

## The Biological Monitoring of Urinary 1-hydroxypyrene by PAH Exposure Among Smokers

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**ABSTRACT:** This study examines changes in the urinary 1-hydroxypyrene (1-OHP) concentration as a polycyclic aromatic hydrocarbon (PAH) metabolite related to smoking among eight volunteers. The study subjects consisted of 8 participants (2 smokers, 4 ex-smokers and 2 non-smokers; mean age 23, range 21 ~ 24 years old) in a smoking cessation program. Three urine samples were collected each week for 4 weeks from each participant to give a total of 96 urine samples. None of the participants smoked during the study period. Levels of urinary 1-OHP, as an internal dose marker of smoke containing PAHs, were measured by high performance liquid chromatography (HPLC) fluorescence detector. Urinary 1-OHP levels in the ex-smokers were decreased during the 4 weeks study period, due to ambient sources of PAH exposure. Thus, smoking cessation was only expected to result in reduction of urinary 1-OHP. Urinary 1-OHP levels were significantly correlated with the amounts of cigarettes smoked, as assessed by the questionnaire (Spearman's correlation coefficient,  $r=0.23$ ,  $P=0.03$ ). This study suggested that urinary 1-OHP can be a potentially useful biomarker of smoke.

**Key words:** Biomarker, Polycyclic aromatic hydrocarbons, 1-hydroxypyrene, Cigarette smoking

### INTRODUCTION

Cigarette smoking is the most preventable risk factor for health problems such as lung cancer (Hausmann, 2007), coronary heart disease (Noda *et al.*, 2008) and chronic obstructive pulmonary disease (Petty, 2006). The smoking rate in Korean men is the highest among Organisation for Economic Cooperation and Development (OECD) countries (OECD, 2006). Cigarette smoking is the cause of 45 % of all hospitalized patients with cardiovascular disease in Korea (Sohn *et al.*, 2008). The carcinogens in tobacco smoke are significantly related to the risk of head and neck cancer (Lacko *et al.*, 2009), and lung cancer (Bhutani *et al.*, 2008; Church *et al.*, 2009). Kidney diseases are also higher in smokers than in ex-smokers and non-smokers (Yoon *et al.*, 2009). Polycyclic aromatic hydrocarbons (PAHs) are one class of chemical compounds generated during cigarette smoking (Hoffmann and Hoffmann, 1997). About 10 carcinogens in PAHs are included in the cigarette smoke inhaled by smokers (Ding *et al.*, 2007). Urinary 1-hydroxypyrene (1-OHP), a metabolite of pyrene, was proposed as a biological marker of exposure to PAHs in humans and their measurements have been useful for exposure monitoring

(Jongeneelen, 2001). Urinary 1-OHP was widely used as biomarker of human exposure to PAHs. Many studies have investigated urinary 1-OHP in humans (Castano-Vinyals *et al.*, 2004; Jongeneelen, 2001), such as the dietary intake of PAHs and smoking (Suzuki and Yoshinaga, 2007). The difference in urinary levels of 1-OHP has also been compared in smokers and non-smokers (Heudorf and Angerer, 2001). In one previous study that monitored the smoking cessation of 17 smokers at various times, 1-OHP was decreased from 3 days after cessation (Carmella *et al.*, 2009). Urinary 1-OHP was decreased after smoking cessation during 26 weeks (Hecht *et al.*, 2004). Another study reported that urinary levels of 1-OHP and 2-naphthol decreased after smoking cessation (Ichiba *et al.*, 2006). However, the change of 1-OHP in urine hasn't been monitored for ex-smokers during long-term in Korean. Therefore, in this study, we measured the change in urinary 1-OHP as PAH metabolites in smokers, ex-smokers and non-smokers.

### MATERIALS & METHODS

Acetonitrile and 1-OHP were purchased from Wako (Osaka, Japan).  $\beta$ -Glucuronidase/sulfatase (G-

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0876) was purchased from Sigma Company (St. Lorraine, Mo. U.S.A). Other materials were 99% purified. The study subjects consist of 8 participants in a smoking cessation program. The subjects consisted of 7 men and one woman who were 2 smokers, 4 ex-smokers, and 2 non-smokers, with a mean age of 23 (range 21-24) years. First morning void urine samples were collected three times a week for four weeks (August, 2004) in order to investigate the levels of urinary 1-OHP. These urine samples were frozen at -76°C until the analysis. Information on demographic characteristics, smoking status, alcohol consumption, and food consumption were collected using a self-administered questionnaire.

To analyze urinary 1-OHP, urine samples were buffered with sodium acetate (0.2M, pH 5.0) and hydrolyzed enzymatically using β-glucuronidase/sulfatase for 16 hours at 37°C in a shaking water bath. After hydrolysis, acetonitrile was added and treated samples were centrifuged at 1,000 rpm for 10 min. A high-performance liquid chromatography (HPLC) system consisting of a pump (Hitachi L-6210, Tokyo, Japan), and fluorescence detector (Hitachi LaChrom L-7480, Tokyo, Japan) was used. A 250mm×4.6mm column (J'sphere ODS-H80; YMC, Wilmington, NC, USA) was used to determine levels of urinary 1-OHP, using a 60% acetonitrile and a 40% filtered water mobile phase and excitation/emission wavelengths of 242/388 nm. The limitation of detection was 0.05mg/ml and the coefficient variation was under 10 %.

The means of urinary 1-OHP concentrations in the smokers, non-smokers and ex-smokers were compared with analysis of variance (ANOVA). Spearman's correlation coefficient was used for the smoking amount by questionnaire and urinary 1-OHP from all participants. All statistical analyses were performed with the SPSS statistical package version 12.0 and statistical significance was defined as  $P < 0.05$ .

**RESULTS & DISCUSSION**

The metabolites of pyrene may play a significant role in the urinary excretion kinetics of 1-hydroxypyrene (1-OHP) (Bouchard *et al.*, 2002). The mean half-life of the urinary excretion of 1-OHP was 29 hours in 17 Chinese workers (Huang *et al.*, 2007), and 18.6 h in coke oven workers (Lu *et al.*, 2002). The polycyclic aromatic hydrocarbons (PAHs) exposure was evaluated by the levels of 1-OHP and 2-naphthol in the smoker's urine in a personal computer games room (Kim *et al.*, 2004). The result of that study found that the levels of urinary 1-OHP increased with increased duration of game room use in Korea (Kim *et al.*, 2004).

This study monitored the levels of urinary 1-OHP as a biomarker of PAH exposure in young Korean smok-

ers, non-smokers and ex-smokers during 4 weeks. Information on demographic characteristics such as age, sex, diet and smoking status was collected using a self-administered questionnaire (Table 1). The duration of smoking in the 2 smokers and 4 ex-smokers was 6 and 4 years, and the pack years were 10 and 7 per day, respectively. Grilled meats were consumed twice a week among smokers, once a week among ex-smokers and three times a week among non-smokers (Table 1).

**Table 1. Demographic characteristics of the study subjects**

Categories	Smoker	Ex-smoker	Non-smoker
N	2	4	2
Mean age (yr)	24	24	21
Duration of smoking (yr)	6	4	-
Pack years (per day)	10	7	-
Consumption of grill meat (per week)	2	1	3
Drinking amount (bottles/week)	2	1	1

Levels of urinary 1-OHP were  $0.20 \pm 0.17$  μmol/mol creatinine in the 2 smokers (urine sample n=24),  $0.06 \pm 0.06$  μmol/mol creatinine in the 4 ex-smokers (urine sample n=48) and  $0.14 \pm 0.14$  μmol/mol creatinine in the 2 non-smokers (urine sample n=24) ( $P < 0.01$  by ANOVA) (Fig. 1). The smokers had the highest levels of urinary 1-OHP and the ex-smokers the lowest. The levels of urinary 1-OHP had decreased in this study significantly. The levels of urinary 1-OHP were also significantly correlated with the amount of cigarettes smoked per day for smokers and ex-smokers (Spearman's correlation coefficient  $r = 0.23$ ,  $P = 0.03$ ) (Fig. 2). In previous study, the urinary 1-OHP of the smokers was significantly reduced by reducing the number of cigarettes per day (Hecht *et al.*, 2004). It was reported that after 3 days of cessation, the urinary 1-OHP of some smokers was decreased significantly (Carmella *et al.*, 2009). In another study, 1-OHP was decreased after nicotine patches were attached on some tobacco smokers (Hatsukami *et al.*, 2004). Ichiba and colleagues reported that they measured 1-OHP, 2-naphthol and 1-naphthol as PAH metabolites before and after smoking cessation. In the result, the levels of all markers were decreased by 19~15% after smoking cessation (Ichiba *et al.*, 2006). The concentrations of 1-OHP, the biomarker of cigarette smoke exposure, were not significant different between of passive smokers and the non-smok-

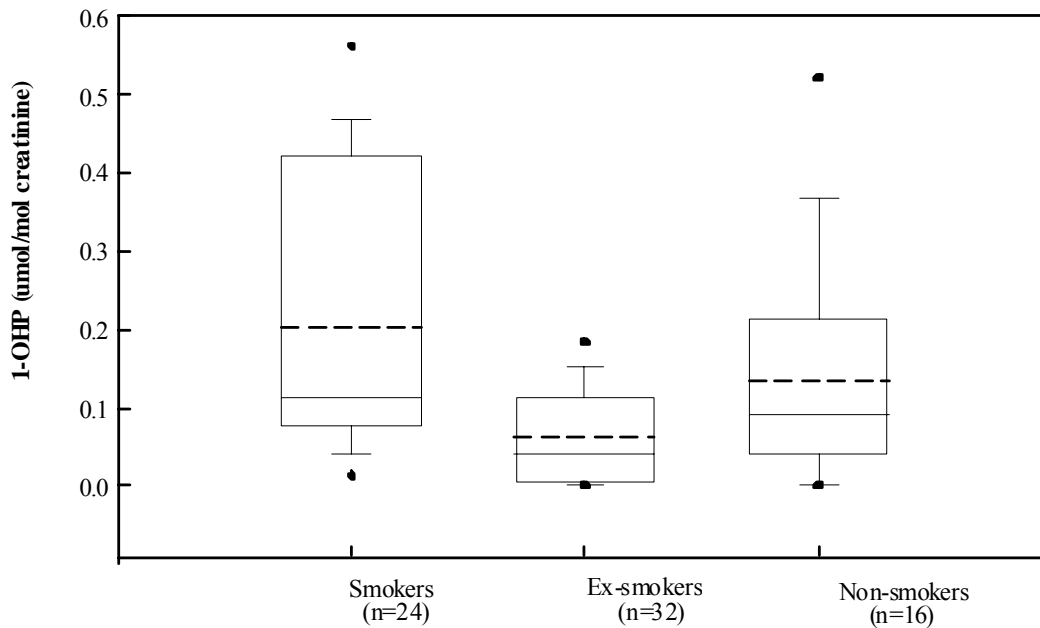


Fig. 1. Levels of urinary 1-hydroxypyrene among the 2 smokers (24 urine samples), 4 ex-smokers (n=32) and 2 non-smokers (n=16) ( $P < 0.01$  by ANOVA test). Short dash is median, line is mean and block dots are 5<sup>th</sup>/95<sup>th</sup> percentiles

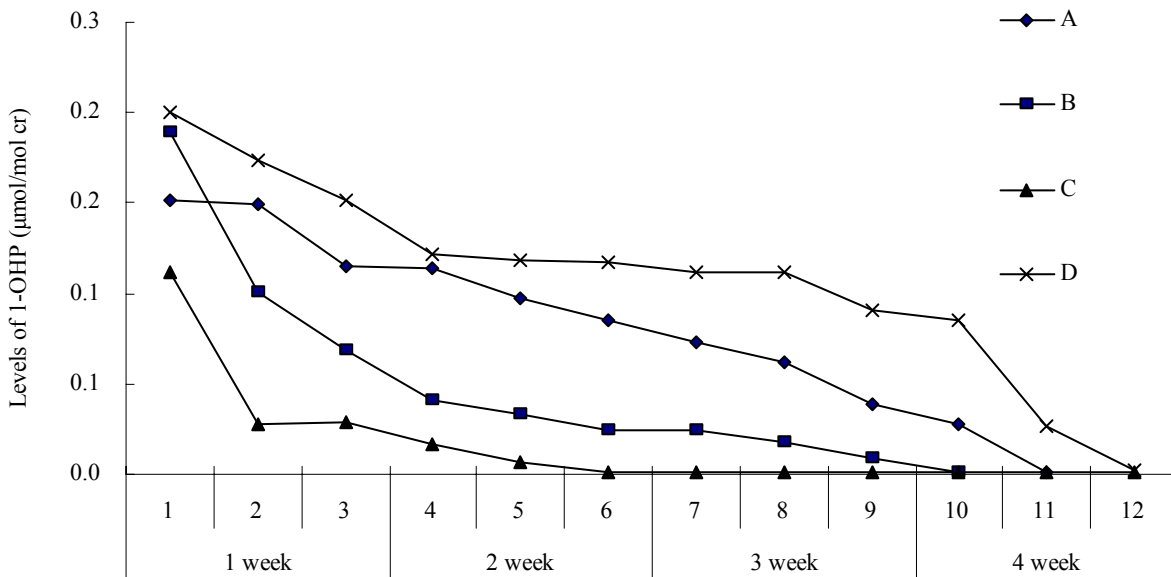


Fig. 2. Decrease of urinary 1-hydroxypyrene levels through time series among the 4 ex-smokers

ers (Zielińska-Danch *et al.*, 2006). Most of the smokers had higher a urinary level of 1-OHP than non-smokers (Dor *et al.*, 2000). However, urinary 1-OHP of the ex-smokers slowly decreased during 1~4 weeks, because they ate grilled meat and may have been exposed to ambient sources such as the vehicle exhaust in the present study.

The primary advantage of this study was that previous studies in Korea never monitored the urinary levels of 1-OHP in ex-smokers. We observed a change of the biomarker after long-term smoking cessation among Koreans. The levels of urinary 1-OHP expo-

sure to PAH from tobacco smoking were significantly correlated. The study limitations were the restriction of the participants' age to those in their 20s and the small sample size.

**CONCLUSION**

The levels of urinary 1-OHP of the ex-smokers had decreased significantly after smoking cessation. And, urinary 1-OHP levels were statistically correlated with the smoking amounts. Therefore, urinary 1-OHP as a PAH metabolite was a strong predictor of cigarette smoking. However, future study with a larger study sample is required.

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