Seasonal and Regional Contributors of 1-Hydroxypyrene among Children near a Steel Mill

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Abstract

Urinary 1-hydroxypyrene (1-OHP) is a biomarker of exposure to polycyclic aromatic hydrocarbons (PAH). Effect of residence on children’s PAH exposure was reported among children living near a polluted area. Instead of a snapshot assessment, however, a temporal history of exposure characteristics needs to be assessed in the studies of chronic disease development such as cancer. The urinary 1-OHP measurements were repeated to determine regional effect of ambient air pollution on 1-OHP levels over extended periods. Two sites were chosen: (a) one site located near the steel mill (“nearby” site) and (b) the other site located at a longer distance from the mill (“remote” site). Spot urinary 1-OHP levels were measured from 72 children for 3 consecutive days per month, repeated over 9-month period. Compared with remote site, the nearby site had increased the urinary 1-OHP level by 62.3% [95% confidence interval (95% CI), 39.8-88.3%]. Other statistically significant factors that contributed to the level include sex [16.5% (95% CI, 1.2-34.1%) higher for girls than boys], consumption of charbroiled meat [16.5% (95% CI, 1.1-34.2%) higher], and an increase in PM10 [10.1% (95% CI, 4.8-15.7%) higher for the interquartile range increment]. Controlling for covariates, the 1-OHP levels were increased in the summer and fall compared with winter. The magnitude of the effects of both seasons had diminished after adjusting for PM10. This is the first report providing seasonal and regional contributors to environmental PAH exposure, assessed by urinary 1-OHP, with higher 1-OHP levels during summer when ambient pollution was also high. (Cancer Epidemiol Biomarkers Prev 2009;18(1):96–101)

Introduction

Polycyclic aromatic hydrocarbons (PAH) have raised much public concern because some congeners are listed as group 1 (i.e., “carcinogenic to humans”) by IARC (1). Epidemiologic studies have shown increased lung cancer risks for employees exposed to PAHs (2–6) as well as for residents of nearby neighborhoods (7, 8). Various adverse health effects also have been reported among children residing near a known PAH source: decline of lung function (9), growth-retarded infant (10), and delay in child’s neurodevelopment from prenatal exposure (11).

PAHs are released as a mixture into the environment in a variety of ways, and the exposure properties of these compounds vary depending on the source. Routes of human exposure to PAHs occur via inhalation, ingestion, and dermal contact because these pollutants are ubiquitous in living environments. Sources of PAHs are mainly cigarette smoke, wood-burning ovens and fireplaces, vehicle exhaust, and consumption of grilled and smoked foods (1). Occupational exposure generally occurs in the setting of coal-fired utilities, steel plants, and waste incineration facilities.

To provide information on the uptake of PAHs from various sources, pathways of exposure, and health risks, urinary 1-hydroxypyrene (1-OHP), as a metabolite of pyrene, has been widely used as the most relevant biomarker of PAH exposure in occupational and nonoccupational settings (12–14). Despite of its wide usage in biomonitoring studies for public health, essential questions about temporal variability should be addressed before applying urinary 1-OHP to the study on PAH-related chronic diseases such as cancer. Urinary 1-OHP levels reflect relatively recent exposures, especially within a few days before urine collection due to its short half-life (15). Therefore, information on the variation of long-term PAH exposure and its contributing factors are necessary for epidemiologic studies of chronic diseases and ensuing health policy. However, little information on the seasonal variation of PAH exposure and its contributing factors are available.

This study was designed to provide information on the seasonal and regional variation of PAH exposure among children living at two separate sites from a steel mill assessed by repeated urine samples over 9 months. The design can provide the insight for further understanding of the role of contributing factors on the long-term variation of biological PAH exposures.

Materials and Methods

Study Site and Subject. A total of 956 school children participated in biomonitoring part of the PAH exposure assessment study (16). Among the participants, 72 children agreed to take part in the repeated PAH
biomarker assessment. This repeated prospective sampling was done from March to December of 2004, except for the school vacation period of January, February, and August. As the urine samples were collected during the attendance of school, study was unfeasible during the vacation. Usually, winter vacation is longer than the one in summer in Korea. Each month, a spot urine sample was collected every morning for 3 consecutive days, and this once-a-month, 3-d sampling was repeated for 9 mo. Each subject, then, could have up to 27 spot urine samples during the study period.

The study site was in the neighborhood of a steel mill located in the southern part of Korea. Two sites were chosen based on wind direction and distance from the steel plant: “nearby” site adjacent to the northern boundary of the plant complex and “remote” site, as a reference area, located on the same wind direction but about 6 km away from the plant. Participants of nearby site were consisted of 36 students from one elementary school (n = 24) and one junior high school (n = 12), whereas those of remote site were consisted of 36 students from another elementary school (n = 24) and another junior high school (n = 12). We hold meetings at each school to explain about the study objectives, sampling methods, and questionnaire guidelines. We distributed on the eve of the sampling day the guideline sheets, individually labeled questionnaires, and labeled sterile sampling tubes for boys and cups for girls. The exposure assessment questionnaires asked about activities and specific exposures such as diet with high PAH contents and indoor [secondhand smoke (SHS) exposure] and/or outdoor sources of exposure (transportation, time spent for the transportation, and location of outdoor play) during 1 d before the urine sampling. Children filled them out at home and, if needed, parents or guardians helped them. They were instructed to get the first spot urine samples next morning and bring back the samples together with the completed questionnaires to school for 3 consecutive days. We collected the urine samples and questionnaires each morning when the children attended school. Children were 7 to 15 y old with the mean age of 11.1 y. Thirty-two of 72 participants were females. All participants provided written informed consent before study participation.

Determination of Urinary 1-OHP. The first spot urine samples in the morning were collected in a sterile sampling tube. The collected urine samples were stored at freezer (−20°C) in school infirmaries less than 3 d, and then the frozen samples with packed ice were sent to the laboratory for the storage at deep freezer (<−20°C) until the final analysis. The 1-OHP in urine was analyzed by a reverse-phase high-performance liquid chromatography method with enzymatic hydrolysis using β-glucuronidase/arylsulfatase (17) as previously described (16). Excitation and emission wavelengths were 242 and 388 nm, respectively. A reverse-phase C18 column (250 mm × 4.6 mm, 5 μm, Supelco) was used for the segregation of 1-OHP. The detection limit of the method was 0.021 nmol 1-OHP/L urine. Urinary creatinine, as a marker of renal clearance, was measured according to the Jaffé method (18) and used for the correction of urinary concentration. The concentrations of 1-OHP were expressed as μmol/mol creatinine.

Ambient Particulate Matter Assessment. Some previous studies used particulate pollutants as a proxy measure of ambient PAHs (16, 19). Recent study showed that >90% of the ambient PAHs measured at different regions of Korea were captured in PM10 fraction (20). Due to the lack of available data about ambient PAHs at the studied sites, we used the mean PM10 levels with a lag of 1 to 7 d before the urine sampling as the proxy indices of atmospheric PAH pollution. The Ministry of Environment of Republic of Korea is operating an ambient air monitoring station at the nearby site. The data on particulate matter <10 μm in aerodynamic diameter (PM10) were measured every 5 min throughout the year using β-ray absorption method.3

Statistical Analysis. All the statistical analyses were done using the Statistical Analysis System software, version 9.1 (SAS Institute). Due to the skewed distribution, log10-transformed creatinine-adjusted 1-OHP concentrations were used to better approximate the normality assumption. To compare the differences in sex and age distributions between the two study groups, χ2 test and Student’s t test were adopted. The difference in the mean 1-OHP level between the two study groups was tested by Student’s t test after log transformation. In addition, descriptive statistics were calculated, especially monthly mean, SD, geometric mean (GM), and geometric SD (GSD) of 1-OHP.

Two-day lag was selected in testing the effects of PM10 on 1-OHP levels based on graphical inspections of different lag periods and indicators of goodness of fit in the Proc Mixed models (Akaike information criterion, Bayesian information criterion, and corrected Akaike information criterion) as well as the reports of short half-lives of 1-OHP (14).

To examine the seasonal and regional variations of urinary 1-OHP levels and the effects of other contributing factors, we did multivariate mixed model analysis using log-transformed 1-OHP data. The intraindividual regression coefficients were considered to be random. Individual-level covariates, that is, demographic factors (age and gender), dietary source (charbroiled meat), indoor source [environmental tobacco smoke (ETS)], outdoor source (ambient PM10), and season, were treated as fixed effects. Dummy variables were used to represent nominal variables, such as gender (boy versus girl), region (remote versus nearby), ETS, and diet. Seasons were categorized into spring (March to May), summer (June to July), fall (September to November), and winter (December) according to the classification by Korean Meteorological Administration.4 Spring, summer, and fall were included into the model using winter as the reference.

We estimated the percent change in creatinine-adjusted 1-OHP levels by each nominal variables as (10β × 1) × 100% with 95% confidence intervals (95% CI) of [10β ± 1.96 × SE] − 1] × 100% and for PM10 lag 2 as (10β × 10QR − 1) × 100% with 95% CI of [10QR × (β ± 1.96 × SE) + 1] × 100%, where β and SE are the estimated regression coefficient and its SE. We also estimated the percent change in 1-OHP levels associated with study region after stratifying by season.

3 http://www.airkorea.or.kr
4 http://www.kma.go.kr/

Table 1. General characteristics of study subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Overall, n (%)</th>
<th>Nearby site, n (%)</th>
<th>Remote site, n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girl</td>
<td>32 (44)</td>
<td>17 (53)</td>
<td>15 (47)</td>
<td>0.27</td>
</tr>
<tr>
<td>Boy</td>
<td>40 (56)</td>
<td>21 (53)</td>
<td>19 (47)</td>
<td></td>
</tr>
<tr>
<td>Age, y (mean ± SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-OHP (μmol/mol creatinine)</td>
<td>0.050 ± 2.435</td>
<td>0.062 ± 2.112</td>
<td>0.040 ± 2.647</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*P* value was calculated by χ² test.  
† *P* values were calculated by Student's *t* test after log transformation.  
‡ GM ± GSD.

Results

**Descriptive Analysis.** Out of total 1,944 (72 subject × 3 days × 9 months) originally scheduled urine samples, 1,497 (77%) samples were actually collected. The number of urine samples finally available was 6 to 27 samples per subject. Twenty-two urine samples were excluded because of the errors in analytic procedure. The GM of urinary 1-OHP concentration for the total urine samples was 0.050 μmol/mol creatinine. When stratified by region, it was 0.062 μmol/mol creatinine for the nearby site and 0.040 μmol/mol creatinine for the remote site. The 1-OHP levels of nearby children were significantly higher than those of remote children (*P* < 0.0001), as shown in Table 1.

The monthly mean, SD, GM, and GSD of the creatinine-corrected 1-OHP levels by study sites are shown in Table 2. The overall monthly means of 1-OHP were higher for nearby site than remote site, but the absolute levels and differences varied by seasons. The difference between study sites was the largest during summer (GM = 0.087 μmol/mol creatinine for nearby site; GM = 0.036 μmol/mol creatinine for remote site).

**Lag Effects of PM10 as a Proxy Measure of Ambient PAHs.** Monthly variation of creatinine-adjusted 1-OHP and ambient PM10 levels are shown in Fig. 1. The monthly variation of 1-OHP followed a similar track of PM10 variation. The ambient PM10 levels were higher in June and July, and 1-OHP levels were also higher during the same periods.

The ambient monitoring data of PM10 were used as a proxy measure of ambient PAHs, and mixed-effects models incorporating PM10 were analyzed with 1 to 7 days of lags before the urine sampling date. The daily mean PM10 levels of 2, 3, and 7 days before survey (lag 2, 3, and 7) showed significantly positive associations with the creatinine-adjusted 1-OHP. Considering the distribution of different lag effects, the 2-day lag model was chosen as the most appropriate for evaluating the effect of air pollution on urinary 1-OHP level as shown in Fig. 2.

**Model of Contributing Factors.** Table 3 presents the estimated percent changes of 1-OHP for potential covariates. The urinary 1-OHP concentration increased by 17.6% (95% CI, 2.1-35.3%; *P* = 0.022) for girls compared with boys, by 17.6% (95% CI, 1.9-35.7%; *P* = 0.027) for charbroiled meat consumption, and by 34.0% (95% CI, 15.1-55.9%; *P* < 0.0001) for nearby children compared with remote children as shown in model I. Urinary 1-OHP concentrations in summer and fall were significantly higher than those of winter (model II): a 40.9% (95% CI, 20.6-64.7%; *P* < 0.0001) increase for summer and a 37.8% (95% CI, 18.6-60.2%; *P* < 0.0001) increase for fall compared with winter levels. The seasonal effects somewhat decreased after adjusting for south wind from a steel mill (model III): 30.2% (95% CI, 9.9-54.3%; *P* = 0.002) for summer and 34.0% (95% CI, 15.1-55.9%; *P* = 0.0002) for fall. The 1-OHP level increased by 10.1% (95% CI, 4.8-15.7%; *P* < 0.021) for the interquartile range (IQR) increment of PM10 lag 2 (model IV). The magnitudes of the percent changes for both summer and fall also diminished after adjusting for PM10 lag 2. Especially, the percent change by summer effect dropped to 20.4% (95% CI, 1.2-43.2%; *P* = 0.036).

Table 2. Means and SDs of creatinine-adjusted urinary 1-OHP levels by month and season

<table>
<thead>
<tr>
<th>Season</th>
<th>Month</th>
<th>Nearby site</th>
<th>Remote site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>Spring</td>
<td>March</td>
<td>105</td>
<td>0.061</td>
</tr>
<tr>
<td></td>
<td>April</td>
<td>101</td>
<td>0.062</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>93</td>
<td>0.070</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>299</td>
<td>0.064</td>
</tr>
<tr>
<td>Summer</td>
<td>June</td>
<td>84</td>
<td>0.107</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>88</td>
<td>0.113</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>172</td>
<td>0.110</td>
</tr>
<tr>
<td>Fall</td>
<td>September</td>
<td>74</td>
<td>0.072</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>70</td>
<td>0.087</td>
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<tr>
<td></td>
<td>November</td>
<td>79</td>
<td>0.079</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>223</td>
<td>0.079</td>
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<tr>
<td>Winter</td>
<td>December</td>
<td>84</td>
<td>0.075</td>
</tr>
</tbody>
</table>


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Discussion

To explore the chronic variation of environmental exposure to PAHs, biomonitoring with multiple measurements, at different time points of day, months, and seasons, can be adopted. Such a strategic design, to our knowledge, has not been yet reported in the literature. Repeated sampling design may have affected sample collection compliance. The collection rate was 77% of the total scheduled urine samples (1,944). During the overall study period, daily collection rate was 58% to 99% of the possible 72 urine samples. Although sampling rate had somewhat varied by season (88% for spring, 76% for summer, 65% for fall, and 72% for winter), there was no trend after the initial decline.

Our study focused primarily on environmental exposure to PAHs from a point industrial source of steel mill, whereas other studies concentrated on traffic and/or dietary exposures to PAHs (9, 21–24). Similar to our study, the spatial effect by different distances from a point source was reported by the study from Ukraine (12). The environmental exposure to PAHs at the steel mill site was compared with that in capital city, but only one cross-sectional measurement was taken without considering dietary PAH intake. Our study was to determine spatial differences in chronic exposure levels using repeated samples to account for the temporal variation.

Several recent studies among children have shown that diet is the most distinct contributor to 1-OHP levels (21, 22). Among the dietary food items, the ingestion of charbroiled meat is one of the main sources of PAH exposure (1, 25). Whereas other Korean study reported no relationship (19), significant contributions of dietary intake to the 1-OHP level were found in this study based on dietary questionnaire after adjusting for other intra-individual variation.

Several studies examined the effect of gender on children’s 1-OHP concentrations (12, 15, 23, 26). The gender effect was inconsistent. Several studies reported higher 1-OHP levels for girls (12, 16), whereas others reported higher levels for boys (15, 26). This study found significantly higher level of 1-OHP for girls when creatinine-adjusted log-transformed values were analyzed (estimate = 0.087; \( P = 0.031 \)), whereas the difference was not statistically significant with creatinine-unadjusted values (estimate = 0.004; \( P = 0.941 \)). Boys, especially those in junior high schools, are bigger and more masculine than girls and, therefore, excrete more creatinine in urine, resulting in the smaller 1-OHP values when adjusted with creatinine. In this way, the differences in body mass might have contributed to this gender-related difference in creatinine-adjusted log-transformed values. In addition, as we did not take account of the amount of intake, the difference in the frequency and amount of charbroiled foods between boys and girls may also have influenced this gender-related difference.

SHS from household members can be an important contributor to PAH exposure. Dutch studies showed the contribution of SHS in nonoccupationally exposed male adults (27) and children (28), whereas several studies (26, 29) did not. Ukraine study showed an effect of SHS among total group of children but not within unexposed and exposed group (12). In our results, exposure to SHS did not contribute to 1-OHP levels. Inaccurate estimation of the SHS based on questionnaire, and the amounts and conditions of SHS in South Korea, especially housing structures, ventilations under different weather conditions, and the change of parent’s smoking behavior at home, may have influenced the results.

Because the ambient PAH data were not available at the study site, we were not able to assess the direct contribution of ambient PAHs to 1-OHP levels. Ambient \( \text{PM}_{10} \) was used as a proxy measure of ambient PAH exposure. More than 70% of PAHs are adsorbed onto suspended particles at ambient temperatures (20). Lighter PAHs with two to three benzene rings are mostly present in gas phase, whereas those with four or more
Contributors of 1-Hydroxypyrene among Children

Table 3. Estimated percent changes (95% CIs) in 1-OHP (μmol/mol creatinine) levels and four-random effects models for contributing factors

<table>
<thead>
<tr>
<th></th>
<th>Model I</th>
<th>Model II</th>
<th>Model III</th>
<th>Model IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>−1.7 (−4.5 to 1.3)</td>
<td>−1.4 (−4.3 to 1.5)</td>
<td>−1.5 (−4.4 to 1.4)</td>
<td>−1.6 (−4.4 to 1.4)</td>
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<tr>
<td>Gender</td>
<td>Boy</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
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<tr>
<td></td>
<td>Girl</td>
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<td>Reference</td>
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<tr>
<td>ETS</td>
<td>No</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
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<tr>
<td></td>
<td>Yes</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
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<tr>
<td>Food</td>
<td>No</td>
<td>Reference</td>
<td>Reference</td>
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<td></td>
<td>Yes</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
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<tr>
<td>Region</td>
<td>Remote</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
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<tr>
<td></td>
<td>Nearby</td>
<td>62.5 (40.2-88.5)</td>
<td>62.1 (39.7-88.2)</td>
<td>62.4 (40.0-88.5)</td>
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<tr>
<td>Season</td>
<td>Spring</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>40.9 (20.6-64.7)</td>
<td>30.2 (9.9-54.3)*</td>
<td>20.4 (1.2-43.2)*</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>37.8 (18.6-60.2)</td>
<td>34.0 (15.1-55.9)*</td>
<td>36.1 (16.9-58.3)*</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>South wind (%)</td>
<td></td>
<td></td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>PM10 lag 2</td>
<td></td>
<td></td>
<td>Reference</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Coefficients are expressed as percent changes in 1-OHP–associated contributing factors: model I included age (years), gender, ETS, and charbroiled meat consumption (yes/no); model II included model I plus season; model III included model II plus south wind; and model IV included model III plus PM10 lag 2.

* P < 0.05.

† Percent change (95% CI) based on IQR (75-25%) of PM10 lag 2.

rings, including carcinogenic PAHs such as benzo(a)pyrene, are mainly found in particle phase (30). The majority of PAHs exist predominantly on ultrafine particles of <0.1 μm in diameter in Los Angeles (31) or fine particles of 0.1 to 2.5 μm in diameter in China (32). More than 60% of ambient PAHs were in particles with diameter of <3 μm and >90% of PAHs were in particles with diameter of <9 μm at studied sites in Korea (20). The concentration of airborne pyrene in urban fine particles was the highest during summer (32) and 43% of total 16 PAHs, the highest value among the measurements, was pyrene during summer in Ulsan, Korea (33).

Over the study period, ambient PM10 had ranged from 30.29 to 88.66 μg/m³ (mean, 51.32 μg/m³) during the 1 week before urine collection. Except for September, October, and December, the mean values of PM10 exceeded the air quality standard of the annual mean of Korean Ministry of Environment (50 μg/m³) and United Kingdom (40 μg/m³). In a Ukraine study (12), much higher annual level of particulate (200 μg/m³) was reported, and even the level in their control area (100 μg/m³) was higher than in our study. Very high level of ambient pollution may have increased the level of 1-OHP among Ukraine children, but beyond this ecological association, no further temporal or spatial variation was examined. In this study, short-term contribution of ambient PM10 could be captured by the repeated measurements over 3 days.

In addition to the short-term variation, we observed significant seasonal variations in 1-OHP levels among children, especially higher during summer and fall compared with winter. Very few studies reported the seasonal variations in biomonitoring. Higher levels of 1-OHP during winter were found among occupational employees (34, 35). One study found the seasonal differences only among nonsmoking employees (34), whereas the other study attributed the seasonality to the variation in sources such as residential heating and higher traffic density during winter time in urban area (35). In our study, seasonal variation could be explained largely by ambient PM10 levels and possibly by wind directions (models II, III, and IV in Table 3). Differences in indoor residence time and outdoor activities between different seasons might have also contributed to this seasonal variation.

This is also consistent with our results on the contribution of regional effects for different seasons (Fig. 3). Regional effect by season was calculated as the percent change in creatinine-adjusted 1-OHP levels by region as (10β − 1) × 100% with 95% CI of [10β ± 1.96 × SE] − 1) × 100%, where β and SE are the estimated regression coefficient and its SE in a

Figure 3. Estimated percent change in creatinine-adjusted 1-OHP levels for region by season. The covariates adjusted for age, sex, ETS, and charbroiled meat consumption. Each triangle and bar represents the percent change and 95% lower and upper confidence intervals.
multivariate model analysis. Overall, the regional effect was the largest during the summer (Fig. 3) when the relative contribution of PM$_{2.5}$ to the I-OHP was also the largest compared with other seasons (models II and IV in Table 3). These results suggest that when seasonal variation in the exposure level is suspected, the assessment of chronic exposure to the ambient PAHs should cover at least two different periods, including seasons of high and low exposures.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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