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Effects of Lifestyle on Urinary 1-hydroxypyrene Concentration

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Abstract: Effects of Lifestyle on Urinary 1-hydroxypyrene Concentration: Toshihiro KAWAMOTO, *et al.* Department of Environmental Health, School of Medicine, University of Occupational and Environmental Health, Japan—This study aimed to clarify the variation of urinary excretion of 1-hydroxypyrene, which is a major metabolite of pyrene, in relation to lifestyle, including factors such as diet and smoking. The study subjects were 251 workers (male: 196, female: 55, mean age: 44.3) who were not occupationally exposed to PAHs. Urine specimens were collected from 8:00 a.m. to 11:00 a.m. and their 1-hydroxypyrene concentrations were determined by HPLC. A questionnaire was distributed in order to learn gross aspects of the subjects' lifestyles, i.e., smoking, alcohol consumption, coffee/black tea intake, and dietary habits. Multiple linear regression analysis revealed that cigarette consumption most strongly affected the 1-hydroxypyrene level in urine, followed by dietary balance. The urinary 1-hydroxypyrene concentrations of smokers were about 2 times higher than those of non-smokers. Subjects who ate more meat and/or fish excreted 1.5-2 times more 1-hydroxypyrene in urine than those who ate more vegetables.
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Key words: 1-Hydroxypyrene, Smoking, PAH (polyaromatic hydrocarbon), Biological monitoring, Biomarkers, Dietary balance, Meat, Vegetable, Fish, Alcohol

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Urinary 1-hydroxypyrene, a major metabolite of pyrene, is a useful biomarker for assessing exposure to environmental polyaromatic hydrocarbons (PAHs), especially in workers with high occupational exposure, such as workers in coke plants[1)]. However, the use of urinary 1-hydroxypyrene to evaluate individual exposure to air pollution levels of PAHs is problematic. PAHs are taken into our bodies not only from ambient air but also from smoking, food, and other sources. Van Rooji *et al.* [2)] estimated the contribution of different sources to daily pyrene intake by distributing a detailed questionnaire to volunteers who were not occupationally exposed to PAHs and reported that the consumption of food products and active smoking accounted for 99% of total pyrene intake. However, it is not clear how such exposure affects urinary 1-hydroxypyrene levels. Therefore, using a simple questionnaire, we studied the effects of lifestyle factors such as smoking, drinking, dietary habits and coffee/tea consumption on the urinary 1-hydroxypyrene level in a relatively large population who were not occupationally exposed to PAHs.

Methods

Chemicals

1-Hydroxypyrene was purchased from Aldrich Chemical Co. (Milwaukee, WI). Beta -glucuronidase/sulfatase (type H-2, from Helix pomatia: β-glucuronidase activity, 107,200 units/ml and sulfatase activity, 4,500 units/m*l*) was purchased from Sigma Chemical Co. (St. Louis, MO).

Study subjects

The study subjects were 251 workers (male: 196, female: 55, mean age: 44.3, range: 18–71) who lived in Kitakyushu city and its surrounding area. They were not

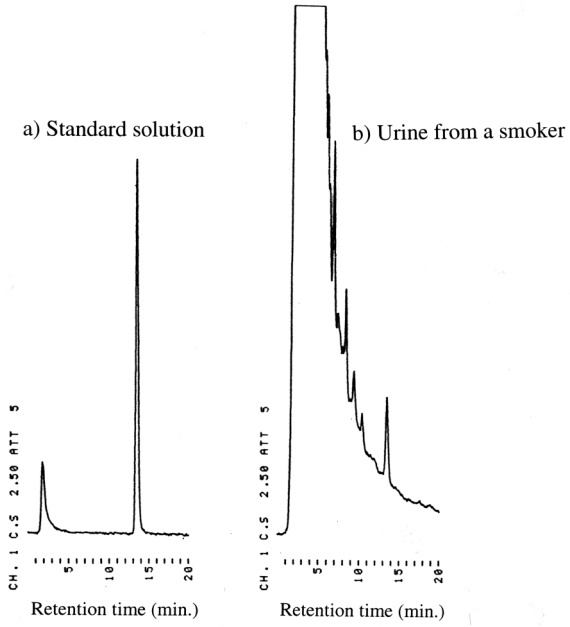

Fig. 1. Chromatograms of a standard solution (a) and a urine sample from a smoker (b). The retention time of 1-hydroxypyrene is around 13.5 min.

occupationally exposed to PAHs and gave their informed consent in health examinations. A questionnaire was used to establish gross aspects of lifestyle, i.e., smoking status, alcohol consumption, and intake of coffee or black tea, besides gender and age. Especially, the number of cigarettes smoked within 24 h before sampling was also asked in the questionnaire. The consumption of greasy or salty food, sweets, fruits, vegetables, meat, and fish was also investigated in order to learn the subjects' dietary habits. This study was approved by the ethics committee of medical care and research of the University of Occupational and Environmental Health.

Measurement of urinary 1-hydroxypyrene

Urine specimens were collected from 8:00 a.m. to 11:00 a.m. and were stored at –20°C until analysis. The urinary 1-hydroxypyrene concentration was determined by the method described by Taguchi *et al.*[3] with a minor modification. Urine specimens were treated with β-glucuronidase in sodium acetate buffer (pH 5.0) for 12 h, and mixed with acetonitrile. After centrifugation, 100 μl of the supernatant was subjected to HPLC (Hitachi: L-7200 autosampler, L-7100 intelligent, L-7300 column oven) with an L-7480 fluorescence detector and L-7500 chromato integrator. The column was a TOSOH TSK gel ODS-80^{TM} (reverse phase), and the mobile phase was 65% acetonitorile and 35% water. The wavelengths of excitation and emission were 242 nm and 388 nm, respectively.

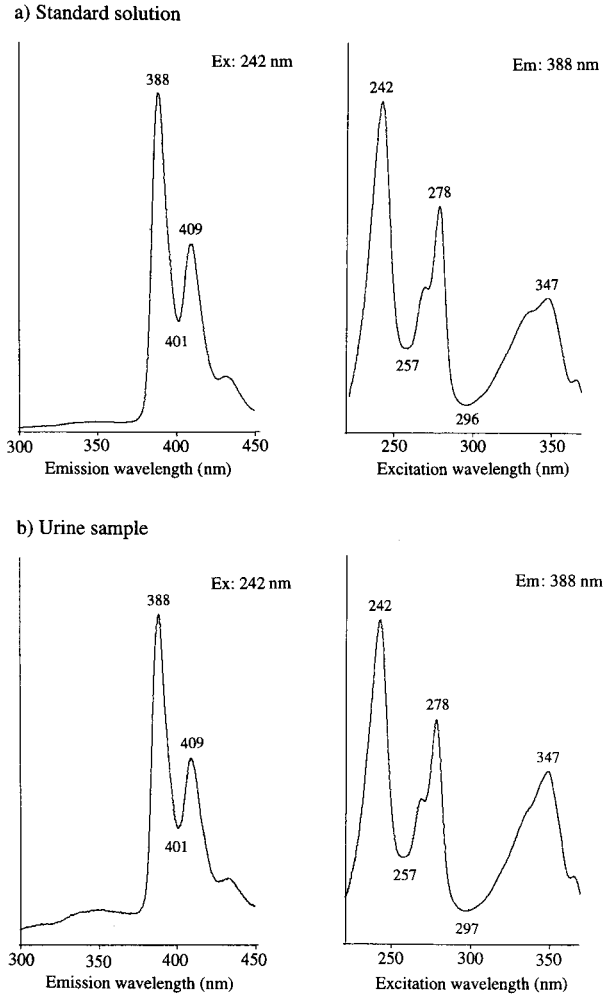

Fig. 2. Emission wavelength scan with excitation at 242 nm, and excitation wavelength scan with emission at 388 nm of the HPLC peak of the standard solution (a) and of the HPLC sample fraction with the same retention time from the urine sample (b).

Statistical analysis

Lifestyle data were quantified as shown in Table 1. Two dummy variables, X1 and X2, are created to index three categories of dietary balance. Multiple linear regression analysis was applied to investigate the effect of lifestyle in determining the urinary levels of log-transformed 1-hydroxypyrene. Two-way ANOVA was also carried out to compare dietary effects. Multiple comparisons were done by Fisher's PLDS with one way ANOVA.

Results

Simple 1-hydroxypyrene analysis

Figure 1 shows chromatograms of a standard sample

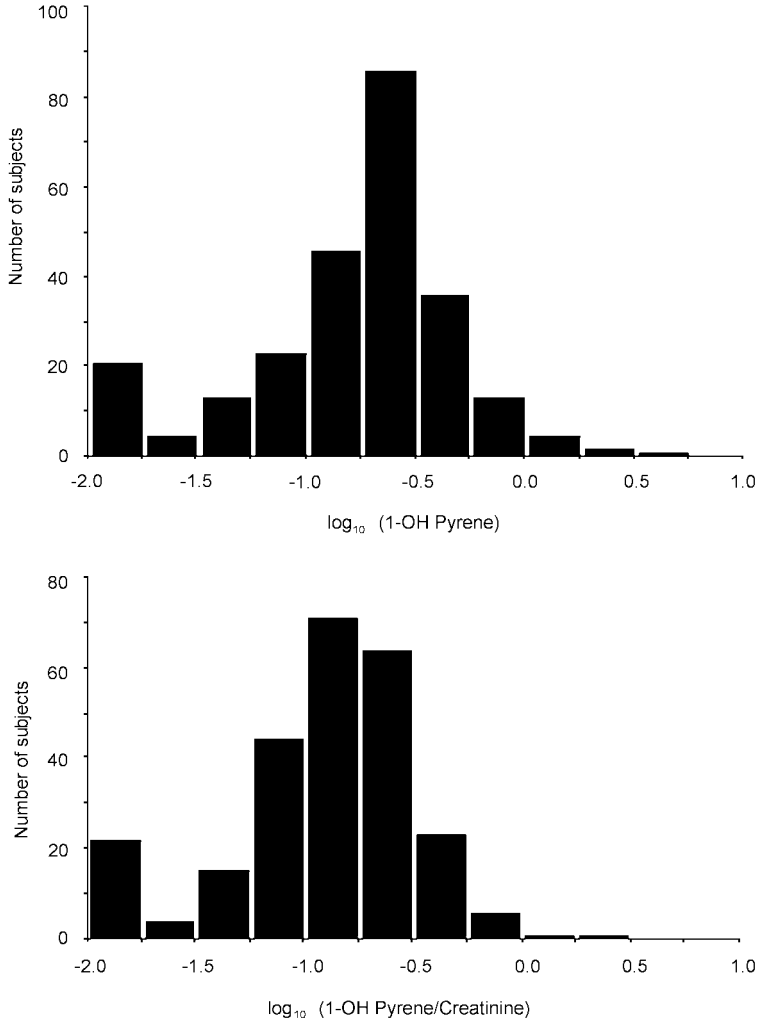

Fig. 3. Distribution of urinary 1-hydroxypyrene concentrations after log-transformation.

and a urine sample from a smoker. The emission and excitation wavelength scans of the HPLC peak of the standard sample and the HPLC fraction with the same retention time from the urine sample were identical (Fig. 2). The detection limit was 0.02 $\mu g/l$. The samples with measurement results below the detection limit were assigned a value of half of the detection limit. As the distribution of urinary 1-hydroxypyrene levels was almost logarithmically normal both with and without creatinine correction (Fig. 3), statistical analyses were carried out after log-transformation. The between-day relative standard deviation (CV) was 10.7% at 1.0 $\mu g/l$.

Photodegradation of 1-hydroxypyrene was studied by exposing the 1-hydroxypyrene solution to artificial light (fluorescent lamp) in an experimental room (Fig. 4). The 1-hydroxypyrene concentration was reduced exponentially over time with a half-life of approximately

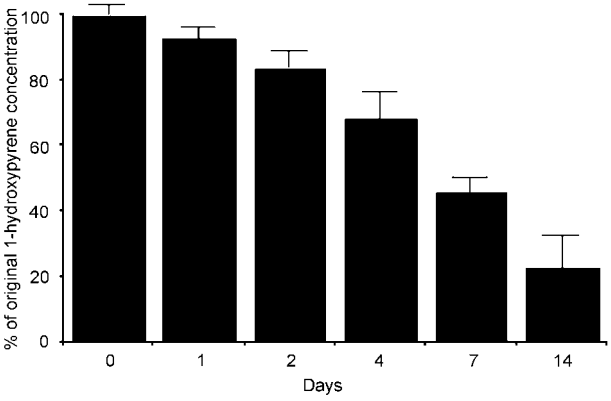

Fig. 4. Photodegradation of 1-hydroxypyrene (2 ng/m*l* in 50% acetonitrile) on an experimental bench under artificial light under the usual sample handling conditions.

Table 1. Quantification of gender and lifestyle data, and characteristics of subjects

Variable	Quantification	Number of Subjects
Gender	Male=0	196
	Female=1	55
Age	Age	251
Smoking	Non-smoker=0	137
	Smoker=Number of cigarettes smoked within 24 h	114
Coffee and tea	Usually not taken=0	39
	0–1 cup/d=1;	49
	1–2 cups/d=2	117
	>3 cups/d=3	46
Alcohol consumption	Non-drinker=0	78
	0–1 d/wk=1	53
	2–4 d/wk=2	40
	>5 d/wk=3	80
Dietary balance		
Dummy valuable (X1)	Eats more vegetables=0; Eats more meat or fish=0	85
	Eats meat or fish, and vegetables in moderate proportions=1	166
Dummy valuable (X2)	Eats more vegetables=0; Eats meat or fish, and vegetables in moderate proportions=0	224
	Eats more meat or fish=1	27
Greasy food	Does not consume large amounts of greasy foods=0	117
	Consumes large amounts of greasy foods=1	134
Salt	Does not eat salty foods=0	112
	Eats salty foods=1	139
Sweets and fruits	Seldom eats=0	130
	Frequently eats=1	121

7 d. Although the half-life of 1-hydroxypyrene photodegradation would vary according to the intensity of the room light, the treatment of urine samples for analysis is not likely to cause a significant decrease of the 1-hydroxypyrene level except for the step of overnight enzymatic digestion. Therefore, only the incubation with β-glucuronidase was done in the dark.

Effects of lifestyle on concentration of urinary 1-hydroxypyrene

Table 1 shows the characteristics of the subjects. Multiple linear regression analysis of lifestyle factors and urinary 1-hydroxypyrene levels revealed that cigarette smoking and dietary balance (dummy valuable X2; whether more meat and/or fish, or more vegetables were consumed) were significantly related to the urinary 1-hydroxypyrene concentration (Table 2). The great majority of the variance was found to be due to cigarette smoking. Urinary 1-hydroxypyrene levels according to smoking status and dietary balance are shown in Table 3. The urinary 1-hydroxypyrene concentration of smokers was about 2 times higher than that of non-smokers ($p<0.001$). Subjects who ate more meat and/or fish excreted 1.5–2 times more 1-hydroxypyrene in urine than those who ate more vegetables ($p<0.05$).

Discussion

In this study, the 1-hydroxypyrene concentration in urine was analyzed by the method described by Taguchi *et al.*[3] with a minor modification. In most reports, 1-hydroxypyrene has been measured using the method developed by Jongeneelen[4], in which the samples are cleaned using a Sep Column and concentrated by evaporation. Compared to that method, our method is simpler, and is more convenient for analyzing a large number of samples in an epidemiological study because of its simplicity. Its reproducibility is also good because pretreatment consists of only one step (glucuronidase treatment). The detection limit of this method did not cause severe problems in analyzing the 1-hydroxypyrene levels of the subjects in the present study. The percentage of samples with 1-hydroxypyrene levels under the detection limit was only 8% (21 out of 251 samples). Therefore, we consider that this method is useful for the measurement of urinary 1-hydroxypyrene levels from subjects exposed to ambient air.

In the present study, the urinary 1-hydroxypyrene concentration of smokers was 0.170 (2.67) μg/g creatinine

Table 2. Multiple linear regression analysis of lifestyle factors in determining urinary concentration of log-transformed 1-hydroxypyrene

	Log(1-OHpyrene)[a]				Log(1-OHpyrene/C)[b]			
	Regression Coefficient	Standard Error	Standard Regression Coefficient	*p*-Value	Regression Coefficient	Standard Error	Standard Regression Coefficient	*p*-Value
Gender	–0.087	0.084	–0.072	0.297	0.001	0.074	0.001	0.985
Age	–0.000	0.003	0.003	0.962	0.004	0.003	0.101	0.118
No. of cigarettes	0.014	0.003	0.314	<0.001**	0.013	0.003	0.346	<0.001**
Coffee and tea	0.020	0.035	0.038	0.562	0.028	0.031	0.059	0.359
Alcohol	–0.003	0.029	–0.008	0.916	0.011	0.026	0.029	0.685
Dietary balance (X1)	0.163	0.101	0.152	0.106	0.114	0.089	0.119	0.202
Dietary balance (X2)	0.264	0.113	0.221	0.021*	0.226	0.100	0.212	0.025*
Greasy food	0.024	0.064	0.023	0.711	–0.034	0.056	–0.037	0.543
Salt	–0.001	0.064	–0.001	0.990	0.005	0.056	0.006	0.928
Sweets and fruits	–0.026	0.067	–0.025	0.698	–0.072	0.059	–0.078	0.226
Intercept	–1.116	0.213	–1.116	<0.001	–1.388	0.188	–1.388	<0.001
R-square	0.155				0.172			
p-value	<0.001				<0.001			

[a]Logarithm of 1-OHpyrene (*μg/l*), [b]Logarithm of 1-OHpyrene/creatinine (*μg*/g creatinine). * $p<0.05$; ** $p<0.001$.

Table 3. The effects of smoking and dietary balance on urinary 1-hydroxypyrene concentration

	Uncorrected (*μg/l*)								
	Smoker			Non-smoker			Total		
Dietary balance	N	G.M.[a] (C.I.)[c]	G.S.D.[b]	N	G.M.[a] (C.I.)[c]	G.S.D.[b]	N	G.M.[a] (C.I.)[c]	G.S.D.[b]
Eats more meat or fish	25	0.356 (0.078–1.347)	2.07	33	0.137 (0.026–0.736)	2.36	58	0.199 (0.034–1.173)	2.48
Eats both in moderate proportions	77	0.224 (0.022–2.272)	3.26	89	0.113 (0.012–1.034)	3.09	166	0.156 (0.015–1.634)	3.32
Eats more vegetables	12	0.138 (0.007–2.720)	4.58	15	0.075 (0.009–0.634)	2.97	27	0.099 (0.008–1.280)	3.70
Total	114	0.231 (0.024–2.218)	3.17	137	0.114 (0.014–0.926)	2.92	251	0.157 (0.016–1.530)	3.20

[a] Geometric mean,[b] Geometric standard deviation,[c] 95% confidence internal, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

	Correction for creatinine (*μg*/g creatinine)								
	Smoker			Non-smoker			Total		
Dietary balance	N	G.M.[a] (C.I.)[c]	G.S.D.[b]	N	G.M.[a] (C.I.)[c]	G.S.D.[b]	N	G.M.a (C.I.)[c]	G.S.D.[b]
Eats more meat or fish	25	0.244 (0.093–0.637)	1.63	33	0.099 (0.021–0.471)	2.21	58	0.146 (0.030–0.716)	2.25
Eats both in moderate proportions	77	0.158 (0.021–1.170)	2.77	89	0.087 (0.012–0.648)	2.78	166	0.115 (0.014–0.922)	2.89
Eats more vegetables	12	0.125 (0.008–1.888)	3.99	15	0.064 (0.008–0.494)	2.83	27	0.086 (0.008–0.959)	3.41
Total	114	0.170 (0.024–1.182)	2.69	137	0.087 (0.116–0.588)	2.65	251	0.118 (0.015–0.899)	2.82

[a]Geometric mean, [b]Geometric standard deviation, [c]95% confidence internal, *$p<0.05$, **$p<0.01$, ***$p<0.001$.

(0.088 μmol/mol creatinine), and that of non-smokers was 0.087 (2.65) μg/g creatinine (0.045 μmol/mol creatinine), expressed as geometric means (geometric standard deviations). These urinary 1-hydroxypyrene levels in Japanese are very similar to the Korean levels reported in our previous studies, that is, 0.04 (1.81) μmol/mol creatinine in smokers and 0.03 (1.71) μmol/mol creatinine in non-smokers among male university students[5], and 0.025 μmol/mol creatinine in Korean people (age: 36.5 +/– 11.1 yr, male: 63.3 %, smokers: 38.9%) who were not occupationally exposed to PAHs[6].

Van Rooji *et al.*[2] reported that smokers excreted on average 0.25 (range; 0.17–0.76) μmol/mol creatinine and non-smokers on average 0.12 (0.08–0.68) μmol/mol creatinine in a study examining 76 Dutch male volunteers. In smoking and non-smoking German housewives, the medians of urinary 1-hydroxyprene were 0.25 μmol/mol creatinine and 0.08 μmol/mol creatinine, respectively[7]. Van Rooji *et al.*[2] also reviewed earlier studies and concluded that the median baseline 1-hydroxypyrene concentrations in the urine of male smokers and non-smokers were 0.17–0.76 μmol/mol creatinine and 0.08–0.68 μmol/mol creatinine, respectively. These values are higher that those in Japanese and Koreans. The effect of the analytical methods on the measurement results is not clear at the present time. However, van Rooji *et al.* reviewed data obtained in the Netherlands, Turkey, China, Denmark and Belgium. As Japanese and Korean people eat less meat compared to these other nationalities, the difference in baseline 1-hydroxypyrene levels might have been caused by differences in dietary habits.

The present study confirms the findings of earlier studies[6, 8] that the smoking of cigarettes significantly increases the urinary 1-hydroxypyrene concentration, while alcohol consumption does not affect the urinary 1-hydroxypyrene concentration.

The relative contribution of different sources to daily pyrene intake has been estimated in human volunteers who were not occupationally exposed to PAHs[2]. Mainstream smoking and foods containing PAHs accounted for some 99% of the total pyrene intake, while the contributions of ETS and indoor/outdoor ambient air were insignificant. The variation of the level of urinary 1-hydroxypyrene explained by smoking and foods was 66% and 2%, respectively.

In the present study, the urinary 1-hydroxypyrene level was affected mainly by cigarette smoking. Dietary balance, that is, the ratio of meat/fish to vegetables, was also related to these levels. In the present study, we asked subjects 4 questions concerning dietary habits, i.e. dietary balance, greasy food, salt and sweet/fruits. There were no criteria for how the subjects should answer: they answered these questions as they felt appropriate. However, it is surprising that urinary 1-hydroxypyrene levels differed by 2–3 times among people with different dietary habits which were classified according to a very simple question, that is, dietary balance.

The urinary concentrations of some metabolites other than 1-hydroxypyrene are also affected by lifestyle. The background level of urinary hippuric acid is reduced by alcohol consumption. The urinary phenol concentration is reduced by coffee and tea intake, and the *o*-cresol concentration is increased with the number of cigarettes smoked[9]. However, dietary habits, for example, dietary balance and the intake of greasy food, salt, sweets and fruits, are not related to the urinary background levels of hippuric acid, phenol, or *o*- or *p*-cresols.

1-Hydroxypyrene is formed from pyrene by reactions catalyzed by cytochrome P450s. We have studied the 1-hydroxylation of pyrene by 10 isoforms of human cytochrome P450s expressed from cloned cDNAs[10]. CYP1A1 exhibited the highest activity at both 0.5 and 50 μM pyrene, followed by CYP1B1 and 1A2, whereas other isoforms, including CYP2A6, 2C8, 2C9*1, 2C19, 2D6, 2E1 and 3A4, showed very low or undetectable rates of 1-hydroxylation.

CYP1A1 is reported to have polymorphic sites in exon 7 and in the 3'-flanking region. An isoleucine-valine transition caused by a point mutation in exon 7[11] has been found to cosegregate with a point mutation in the 3'-flanking region, which causes the generation of an MspI restriction site. It is suspected that CYP1A1 polymorphism causes changes in monooxygenation activity that alter the rate of formation of 1-hydroxyprene from pyrene. Recently, a relationship between CYP1A1 polymorphism and urinary 1-hydroxypyrene levels has been reported. Wu *et al.*[12] showed a significant increase in urinary 1-hydroxypyrene excretion in coke-oven workers with the CYP1A1 MspI homozygous variant genotype. Merlo *et al.*[13] studied the urinary excretion of 1-hydroxypyrene in 94 traffic police officers and 52 control subjects exposed to indoor air pollution. No significant role of any metabolic polymorphism of CYP1A1 (MspI) was detected. Our recent studies[6, 8] showed no differences in urinary 1-hydroxypyrene levels according to CYP1A1 polymorphism, either. One reason why the results are inconsistent even after the adjustment for smoking and occupational exposure may be that the differences of 1-hydroxypyrene formation from pyrene due to CYP1A1 polymorphism are concealed by the wide variation of pyrene intake from various sources such as dietary intake.

In conclusion, we clarified that the urinary 1-hydroxypyrene level was strongly affected by smoking. It is also noteworthy that the answer to the simple question of whether subjects eat more meat and fish or more vegetables may also be a good indicator for predicting urinary 1-hydroxypyrene levels of subjects who are not exposed to PAHs occupationally.

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