Arbor, MI), previously fired at 400°C for 60 min to remove organic contaminants, were used. Collected filters were placed in glass Petri capsules, protected from light, and stored at −20°C until analysis.

PAH Analyses. Four ml of toluene (Merck, Darmstadt, Germany) were added to each loaded filter, which was placed in a glass beaker and sonicated for 15 min. Solvent was collected, and 3 ml of toluene were added to the filter for a second sonication. The two toluene fractions were pooled and concentrated by mild heating (45°C), under a nitrogen flow, to about 1 ml. Toluene was quantitatively transferred to a column filled with 3 ml of silica (Bond Elut SI, Analyticchem International, Harbor City, CA) almost to dryness, by a gentle nitrogen flow. The residue was dissolved by 100 μl of methanol (HPLC grade). With a 50-μl loop, the purified extract was injected in a C18 reverse-phase column thermostated at 30°C (length, 15 cm; internal diameter, 4.6 mm; Supelcosil LC-PAH; Supelco Inc., Bellefonte, IA). The elution was done by gradient of methanol and water: from 0 to 2 min, methanol 70%; from 2 to 10 min, methanol from 70 to 100%; and from 10 to 30 min, methanol 100%. Solvent flow, 1 ml/min. The PAH analysis was carried out by a fluorescence detector (excitation, 340 nm; emission, 425 nm; LS 1, Perkin-Elmer Corp., Norwalk, CT).

Quality Controls. Quantitative analysis was carried out by external standards, using a certified PAH mixture by Supelco (Supelpreme PAH Mix, Supelco, Inc., Bellefonte, IA) as a standard. The method recovery was evaluated by the addition method using four airborne samples simultaneously collected in the same place by four personal samplers. The calculated recoveries for B(a)P, B(k)F, and B(b)F were, respectively: 99%, 89%, and 93%. The accuracy and precision for B(a)P were, respectively: ±10% and ±5%.

Determination of Urinary 1-OH-P

Urine samples were analyzed according to the method developed by Jongeneelen et al. (16).

Chemicals. 1-OH-P was synthesized by Janssen (Beerse, Belgium). β-Glucuronidase and arylsulfatase was obtained from Boehringer Mannheim (Mannheim, Germany). HPLC-methanol was from Fluka (Buchs, Switzerland). A creatinine standard (100 mg/ml) was obtained from Beckman (Brea, CA).

Enzymatic Hydrolysis. An aliquot of urine (10 ml) was added to 0.1 m acetic buffer (pH 5.0) to a total volume of 30 ml and adjusted to pH 5.0 with 2 m hydrochloric acid. This mixture was incubated overnight with 12.5 μl of β-glucuronidase and arylsulfatase at 37°C.

Extraction Procedure. A sample enrichment and purification cartridge packed with C18 reverse-phase liquid chromatographic material (Sep-Pak C18 cartridge; Waters, Milford, MA) was used for extraction of the metabolites on a Waters MilliLab workstation. The cartridge was primed with 5 ml of methanol,
followed by 10 ml of distilled water, and the hydrolysed sample was passed through the cartridge at approximately 10 ml/min. The cartridge was subsequently washed with 8 ml of distilled water. The retained metabolites were eluted with 4 ml of methanol.

**Calibration Procedure.** Samples of hydrolysed urine of non-exposed persons (blank urine) were spiked with the given analyte. These calibration samples were treated as described above. At least five different concentrations across the working range were measured. Reagent blanks were used to monitor for interference.

**Reverse-Phase HPLC Analysis.** The analysis was conducted on a HPLC (Waters) gradient system controlled by a microprocessor program, Millennium 2010 Chromatography Manager (Waters). A fully automated sample injector was filled under slight pressure with a 50-μl sample from sealed 4-ml vials and subsequently injected on a C18 Novapack column (5 μm 150 × 3.9 mm; Millipore, Milford, MA). The column temperature was 40°C, and the flow rate 0.8 ml/min. The solvent gradient was as follows: 5 min in methanol-water at a 46:54 ratio; a linear gradient for 35 min to a methanol:water ratio of 94:6; and holding phase for 15 min, followed by 14-min equilibration. The chromatograph was equipped with a fluorescence detector (Type LC 240, Perkin-Elmer Corp., Beaconsfield, United Kingdom). The excitation was set to 242 nm. Peak areas were used for quantification, and all 1-OH-P areas were correlated to their respective creatinine levels.

**Creatinine in Urine.** Every urine sample was analyzed for creatinine level. This was measured on a Beckman optical reader with sodium picrate and related to the reading of the Beckman creatinine standard.

**Genotype Analyses**

Both GSTM1 and GSTT1 were coamplified together with CYP1A1, with CYP1A1 acting as a control for amplification in the event of GST-null genotypes. Amplification of CYP1A1 and GSTM1 sequences from genomic WBC DNA was accomplished in a reaction that consisted of an initial melt (94°C for 5 min) and 35 cycles of melting (94°C for 1 min), annealing (55°C for 1 min), and synthesis (72°C for 1 min) with a final extension (72°C for 7 min). The reaction mixture (100 μl) contained human genomic DNA (100 ng), primers for CYP1A1 and GSTM1 (0.15 μm each: 5'-TAGGAGTCTTGTTGTCATCTAC-3' and 5'-CAGTGAAGGGTGTTAGCGCCGT-3' for CYP1A1, and 5'-GCCATCTTGTGCTACATGCCC-3' and 5'-GCACTGCTAAGACACCTCACG-3' for GSTM1), Tris-HCl (10 mM; pH 8.3), KCl (50 mM), MgCl₂ (1.25 mM), deoxyribonucleotide triphosphates (200 μM), and Taq polymerase (2.5 units; Perkin-Elmer Corp.). For GSTT1, the amplification reaction consisted of an initial melt (94°C for 6 min) and 35 cycles of melting (94°C for 1 min), annealing (60°C for 1 min), and synthesis (72°C for 1 min), with a final extension (72°C for 10 min). The reaction mixture (100 μl) contained human genomic DNA (100 ng), primers for GSTT1 (0.15 μm each: 5'-TTCCCTACTGTGCTCCATCTC-3' and 5'-TCACG-GATCATGCGACAGCA-3') and for CYP1A1 (see above), Tris-HCl (10 mM; pH 8.3), KCl (50 mM), MgCl₂ (1.25 mM), deoxyribo- nucleotide triphosphates (200 μM), and Taq polymerase (1 unit; Perkin-Elmer Corp.). Following amplification, a portion of the reaction mixture (40 μl) was subjected to electrophoresis in agarose gels (2%). In the case of GSTM1 functional homozygotes, two bands were expected (694 bp for GSTM1 and 340 bp for CYP1A1) of approximately equal intensity; for heterozygotes, two bands were also expected (694 and 340 bp), of which the 694-bp band was approximately half the intensity of the 340-bp band; the null phenotype was signaled by only one band (340 bp). Another portion of the reaction mixture (40 μl) was subjected to restriction analysis [1 unit of MspI, 50 mM NaCl, 10 mM Tris-HCl (pH 7.9), 10 mM MgCl₂, 1 mM DTT, and 37°C, for 2 h] using agarose gel (1%) electrophoresis. For the CYP1A1 polymorphism, the major MspI allele was the unrestricted 340-bp band; the minor allele was signaled by cleavage products (130 and 210 bp). For GSTT1, all lanes contained the unrestricted 340-bp CYP1A1 control band. The GSTT1 functional homozygote was characterized by a strong band (500 bp);
### Table 2  Mean concentrations of 1-hydroxypyrene (μmol/mol creatinine) and their SD in the urine from referent subjects and traffic police officers by selected covariates

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Referents</th>
<th>Police officers</th>
<th>All subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Mean (SD)</td>
<td>No.</td>
</tr>
<tr>
<td>Exposure to PAHs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking habits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>32</td>
<td>0.067 (0.050)</td>
<td>57</td>
</tr>
<tr>
<td>≤15 cigarettes/day</td>
<td>7</td>
<td>0.130 (0.107)*</td>
<td>20</td>
</tr>
<tr>
<td>&gt;15 cigarettes/day</td>
<td>4</td>
<td>0.265 (0.148)*</td>
<td>12</td>
</tr>
<tr>
<td>Environmental tobacco smoke (nonsmokers only)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>22</td>
<td>0.063 (0.569)</td>
<td>8</td>
</tr>
<tr>
<td>≤4 h/day</td>
<td>8</td>
<td>0.068 (0.032)</td>
<td>26</td>
</tr>
<tr>
<td>&gt;4 h/day</td>
<td>2</td>
<td>0.095 (0.021)</td>
<td>23</td>
</tr>
<tr>
<td>Host factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females*</td>
<td>16</td>
<td>0.106 (0.110)</td>
<td>9</td>
</tr>
<tr>
<td>Males</td>
<td>27</td>
<td>0.082 (0.057)</td>
<td>80</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>21</td>
<td>0.102 (0.086)</td>
<td>46</td>
</tr>
<tr>
<td>≥35 years*</td>
<td>22</td>
<td>0.092 (0.102)</td>
<td>43</td>
</tr>
<tr>
<td>CYP1A1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major</td>
<td>33</td>
<td>0.109 (0.101)</td>
<td>69</td>
</tr>
<tr>
<td>Minor*</td>
<td>10</td>
<td>0.061 (0.038)</td>
<td>20</td>
</tr>
<tr>
<td>GSTM1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Functional</td>
<td>20</td>
<td>0.121 (0.124)</td>
<td>46</td>
</tr>
<tr>
<td>Null</td>
<td>23</td>
<td>0.083 (0.054)</td>
<td>43</td>
</tr>
<tr>
<td>GSTT1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Functional</td>
<td>34</td>
<td>0.100 (0.098)</td>
<td>69</td>
</tr>
<tr>
<td>Null</td>
<td>9</td>
<td>0.101 (0.084)</td>
<td>20</td>
</tr>
<tr>
<td>Seasonality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>June through July</td>
<td>10</td>
<td>0.096 (0.130)</td>
<td>21</td>
</tr>
<tr>
<td>October through November</td>
<td>11</td>
<td>0.084 (0.056)</td>
<td>25</td>
</tr>
<tr>
<td>January through March</td>
<td>14</td>
<td>0.109 (0.102)</td>
<td>28</td>
</tr>
<tr>
<td>April through May</td>
<td>8</td>
<td>0.088 (0.067)</td>
<td>15</td>
</tr>
<tr>
<td>All subjects</td>
<td>43</td>
<td>0.097 (0.094)</td>
<td>89</td>
</tr>
</tbody>
</table>

*Subjects with complete data for all covariates.

*Statistically significant difference (P < 0.05) between covariate levels according to the t test (for dichotomous covariates) and the Duncan multiple-range test (for polychotomous covariates) performed on log-transformed data.

*Significantly higher level (P < 0.05) in traffic police officers compared to referents.

*Homozygous and heterozygous genotypes combined.

*Significantly higher than June through July and October through November.

*Significantly higher than October through November.

*Heterozygote had a weak band of the same size, and no band appeared for the null.

### Statistical Analysis

Ambient air levels of PAHs and urinary levels of 1-OH-P were log transformed to meet normality assumptions to contrast mean values in police officers and referents and to investigate the role of categorical and ordinal covariates. Arithmetic and geometric mean values and their SD, median values, and the range of observed values are reported as measures of the data central location and dispersion. Univariate statistical analyses are based on the t test and the Duncan’s multiple range test statistics for dichotomous and polychotomous covariates, respectively. The latter test is used to account for the effect of multiple comparisons on the type I error (i.e., the α level). Simple regression analysis was used to investigate the relationships between continuous covariates and the urinary excretion of log-transformed 1-OH-P. Multiple regression analysis was applied to investigate the relative effect of single covariates and their potential statistical interactions in determining the urinary level of log-transformed 1-OH-P. The use of log-transformed data as response variables in multiple regression analysis entails the application of a multiplicative model to explain the observed variability (39). In this framework, the following regression model has been generated:

\[
\log(1\text{-OH-pyrene}) = \beta_0 + \beta_1 X_1 + \cdots + \beta_p X_p
\]

where \(X_s\) are the explanatory covariates and their possible interaction terms and \(\beta_s\) are unknown regression parameters modulating their effects on the response variable to be estimated from data by least-squares method. Continuous explanatory covariates were categorized according to the 50th percentile of the frequency distribution of age (years), exposure to ETS (h/day), and cigarettes smoked per day and the 33.3rd and 66.6th percentiles of B(a)P air level. For each explanatory covariate, a set of dummy variables was created to represent the contrast between the reference and the other levels taken by the explanatory covariates. In this setting, the exponentiation of the estimated regression parameters (i.e., \(e^{\beta_s}\)) gives rise to the statistical index MR. The MR is the ratio of the expected median value of the response variable for a given level of a
given explanatory covariate \((X_i)\), the expected median value in the reference level of that covariate adjusted for the confounding effect of other covariates included in the regression model. The exponentiation of the intercept term \(\beta_0\) is the expected median value taken by the response variable when all of the covariates included in the model are set to the reference level (39). The statistical significance of the explanatory covariates was tested according to the \(F\) distribution. Plots of residual, leverage, and influence measures were used as diagnostic quantities to evaluate goodness of fit of models to data (40). The statistical analyses were performed with the SPSS for Windows 95 statistical package (41) and the Generalized Linear Model (GLIM) statistical software (42).

### Results

Age and sex distribution of the study subjects; their smoking habits, including the number of cigarettes smoked per day; the frequency distributions for CYP1A1, GSTM1, and GSTT1 genetic polymorphisms; and measures of central location and frequency distributions for CYP1A1, GSTM1, and GSTT1 genetic polymorphisms; and measures of central location and frequency distributions for CYP1A1, GSTM1, and GSTT1 metabolic polymorphisms.

The concentrations of 1-OH-P detected in urine collected at the end of a typical morning work shift from referents and police officers were shown in Fig. 2 and in Table 2 according to selected covariates. Univariate statistical analyses performed on log-transformed data showed that urinary 1-OH-P excretion in traffic police workers (0.140 μmol/mol creatinine; SD, 0.152) was increased nonsignificantly compared to referent subjects (0.097 μmol/mol creatinine; SD, 0.094).

Significantly increased levels of urinary 1-OH-P were observed in cigarette-smoking subjects compared to nonsmokers in both the police officers and referents \((P < 0.05)\). As it is shown in Table 2, 2-fold and 4-fold higher 1-OH-P urinary excretions were reported for police officers and referents who smoked \(\leq 15\) and \(>15\) cigarettes/day compared to nonsmokers. Investigation of the nature of the relationship between 1-OH-P excretion and the number of cigarettes smoked per day (Fig. 3) showed that the number of cigarettes smoked is a good predictor of 1-OH-P urinary levels in police officers and referents. The proportion of variation of 1-OH-P explained by cigarettes

### Table 3

<table>
<thead>
<tr>
<th>Covariate</th>
<th>MR</th>
<th>95% CI</th>
<th>(F) test</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.062*</td>
<td>0.035-0.108</td>
<td>95.17</td>
<td>0.001</td>
</tr>
<tr>
<td>B(a)P (ng/m3)</td>
<td></td>
<td></td>
<td>1.42</td>
<td>0.247</td>
</tr>
<tr>
<td>(&lt;0.061)</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0.61-3.50)</td>
<td>1.096</td>
<td>0.679-1.768</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&gt;3.50)</td>
<td>1.425</td>
<td>0.902-2.252</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking habits</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>1.000</td>
<td></td>
<td>7.04</td>
<td>0.001</td>
</tr>
<tr>
<td>(\leq 15) cig/day</td>
<td>1.840</td>
<td>1.240-2.730</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&gt;15) cig/day</td>
<td>2.053</td>
<td>1.260-3.345</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Environmental tobacco smoke</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unexposed</td>
<td>1.000</td>
<td></td>
<td>2.42</td>
<td>0.094</td>
</tr>
<tr>
<td>(\leq 4) h/day</td>
<td>1.473</td>
<td>0.949-2.287</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&gt;4) h/day</td>
<td>1.691</td>
<td>1.049-2.726</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seasonality</td>
<td></td>
<td></td>
<td>4.60</td>
<td>0.012</td>
</tr>
<tr>
<td>June through November</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April through May</td>
<td>1.485</td>
<td>0.904-2.440</td>
<td></td>
<td></td>
</tr>
<tr>
<td>January through March</td>
<td>1.795</td>
<td>1.223-2.634</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\* MR, median ratio; 95% CI, 95% confidence interval for MR; overall \(F\) test = 3.35 \((P < 0.001)\); \(R^2 = 0.32\); \(F\) test, significance test; \(P\), significance level of the \(F\) test.

\* Estimated baseline median value.

\* 33.3rd and 66.6th percentiles of B(a)P air concentrations.

\* June through July and October through November urine samples (see Table 2) were considered as reference strata.
smoked per day was much higher in referents ($R^2 = 0.31; P = 0.0001$) than in police officers ($R^2 = 0.06; P = 0.01$).

Urinary 1-OH-P levels were slightly increased in referent subjects exposed to ETS (0.143 μmol/mol creatinine) compared to ETS nonexposed referents (Table 2). ETS showed no effect in police officers (Table 2). 1-OH-P levels in nonsmoking and ETS nonexposed police officers were similar to those detected in urine of nonsmoking referents exposed to ETS for more than 4 h a day.

Urinary excretion of 1-OH-P was higher in female than in male subjects (Table 2). The observed difference was statistically significant ($P < 0.05$) when female police officers (0.293 μmol/mol creatinine; SD, 0.0307) were compared to male officers (0.124 μmol/mol creatinine; SD, 0.116) and to female referents (0.106 μmol/mol creatinine; SD, 0.110). Among older subjects (i.e., ≥35 years), police officers showed a higher excretion of 1-OH-P (0.188 μmol/mol creatinine; SD, 0.183) compared to referent subjects (0.092 μmol/mol creatinine; SD, 0.102). This difference reflects the higher proportion of current cigarette smokers among police officers (48%) than among referents (34%) ages 35 or above.

The CYP1A1 MspI, GSTM1, and GSTT1 metabolic polymorphisms were not found to be significantly associated with the urinary excretion of 1-OH-P when investigated within the whole study population and the two study groups (Table 2). However, when the subjects were stratified with respect to their smoking habits (Fig. 4), subjects (in this case, police officers) carrying the CYP1A1 MspI polymorphism exhibited higher levels of excretion of the metabolite if they smoked ≤15 cigarettes/day. No such effect was seen either with nonsmokers or with people exposed to high doses of cigarette smoke (i.e., more than 15 cigarettes/day). A similar but less pronounced effect was seen in the total population, but there were not enough data to examine this phenomenon in the referent group alone. The null GSTM1 and GSTT1 genotypes showed no influence on hydroxypyrene levels at any smoking dose (data not shown).

A seasonality pattern was observed in police officers but not in referent subjects (Table 2). Significantly increased 1-OH-P mean levels ($P < 0.05$) were detected in police officers’ urine samples collected in January through March (0.238 μmol/mol creatinine) and April through May (0.152 μmol/mol creatinine) compared to samples collected in June through July and October through November: 0.110 and 0.099 μmol/mol creatinine, respectively. No differences were observed according to consumption of broiled/grilled meat and fresh fruit and vegetables (data not shown).

Multiple regression analysis (Table 3) confirmed that smoking habits and seasonality (both used as ordinal covariates) were the strongest predictors of 1-OH-P excretion. Environmental exposure to airborne B(a)P and ETS, as well as the host factors included in the regression model (i.e., sex, and the CYP1A1, GSTM1, and GSTT1 genotypes), failed to contribute significantly to the prediction of 1-OH-P in urine. Although B(a)P and ETS were not identified as statistically significant predictors of 1-OH-P by multiple regression analysis, examinations of the estimated MRs reported in Table 3 suggest the possibility of positively graded relationships between exposure to ambient air B(a)P and ETS and urinary excretion of 1-OH-P.

Discussion

The findings of the present study indicate that individual exposure to selected PAHs experienced by traffic police officers during a typical work shift in the city of Genoa, Italy, is clearly higher than that experienced by referent subjects working indoors. The geometric mean of B(a)P air measurements (considered an index compound of atmospheric PAH levels) detected in traffic police officers (3.67 ng/m³) was 70 times higher than that detected in referent subjects (0.05 ng/m³). Similar patterns were observed for B(b)F and B(k)F. The measured outdoor air levels are in line with those detected in Western
was not statistically significant. Conversely, significantly increased mean concentrations of 1-OH-P were detected in the urine of cigarette-smoking subjects compared to nonsmokers, both police officers and referents. Moreover, the number of cigarettes smoked per day was found to be a good predictor of 1-OH-P excretion in urine, confirming the dose-dependent relationship reported by other investigations (26, 27). These findings indicate inhalation of mainstream tobacco smoke as one of the major determinants of 1-OH-P excretion in urine under the condition of environmental exposure described in our study.

Estimates of the amount of pyrene delivered to the lung from one cigarette range between 50 and 270 ng, depending on the different tar content of cigarette brands (15).

The amount of pyrene inspired through normal breathing varies according to pyrene ambient air concentration and the time an individual spends in polluted microenvironments. Because similar concentrations of particulate pyrene and B(a)P have been detected in Genoa, Italy (43), and United Kingdom outdoor urban and suburban air (44), B(a)P air levels can be used as a surrogate of exposure to airborne particulate pyrene. Assuming that 90% of airborne PAH is associated with respirable particles and that 80% of the inhaled particulate PAHs is deposited in the lung, a police officer breathing an average 0.8 m$^3$/h of urban air polluted with 3.6 ng/m$^3$ pyrene (i.e., the median B(a)P level detected in our study) will inhale 10.4 ng pyrene during a typical 5-h workshift (i.e., 90% × 80% × 0.8 × 3.6 × 5). This figure is roughly equivalent to one-fifth of the estimated exposure to pyrene experience by the lung through mainstream smoke from one low-tar cigarette (i.e., 50 ng).

Exposure to ETS was associated with higher 1-OH-P excretion in urine, suggesting that pyrene intake through inhaled cigarette smoke-polluted air contributes to the excretion of 1-OH-P. This seems to be a logical and biologically plausible argument, because airborne concentrations of particulate pyrene in rooms polluted with ETS range between 4.1 and 9.4 ng/m$^3$ (45). Therefore, for the same unit of time and given the airborne PAH concentrations detected in our study, breathing ETS-polluted air will roughly be the same as working in a traffic environment. This reasoning was confirmed by statistical modeling of our data (Table 3) showing similar increased

European cities (43), despite differences in sampling strategy and analysis between data generated by environmental monitoring studies and the present study. Our study failed to detect the typical seasonal variations of PAH concentrations in air reported in the scientific literature indicating 2- to 5-fold higher levels of PAHs in winter than in summer (43). This could be explained by at least three factors: (a) personal sampling, as carried out in our study, is likely to have picked up mostly PAHs generated during incomplete combustion of fossil fuels in motor vehicles (i.e., exhaust emissions) that show little or no seasonal variation; (b) the predominant use of methane gas as domestic heating fuel, with negligible production of PAHs, does not affect significantly the baseline concentrations of PAHs in urban air; and (c) the mild climate of the Ligurian Coast, where Genoa is located, allows a lower daily fuel consumption for domestic heating compared to other European regions.

Although the mean concentration of urinary 1-OH-P was higher in police officers (0.140 μmol/mol creatinine) than in referent subjects (0.097 μmol/mol creatinine), the difference

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**Fig. 4.** Urinary 1-OH-P median concentrations and their 25th and 75th percentile values (whiskers) detected in traffic police officers according to current cigarette smoking habits and CYP1A1 metabolic polymorphism. CYP1A1− and CYP1A1+ major and minor (homozygous and heterozygous genotypes combined) genotypes, respectively; numbers in parentheses, number of subjects.

**Fig. 5.** Urinary excretion of 1-OH-P (mean values; whiskers, 90% CIs) detected in referent subjects and police officers according to their current smoking habits, number of cigarettes smoked per day (cigarettes/day), and level of exposure to ETS (hours/day); results are from univariate analysis.

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**Table 3.** Median B(a)P levels detected in urban air during a typical 5-h workshift (i.e., 90% × 80% × 0.8 × 3.6 × 5). This figure is roughly equivalent to one-fifth of the estimated exposure to pyrene experience by the lung through mainstream smoke from one low-tar cigarette (i.e., 50 ng).
urban urinary 1-OH-P excretion in subjects exposed to ETS for ≤4 h/day (MR, 1.473; 95% CI, 0.949–2.287) and in police officers exposed to Ba(a)P levels >3.50 ng/m³ (i.e., the 66.6th percentile value; MR, 1.425; 95% CI, 0.902–2.252), after adjustment for the effect of the other covariates included in the model.

According to the above-mentioned data on PAH intake through cigarette smoking and inhalation of ETS-polluted air and the individual exposure to airborne PAHs measured in our study populations, we have constructed two semiquantitative indices of exposure to polycyclic compounds that were used to graphically depict the relationship between exposure to PAHs and the urinary excretion of 1-OH-P (Figs. 5 and 6). Both univariate and multivariate analyses showed that 1-OH-P excretion increases with the increase of the exposure index, that the increase in nonsmokers is due to the time spent in ETS-polluted spaces and the exposure to air PAHs experienced by subjects, and that in cigarette smokers such an increase is due to the number of cigarettes smoked per day and the measured individual exposure to Ba(a)P.

The higher urinary excretion of 1-OH-P that was observed in female than in male subjects may reflect sex-related differences in the metabolism of PAHs mediated by endogenous mechanisms. Similar effects have been reported by studies of nontumorous and cancer tissues from females and males (46, 47).

Dietary intake of PAHs is considered to be an important route of exposure to PAHs and cooking practices and food processing may contribute to the amount of PAHs ingested by humans (48, 49). Our study failed to detect any association between 1-OH-P in urine and consumption of broiled/grilled meat and fresh fruit and vegetables. However, the study by Van Rooij et al. (26), which was specifically aimed at investigating the sources of variability in baseline 1-OH-P urinary excretion, indicated dietary pyrene intake to explain only 2% of the variation in 1-OH-P urinary concentrations observed in 76 human volunteers, despite the fact that 53% of the total pyrene daily intake was estimated to be from food. This finding may reflect a daily dietary intake that does not vary within populations recruited in a limited geographical area.

The seasonal variation of 1-OH-P urinary levels observed in traffic police officers, with higher excretion in winter and spring samples than summer and fall samples, is apparently in contrast with the measured lack of seasonal variation of airborne PAH concentrations. Possible explanations for this discrepancy might include seasonal differences in the metabolic pathways leading to formation and excretion of 1-OH-P or a higher respiratory intake of pyrene due to the reported increased concentration of PAHs as particulate matter under low-temperature conditions (50).

The three genetic polymorphisms that were investigated in the study failed to show a significant association with the excretion of 1-OH-P, and their exclusion from the multiple regression model did not modify the effect estimated for the other covariates. Failure to detect a significant role of CYP1A1 and GSTM1 genetic polymorphisms may be explained by the lack of inducibility of CYP1A1 activity in the human liver by cigarette smoking (51, 52), which may reflect the relatively low dose of inducers delivered through smoking to the hepatocytes compared to that delivered to pulmonary cells with high CYP1A1 gene expression.

The effect of the CYP1A1 MspI on metabolite levels at low smoking doses has been observed previously in other studies of this gene (29, 53), as well as with NAT2 (54). One explanation of this effect is that at low doses of exposure, altered activity of the gene product resulting from the polymorphism leads to an observable change in metabolism, whereas for very high exposures, a saturating effect masks any small further increase in metabolism resulting from the presence of the polymorphism. Our findings suggest a type of gene-environment interaction that is more observable at low doses of exposure. The importance of this low-dose effect for polymorphisms in metabolic genes has been discussed (55) and may be a significant factor to be taken into account when applying this biomarker to larger populations of people exposed to low environmental levels of hydrocarbons.

In conclusion, the present study suggests that (given sufficient statistical power) urinary excretion of 1-OH-P may be useful as a biomarker of exposure to airborne PAH concentrations (experienced by traffic police officers) that are several orders of magnitude lower than those reported for coke-oven and aluminum workers (20, 21). The levels of exposure investigated are more typical of the low-level environmental exposure experienced by most people living in Western European and North American cities than the occupationally high-exposure group often used for the assessment of biomarkers of exposure.

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