Association between polycyclic aromatic hydrocarbons exposure and bone turnover in adults

Yuan-Yuei Chen^{1,2,6}, Tung-Wei Kao^{2,3,4}, Chung-Ching Wang², Chen-Jung Wu^{2,3,5}, Yi-Chao Zhou² and Wei-Liang Chen^{2,3}

¹Department of Internal Medicine, Tri-Service General Hospital Songshan Branch, ²Division of Family Medicine, Department of Family and Community Medicine, Tri-Service General Hospital, ³Division of Geriatric Medicine, Department of Family and Community Medicine, Tri-Service General Hospital, School of Medicine, National Defense Medical Center, Taipei, Taiwan, Republic of China, ⁴Graduate Institute of Clinical Medical, College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China, ⁵Division of Family Medicine, Department of Community Medicine, Taoyuan Armed Forces General Hospital, Taoyuan, Taiwan, Republic of China, and ⁶Department of General Medicine, Tri-Service General Hospital, School of Medicine, National Defense Medical Center, Taipei, Taiwan, Republic of China

Correspondence should be addressed to W-L Chen
Email
weiliang0508@gmail.com

Abstract

Background: Cigarette smoking is a risk factor of osteoporosis and bone fracture. Tobacco smoke contains several polycyclic aromatic hydrocarbons. Thus, we hypothesized that environmental polycyclic aromatic hydrocarbon exposure is associated with bone loss and fracture risk. The present study examined the association between polycyclic aromatic hydrocarbon exposure and bone turnover in the general adult population.

Methods: A total of 1408 eligible participants from the National Health and Nutrition Examination Survey (NHANES 2001–2006) were included in this cross-sectional analysis. The levels of urinary N-telopeptide and serum bone-specific alkaline phosphatase, which are biomarkers of bone resorption and formation, respectively, were assessed. Meanwhile, polycyclic aromatic hydrocarbon exposure was evaluated using the concentrations of urinary polycyclic aromatic hydrocarbon metabolites. The association between polycyclic aromatic hydrocarbon exposures and N-telopeptide, and bone-specific alkaline phosphatase levels was assessed using a multivariate linear regression model.

Results: All polycyclic aromatic hydrocarbon metabolites except 3-phenanthrene were significantly associated with increased N-telopeptide levels ($P < 0.05$) after adjustment of relevant covariables. However, no significant relationship was observed between polycyclic aromatic hydrocarbon metabolites and bone-specific alkaline phosphatase levels. This relationship remained significant after the participants were assessed according to sex ($P < 0.05$). Additionally, all polycyclic aromatic hydrocarbon metabolites showed a positive association with N-telopeptide levels in participants aged <60 years ($P < 0.05$).

Conclusion: Polycyclic aromatic hydrocarbon exposure is associated with increased bone resorption among the general adult population in the United States. Further studies must assess the potential mechanisms associated with the adverse effects of polycyclic aromatic hydrocarbon exposure on bone loss.

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Introduction

Polycyclic aromatic hydrocarbons are organic compounds with multiple aromatic rings of only carbon and hydrogen (1). These pollutants are generated via the incomplete combustion of organic matter from either natural or synthetic sources and are widely present in various environment matrices, such as air, soil, and water. In the general population, exposure mainly occurs via inhalation of contaminated air and consumption of contaminated food and water (2). In particular, high exposure can occur via cigarette smoking. polycyclic aromatic hydrocarbon exposure causes various systemic diseases, including cancer (3, 4), obstructive lung diseases (5), metabolic syndrome (6), and cardiovascular diseases (7, 8).

The bone is a dynamic tissue that constantly undergoes remodeling throughout life (9); this is caused by the coupling of bone formation and resorption at various rates balancing bone turnover (10). Epidemiological studies have reported that cigarette smoking contributes to decreased bone mass and osteoporosis (11, 12, 13). Polycyclic aromatic hydrocarbon is a component of cigarette smoke that decreases bone mechanical properties, leading to bone loss (14).

To the best of our knowledge, no study has examined the relationship between polycyclic aromatic hydrocarbon exposure and bone loss in the human population. Thus, the present study investigated the associations between urinary polycyclic aromatic hydrocarbon metabolite and biomarkers of bone turnover in a cross-sectional analysis of a nationally representative general adult sample in the United States.

Methods

Study design

The National Health and Nutrition Examination Survey (NHANES) is a cross-sectional survey on a nationally representative sample that collects demographic, clinical, behavioral, dietary, social, and laboratory data about the health and nutritional status of non-institutionalized individuals in the United States. The National Center of Health Statistics (NCHS) Ethics Review Board approved the study. All participants were requested to sign an informed consent form before the initiation of the study.

Participants

Initially, adults aged ≥ 20 years from the NHANES 2001–2006 were included in the study. After excluding those

with missing information such as demographic and laboratory data, 1408 eligible participants were enrolled for further analysis.

Assessment of bone turnover biomarker levels

N-telopeptide is a biomarker of bone resorption, which is a telopeptide used to evaluate the rate of bone turnover (15). N-telopeptide can be measured in the urine or blood (16). In NHANES, analysis of urinary N-telopeptide levels was performed using a competitive-inhibition enzyme-linked solid-phase immunosorbent assay (Osteomark; NHANES 1999–2001) or the Vitros ECI competitive assay (NHANES 2002). Bone-specific alkaline phosphatase is synthesized by osteoblasts and is involved in the calcification of bone matrix. Moreover, it is a highly specific marker of bone formation. In this study, the bone-specific alkaline phosphatase level in the blood samples was measured using the Beckman Access Ostase assay (Beckman Coulter, LaBrea, CA, USA). The reference range for N-telopeptide was 14–87 nM BCE/mM in adult males and 5–65 nM BCE/mM in premenopausal females. The intra- and inter-assay coefficients of variation were 7.9 and 10.9%, respectively. The reference range for bone-specific alkaline phosphatase was 3.7–20.9 $\mu\text{g/L}$ in adult males and 2.9–14.5 $\mu\text{g/L}$ in premenopausal females. The intra- and inter-assay coefficients of variation were 4.7 and 9.1%, respectively.

Level of urinary polycyclic aromatic hydrocarbons

Urine samples were collected by experienced technicians and were stored at -20°C according to laboratory/medical procedures. Urinary metabolites were quantified by capillary gas chromatography combined with high-resolution mass spectrometry. Details regarding the procedure are available on the NHANES website (17). The urinary hydroxylated polycyclic aromatic hydrocarbon metabolites included 1-naphthalene, 2-naphthalene, 2-fluorene, 3-fluorene, 1-phenanthrene, 2-phenanthrene, 3-phenanthrene, and 1-pyrene.

Measurement of covariates

For causal inferences from observational data, it was important to elucidate confounding, but it was not easy to recognize. A confounder was a factor that was associated with the exposure and with the outcome and was not in the causal path between the exposure and outcome. In Fig. 1, we demonstrated our Directed

Acyclic Graph (DAG) analysis of the study based on the correlations and previous evidences. Factors of interest, such as demographic information, cigarette use, and medical histories, were assessed using the respective questionnaires. The patients were classified according to race: non-Hispanic white and others. The question 'have you ever smoked cigarettes?' was used to assess cigarette use. Patients with medical histories were defined as participants who had been diagnosed with congestive heart failure (CHF), coronary heart disease (CHD), angina, heart attack, and cancer. Laboratory data were collected using the respective standard procedures. Cotinine is the predominant metabolite of nicotine that can be used as a marker of both active smoking and environmental exposure. It is generally preferred over nicotine for such assessments due to its substantially longer half-life. Serum cotinine level was assessed using isotope dilution HPLC/atmospheric pressure chemical ionization tandem mass spectrometry. C-reactive protein (CRP) level was measured using latex-enhanced nephelometry with an automated Behring Nephelometer Analyzer System (Behring Diagnostics, Inc, Somerville, NJ, USA). Serum albumin, total calcium, and glucose levels were assessed using the Beckman Synchron LX20. All data about specimen collection are available on the NHANES dataset.

Statistical analysis

The associations between polycyclic aromatic hydrocarbons and N-telopeptide and bone-specific alkaline phosphatase were evaluated using a linear

regression model, which was adjusted for the following multivariate models. Model 1 included age, sex, and race/ethnicity; Model 2 included Model 1, cotinine, CRP, glucose, total calcium, and albumin levels; Model 3 included Model 2 and history of CHF, CHD, angina, heart attack, and cigarette use. We tested for effect modification by polycyclic aromatic hydrocarbon groups and gender by including interaction terms in the models for the N-telopeptide and bone-specific alkaline phosphatase. Because of the significant findings of the interaction testing, we performed further stratified analyses. A P value ≤ 0.05 was considered statistically significant. Differences in group characteristics were compared using the ANOVA and Bonferroni *post hoc* comparisons. All analyses in the current study were conducted using the Statistical Package for the Social Sciences software version 22.0 (SPSS Inc.) for Windows.

Results

The participants comprised 692 males and 716 females; their demographic and laboratory data are shown in Table 1. The mean ages were 49.25 ± 17.62 years and 47.70 ± 19.26 years for males and females, respectively. The male participants had significantly higher levels of cotinine, glucose, total calcium, and albumin than female participants. In addition, a higher prevalence of cigarette use was observed in the male population than female population.

Table 2 displays the associations between polycyclic aromatic hydrocarbon metabolites as well as N-telopeptide and bone-specific alkaline phosphatase. A positive relationship was observed between all polycyclic aromatic hydrocarbon metabolites, except 3-phenanthrene and N-telopeptide after adjustment of covariables (1-naphthol: $\beta=0.006$, 95% CI=0.004–0.008; 2-naphthol: $\beta=0.021$, 95% CI=0.017–0.025; 3-fluorene: $\beta=0.431$, 95% CI=0.355–0.507; 2-fluorene: $\beta=0.168$, 95% CI=0.134–0.202; 1-phenanthrene: $\beta=0.264$, 95% CI=0.197–0.331; 2-phenanthrene: $\beta=0.503$, 95% CI=0.351–0.655; and 1-pyrene: $\beta=0.157$, 95% CI=0.083–0.230). However, no significant association was observed between polycyclic aromatic hydrocarbon metabolites and bone-specific alkaline phosphatase.

Figures 2 and 3 depict the sex-based relationships between polycyclic aromatic hydrocarbon metabolites as well as N-telopeptide and bone-specific alkaline phosphatase. The positive relationship between polycyclic aromatic hydrocarbon metabolites and

Figure 1

Directed Acyclic Graph. A full colour version of this figure is available at <https://doi.org/10.1530/EJE-19-0750>.

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Table 1 Characteristics of study population.

Variables	Male	Female	P value
Continuous variables, mean (s.d.)			
Age (years)	49.25 (17.62)	47.72 (19.26)	0.002
Glucose (mg/dL)	100.08 (38.96)	93.48 (26.81)	0.004
Total calcium (mg/dL)	9.48 (0.38)	9.37 (0.42)	0.025
Albumin (g/dL)	4.34 (0.30)	4.09 (0.34)	0.013
C-reactive protein (mg/dL)	0.42 (1.04)	0.56 (0.84)	0.110
Cotinine (ng/mL)	71.54 (140.93)	36.92 (102.38)	<0.001
1-Naphthol (μg/L)	6.31 (14.26)	7.62 (25.84)	0.004
2-Naphthol (μg/L)	7.36 (13.38)	5.84 (10.45)	0.003
3-Fluorene (μg/L)	0.46 (0.82)	0.27 (0.56)	<0.001
2-Fluorene (μg/L)	0.90 (1.51)	0.59 (1.28)	<0.001
3-Phenanthrene (μg/L)	0.53 (4.97)	0.32 (1.85)	0.132
1-Phenanthrene (μg/L)	0.27 (0.45)	0.26 (0.80)	0.645
2-Phenanthrene (μg/L)	0.13 (0.21)	0.11 (0.35)	0.618
1-Pyrene (μg/L)	0.15 (0.85)	0.08 (0.17)	0.010
Category variables (%)			
Congestive heart failure	18 (2.6)	13 (1.8)	0.443
Coronary heart disease	42 (6.1)	23 (3.2)	0.776
Angina/angina pectoris	26 (3.8)	16 (2.2)	0.854
Heart attack	34 (4.9)	17 (2.4)	0.279
Cancer or malignancy	55 (7.9)	62 (8.7)	0.881
Cigarette use	421 (60.8)	251 (35.1)	<0.001

N-telopeptide was significantly observed in both sexes after adjustment of pertinent covariables (Fig. 2). Similarly, only 3-phenanthrene was unassociated with increased N-telopeptide level in both sexes. However, no significant association was observed between polycyclic aromatic hydrocarbon metabolites and bone-specific alkaline phosphatase in both sexes (Fig. 3).

Figures 4 and 5 depict the age-based associations between polycyclic aromatic hydrocarbon metabolites and bone turnover markers biomarker levels. All polycyclic aromatic hydrocarbon metabolites were significantly associated with increased N-telopeptide levels in participants aged <65 years ($P < 0.001$) (Fig. 4). In contrast, all polycyclic aromatic hydrocarbon metabolites except 2-naphthol and 3-phenanthrene showed positive relationships with N-telopeptide levels in participants >65 years. However, no significant association was observed between polycyclic aromatic hydrocarbon metabolites and bone-specific alkaline phosphatase in both age groups (Fig. 5).

Discussion

Urinary polycyclic aromatic hydrocarbon metabolites showed a significant association with increased N-telopeptide levels in nationally representative sample of U.S. adult population, independent of sex. This finding

indicated that polycyclic aromatic hydrocarbon exposure induces the process of bone resorption, which causes bone turnover imbalance, leading to bone loss, even in osteoporosis. To the best of our knowledge, this study first examined the relationship between environmental polycyclic aromatic hydrocarbon exposure and bone turnover in a population-based cross-sectional survey.

In this study, polycyclic aromatic hydrocarbon metabolites were associated with increased N-telopeptide levels and not with bone-specific alkaline phosphatase; therefore, they affected bone resorption rather than bone formation. Several epidemiological studies suggest that cigarette smoking is a risk factor of decreased bone mass and osteoporosis. Smokers have significantly lower bone mineral density and higher risk of bone fracture than non-smokers (18, 19). Smoking can increase osteoclastic bone resorption. Increased levels of total and free deoxypyridinoline and C-telopeptide, which are bone resorption markers, are evident in current male smokers than previous smokers or non-smokers (20).

In addition, the levels of urinary N-telopeptide, which is another biomarker of bone resorption, is higher in elderly female smokers than in elderly female non-smokers (21). In a rat model study, Lee *et al.* demonstrated that benzo[a]pyrene, which is a polycyclic aromatic hydrocarbon found in cigarette, causes bone loss based on an *in vivo* study (14). Taken together, polycyclic aromatic hydrocarbons may have detrimental effects on bone turnover that

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Table 2 Association between PAHs and N-Telopeptides.

Variables	Model 1*	P value	Model 2*	P value	Model 3*	P value
	$\beta^{\dagger}$ (95% CI)		$\beta^{\dagger}$ (95% CI)		$\beta^{\dagger}$ (95% CI)	
N-telopeptides						
1-naphthol	0.006 (0.004, 0.008)	<0.001	0.006 (0.004, 0.008)	<0.001	0.006 (0.004, 0.008)	<0.001
2-naphthol	0.018 (0.015, 0.022)	<0.001	0.021 (0.017, 0.025)	<0.001	0.021 (0.017, 0.025)	<0.001
3-fluorene	0.301 (0.238, 0.363)	<0.001	0.424 (0.348, 0.500)	<0.001	0.431 (0.355, 0.507)	<0.001
2-fluorene	0.147 (0.116, 0.179)	<0.001	0.168 (0.134, 0.201)	<0.001	0.168 (0.134, 0.202)	<0.001
3-phenanthrene	0.005 (−0.007, 0.017)	0.450	0.007 (−0.005, 0.019)	0.270	0.007 (−0.005, 0.019)	0.268
1-phenanthrene	0.269 (0.202, 0.336)	<0.001	0.265 (0.198, 0.332)	<0.001	0.264 (0.197, 0.331)	<0.001
2-phenanthrene	0.518 (0.366, 0.669)	<0.001	0.503 (0.351, 0.655)	<0.001	0.503 (0.351, 0.655)	<0.001
1-pyrene	0.162 (0.088, 0.235)	<0.001	0.157 (0.083, 0.230)	<0.001	0.157 (0.083, 0.230)	<0.001
Bone alkaline phosphatase						
1-naphthol	0.001 (0.000, 0.002)	0.173	0.001 (0.000, 0.002)	0.193	0.001 (0.000, 0.002)	0.182
2-naphthol	0.001 (−0.001, 0.003)	0.221	0.001 (−0.001, 0.003)	0.219	0.001 (−0.001, 0.003)	0.203
3-fluorene	0.012 (−0.018, 0.042)	0.428	0.006 (−0.030, 0.042)	0.749	0.008 (−0.028, 0.045)	0.648
2-fluorene	0.011 (−0.004, 0.025)	0.165	0.008 (−0.008, 0.024)	0.320	0.008 (−0.008, 0.025)	0.300
3-phenanthrene	−0.002 (−0.007, 0.004)	0.533	−0.005 (−0.010, 0.001)	0.107	−0.005 (−0.010, 0.001)	0.112
1-phenanthrene	0.028 (−0.004, 0.060)	0.085	0.028 (−0.004, 0.059)	0.084	0.028 (−0.004, 0.059)	0.085
2-phenanthrene	0.062 (−0.009, 0.134)	0.085	0.058 (−0.013, 0.129)	0.107	0.059 (−0.012, 0.130)	0.102
1-pyrene	0.012 (−0.022, 0.046)	0.481	0.013 (−0.021, 0.047)	0.441	0.014 (−0.020, 0.048)	0.431

*Adjusted covariates: Model 1: age, gender, race/ethnicity; Model 2: Model 1 + glucose, total calcium, albumin, C-reactive protein, cotinine; Model 3: Model 2 + history of CHF, CHD, angina, heart attack, cancer, or malignancy, ever smoking; [†]indicates statistical significance

induced bone resorption via potential mechanisms. This result is consistent with our findings; polycyclic aromatic hydrocarbon metabolites were significantly associated with increased levels of N-telopeptide, which reflects the rate of bone resorption.

The physiological mechanisms of polycyclic aromatic hydrocarbon mediated by the aryl hydrocarbon receptor (AhR) are well established (22, 23). This receptor is AhR, a dioxin receptor that is responsible for the toxicity of polyhalogenated hydrocarbons (24). It is a ligand-activated transcription factor, which exists in almost all human organs, such as the heart and liver, and tissues, including those of the ectoderm, muscle, and bone (25, 26, 27). In a previous study, AhR was assumed to play an important role in bone homeostasis maintenance (28). Iqbal *et al.* demonstrated that osteoclastic bone resorption is induced by the interaction between polycyclic aromatic hydrocarbon and AhR via the activation of cytochrome P450 enzymes (29). These chemicals accelerated osteoclast formation in bone cells with benzo[a]pyrene-stimulated bone resorption and osteoclastogenesis *in vivo* (29). Accordingly, we speculated that other polycyclic aromatic hydrocarbons analyzed in this study have contributed to bone resorption via the similar pathways.

The activity of osteoblasts and osteoclasts decreases in bone tissues of elderly individuals (30). Becerikli *et al.* demonstrated that the levels of RANKL and NFATc1, which are markers of osteoclastogenesis, are significantly lower

in older adults (31). Because of reduced bone resorption activity, polycyclic aromatic hydrocarbon metabolites may have a weak impact on N-telopeptide levels of elderly individuals.

The present study has some notable limitations. First, it has a cross-sectional design; therefore, the causal relationships between polycyclic aromatic hydrocarbon and N-telopeptide were not assessed. A long-term observation period should be considered in future studies. Second, N-telopeptide levels were measured in the urine sample, but the time sample collection was not considered; however, it is critical due to significant circadian variations. Patients in previous studies were instructed to fast to reduce these variations (32, 33). Fasting morning samples must be collected for optimal clinical examination. Third, urine samples instead of serum samples were used to assess the biomarker levels of bone resorption in this study. Serum biomarkers have better reproducibility and prevent correcting creatinine levels, which can cause variability in the analysis. In addition, the assessment of C-terminal telopeptides in serum samples using an antibody-specific immunoassay is an accurate method for predicting bone loss and fracture risk (34). An important study strength is that all multivariate statistical models were adjusted for cigarette use to identify the underlying mechanisms in smokers. Moreover, the generalizability of our findings may not be limited to adult populations in the United States.

Figure 2

(A) Association between PAH metabolites and NTx in males. (B) Association between PAH metabolites and NTx in females.

Figure 3

(A) Association between PAH metabolites and BAP in males. (B) Association between PAH metabolites and BAP in females.

Figure 4
(A) Association between PAH metabolites and NTx in <65 years old. (B) Association between PAH metabolites and NTx in >65 years old.

Figure 5
(A) Association between PAH metabolites and BAP in <65 years old. (B) Association between PAH metabolites and BAP in >65 years old.

Conclusion

Our study highlighted the important role of polycyclic aromatic hydrocarbon exposure in the process of bone resorption. Urinary polycyclic aromatic hydrocarbon metabolites were positively associated with increased levels of N-telopeptide, independent of sex. In addition, all polycyclic aromatic hydrocarbon metabolites were significantly correlated with N-telopeptide in participants aged <60 years. The polycyclic aromatic hydrocarbon exposure was found to have a detrimental effect on health, particularly carcinogenic risks. However, its impact on bone loss has not been fully elucidated. Compared with N-telopeptide, bone-specific alkaline phosphatase was not significantly associated with polycyclic aromatic hydrocarbon metabolites. Thus, polycyclic aromatic hydrocarbon metabolites may be involved in the progression of bone resorption. Moreover, future studies exploring the mechanisms underlying the effects of polycyclic aromatic hydrocarbon metabolites on bone remodeling process must be conducted. To the best of our knowledge, this epidemiological study first presented the association between polycyclic aromatic hydrocarbon, a widely distributed environmental pollutant, and bone loss that may result in osteoporosis and fracture.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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Author contribution statement

Yuan-Yuei Chen contributed to the design of the study, was responsible for the management and retrieval of data, contributed to initial data analysis and interpretation, and drafted the initial manuscript. Yuan-Yuei Chen, Tung-Wei Kao, Chung-Ching Wang, Chen-Jung Wu, Yi-Chao Zhou, and Wei-Liang Chen decided upon the data collection methods. Yuan-Yuei Chen and Wei-Liang Chen were also responsible for the data analysis decisions. Wei-Liang Chen conceptualized and designed the study, supervised all aspects of the study, critically reviewed and revised the manuscript, and approved the final manuscript as submitted. All authors meet the ICMJE criteria for authorship.

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