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# Associations of polycyclic aromatic hydrocarbons exposure with mortality and effect modification by folate biomarkers in a prospective population

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ABSTRACT

The associations of folate biomarkers and polycyclic aromatic hydrocarbons (PAHs) in the general population remain unclear. Therefore, this study aimed to examine whether folate biomarkers are associated with PAHs, and whether folate biomarkers can mitigate adverse health outcome caused by PAHs. This prospective cohort study included 11,246 participants from the National Health and Nutrition Examination Survey (NHANES), which documented 1,303 deaths over a mean follow-up of 9.1 years. Multivariable linear regression models were used to examine the relationship between urinary individual PAHs and folate biomarkers. Multivariable Cox proportional hazards regression models were used to calculate hazard ratios and 95 % CIs for the associations of PAHs and folate biomarkers with CVDs mortality and all-cause mortality. We found negative associations between folate in red blood cells (RBC) and urinary 1-Hydroxyphenanthrene (percentage change for a 2·7 fold-increase in folate −4.19 %, 95 % CI -5.80 % to −2.56 %CI), 2-Hydroxyfluorene (−6.66 %, −7.84 % to −5.49 %), 3-Hydroxyfluorene (−5.78 %, −6.77 % to −4.78 %)) and 1-Hydroxynapthalene (−2.75 %, −3.48 % to −2.01 %). The associations between serum folate and PAHs were consistent with those observed for RBC folate, and negative associations were also found between serum folate and 2-Hydroxynapthalene (−4.10 %, −5.26 % to −2.94 %). Within the lowest quartile of folate levels in RBC, there are strong associations of 2-Hydroxyfluorene, 3-Hydroxyfluorene, 1-Hydroxynapthalene, and 2-Hydroxynapthalene with elevated risk of CVDs mortality [HRs (95 % CI) >1]. As folate levels in RBC increase to the third and fourth quartiles, these associations no longer exist [HRs (95 % CI) <1, *P*-interaction<0.05]. The positive associations between urinary PAHs and CVDs mortality are also eliminated as serum folate levels rise [HRs (95 % CI) <1, *P*-interaction<0.05]. Furthermore, we also found higher levels of folate in both RBC and serum can greatly reduce the adverse impact of 1-Hydroxynapthalene on all-cause mortality. Consistent results were also validated in daily dietary folate and the folic acid supplement intake. Our study highlighted a robust negative relationship between urinary PAHs and folate. Additionally, folate was found to effectively mitigate mortality caused by PAHs, although we did not observe a direct reduction in mortality attributable to folate.

## 1. Introduction

The polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants produced by incomplete combustion or pyrolysis of organic matter [1]. Besides ambient air sources (such as industrial waste incineration and coal/wood/waste burning), indoor air sources (such as smoking and cooking), and contaminants in food and water, people are regularly exposed to PAHs [2]. PAHs exert diverse toxic effects on several organs and are associated with cancer [3,4] and cardiovascular diseases [5–7]. Recently, our group found PAHs may lead to premature aging and increased mortality rates [8].

In light of PAHs' widespread exposure, hazardous toxicity, and persistence characteristics, effective measures to reduce their burden in humans are essential. The role of nutrition in mitigating the harmful effects of environmental contaminants has been widely acknowledged [9–12]. Folate (vitamin B9) is of great importance due to its critical role

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in one-carbon metabolism [13], an essential mechanism that supports DNA synthesis, repair, methylation, and cellular function [14]. Research conducted on animals has revealed that antioxidant foods, including folate, have been observed to mitigate DNA damage associated with exposure to PAHs by stimulating the activity of detoxifying enzymes [15,16]. Meanwhile, folate has the potential to inhibit the expression of cyclinD1 protein that is triggered by PAHs, which assists cells in returning to the normal cell cycle [17]. Moreover, previous studies also have found that folate has the capacity to enhance the elimination of environmental chemicals [18,19]. Therefore, folate and PAHs might have antagonistic kinetic effects that lead to an opposing relationship, suggesting folate could counteract the detrimental effects of PAHs [20–23]. Epidemiological studies has primarily been on the association between folate intake and PAHs levels during pregnancy [24]. However, to date, there have been no investigations conducted on the relationship between PAHs and folate with health outcomes in the general population.

To address this research gap, our study examined the associations of urinary PAHs levels and red blood cells (RBC) folate, serum folate, daily dietary folate equivalent intake, and daily folic acid supplementation with the risks of CVDs mortality and all-cause mortality by using a prospective study in the general population.

## 2. Methods

### 2.1. Study design and population

Data for this cohort analysis were retrieved from the National Health and Nutrition Examination Survey (NHANES), conducted by the Centers for Disease Control and Prevention (CDC) in the United States, which uses a multistage probability design, a representative sample of civilian, community-dwelling members of the US population. The design and collection of data have already been described in detail [25].

We retrieved data between 2003 and 2018. Among the 80,312 participants, 11,830 has been examined for urinary PAHs concentrations, urine creatinine, folate in serum and red blood cells, and mortality data. Participants aged less than 18 years old ($N = 214$), pregnant ($N = 317$), and cancer-related ($N = 53$) participants were excluded [26–28]. Finally, 11,246 participants were included in the study. Of these, 10,601 completed the 24-h diet questionnaire twice and responded to folic acid questionnaire. The flowchart was shown in **Fig. S1**.

### 2.2. Outcomes ascertainment

The statistics on follow-up and mortality status were collected by integrating the NHANES data to the records of the National Death Index up until December 31, 2019. All-cause and CVDs mortality (I00-I09, I11, I13, I20-I51) were defined by ICD-10.

### 2.3. Measurements of urinary PAHs metabolites

The morning urine of participants in NHANES was collected using sterile polypropylene tubes, and samples were stored until measurement at −20 °C. In 2003–2008, capillary gas chromatography combined with high-resolution mass spectrometry (GC/HRMS) was used to analyze PAHs metabolites. From 2009 to 2012, isotope dilution capillary gas chromatography-tandem mass spectrometry (GC–MS/MS) was employed. From 2013 to 2016, isotope dilution high performance liquid chromatography-tandem mass spectrometry (on-line SPE-HPLC-MS/MS) was used for analysis [29]. The PAHs with a detection rate below 70 % were eliminated. Finally, we examined five metabolites of urinary PAHs: 1-Hydroxyphenanthrene (1-PHE), 2-Hydroxyfluorene (2-FLU), 3-Hydroxyfluorene (3-FLU), 1-Hydroxynapthalene (1-NAP), and 2-Hydroxynapthalene (2-NAP). Limit of detection and detection rate of PAHs as shown in **table S1**.

### 2.4. Measurements of folate biomarkers

The Quantaphase II Folate radioassay kit was used to determine the folate levels in RBC and serum from 2003 to 2006 [30,31]. Between 2007 and 2010, using a microbiological assay, whole-blood and serum folates were measured, then the folate concentration in RBC were calculated [32,33]. From 2011, the total folate concentration in serum was measured by isotope-dilution high performance liquid chromatography coupled to tandem mass spectrometry. The calculation of RBC folate was based on the measurements of total folate in serum and whole-blood folate concentrations, which were determined using a microbiological test [34–39]. Estimation of daily dietary folate equivalent intake was based on the mean of two 24-h dietary recalls. Whether daily folic acid supplementation used was obtained from 24-h dietary supplement use component.

### 2.5. Covariates ascertainment

Directed Acyclic Graph (DAG) was used to incorporate covariates based on previous causal knowledge, as shown in **Fig. S2**. Data on age, sex, ethnicity (Mexican Americans, non-Hispanic white, non-Hispanic black, and others), education (less than high school, high school or equivalent, college or higher education), marital status(never married, married, separated), drinking(never, low to moderate, heavy), smoking (never, former smoker, current smoker), family poverty to income ratio levels [PIR (0–1.0, 1.1–3.0, >3.0)], self-reported health condition (very good to excellent, good, poor to fair), healthy eating index (HEI) scores [40] (which obtained from 24-h dietary recalls), nephropathy (no, yes) was determined through face-to-face interviews. Physical examinations provided a measure of waist size (cm). Hypertension was defined by self-reported hypertension or taking antihypertensive medications or a three-measurement of mean systolic blood pressure > 140 mm/Hg and/or a diastolic blood pressure > 90 mm/Hg. Diabetes was diagnosed by either self-reported diabetes history, insulin use, or FBG > 126 mg/dL and HbA1c > 6.5 %.

### 2.6. Statistical analysis

Since NHANES has a complex sampling design, all analysis in this study incorporates sample weights, strata, and primary sampling units. R 4.0.5 was used to conduct the statistical analysis.

As baseline covariates, continuous variables were presented as means and standard deviations, and categorical variables as percentages. The concentration of folate in serum, red blood cells, daily dietary equivalent intake and urinary PAHs metabolites were described using the median and interquartile range. In order to account for urine dilution, PAHs metabolites were adjusted using urinary creatinine (Cr). Continuous and categorical variables were compared using *t*-tests, Mann-Whitney *U* tests, and $\chi^2$ tests.

Multivariable Cox proportional hazards regression models were used to calculate hazard ratios (HRs) and 95 % confidence interval (95 % CIs) to examine the associations of PAHs and folate concentrations with CVDs mortality and all-cause mortality. In regards to progressive models, in model 1, we adjusted for fundamental demographic factors. Model 2 further adjusted for additional socioeconomic status, lifestyle behaviors, and self-reported health status. Model 3 further included variables related to disease factors.

Our study examined the percentage change in urinary PAHs levels per 2·7-fold (ie, per unit increase in natural logarithmic transformation) increase in folate biomarkers by using multivariable linear regression models.

We conducted stratified analysis by quartiles of folate concentrations in red blood cells (categorical), serum (categorical), daily dietary equivalent intake (categorical) and whether daily folic acid supplementation used (categorical) to investigate associations of PAHs concentrations (continuous) with mortality in different subgroups. To

quantify the interactions, we additionally included a product term of PAHs concentrations (continuous) and folate concentrations (continuous) in the model.

## 3. Results

The present prospective study included 11,246 participants with 5673 (50.5 %) of men. The mean (SD) age at baseline was 47.3 (18.7) years. During a mean (SD) follow-up of 9.1 (4.0) years, a total of 429 CVDs deaths and 1,303 all-cause deaths occurred (Table 1).

### 3.1. Folate, PAHs, and mortality

In model 1, the levels of folate in serum, and daily dietary folate equivalent intake were associated with a lower risk of CVDs mortality and all-cause mortality. However, after adjusting for potential confounders, the risk of folate biomarkers for mortality was no longer significant (Table 2). The levels of 2-FLU, 3-FLU, 1-NAP, and 2-NAP were found to be significantly associated with a higher risk of CVDs mortality and all-cause mortality in model 1. After adjusting for potential confounders, in model 3, the HRs and 95 % CIs of 2-FLU, 3-FLU, 1-NAP, and 2-NAP were 1.24 (1.10,1.39), 1.16 (1.04,1.29), 1.07 (0.99,1.15), and 1.17 (1.04,1.31) for CVDs mortality. The HRs and 95 % CIs for all-cause mortality were 1.18 (1.10,1.27), 1.13 (1.07,1.21), 1.09 (1.04,1.14), and 1.11 (1.04,1.18) (Table 2).

### 3.2. Folate and PAHs

Covariate-adjusted models showed negative associations between folate in red blood cells and 1-PHE, 2-FLU, 3-FLU, and 1-NAP concentrations in urine. Specifically, a 2·7-fold increase in red blood cell folate concentrations was associated with a 4.19 % (−5.80 %,-2.56 %)

**Table 1**
Baseline characteristics of participants.

| Characteristics | Total | CVDs mortality | *P* value [a] | All-cause mortality | *P* value [b] |
|---|---|---|---|---|---|
| | (*n* = 11,246) | (*n* = 429) | | (*n* = 1303) | |
| Characteristics, No. (%) | | | | | |
| Age, mean (SD), y | 47.3(18.7) | 72.4 (11.3) | <0.001* | 70.1(12.7) | <0.001* |
| Sex | | | <0.001* | | <0.001* |
| Male | 5673(50.5) | 252(58.7) | | 749(57.5) | |
| Female | 5573(49.5) | 177(41.3) | | 554(42.5) | |
| Ethnicity | | | <0.001* | | <0.001* |
| Mexican American | 1914(17.0) | 52(12.2) | | 160(12.2) | |
| Non-Hispanic white | 4921(43.7) | 276(64.3) | | 820(62.9) | |
| Non-Hispanic black | 2324(20.6) | 70(16.3) | | 216(16.5) | |
| Others | 2087(18.7) | 31(7.2) | | 107(8.4) | |
| Education | | | <0.001* | | <0.001* |
| Less than high school | 2999(26.6) | 170(39.6) | | 507(38.9) | |
| High school or equivalent | 2670(23.7) | 111(25.9) | | 329(25.2) | |
| College or above | 5577(49.7) | 148(34.5) | | 467(35.9) | |
| Family income-poverty ratio levels (PIR) | | | <0.001* | | <0.001* |
| 0–1.0 | 2415(21.4) | 86(20.1) | | 262(20.1) | |
| 1.1–3.0 | 4837(43.1) | 241(56.2) | | 713(54.7) | |
| >3.0 | 3994(35.5) | 102(23.7) | | 328(25.2) | |
| Marital status | | | <0.001* | | <0.001* |
| Never married | 2588(23.1) | 26(6.1) | | 91(6.9) | |
| Married | 6356(56.5) | 211(49.2) | | 645(49.5) | |
| Separated | 2302(20.4) | 192(44.7) | | 567(43.6) | |
| Smoking | | | <0.001* | | <0.001* |
| Never | 2439(21.6) | 91(21.2) | | 275(21.1) | |
| Former | 6271(55.8) | 170(39.6) | | 498(38.2) | |
| Current | 2536(22.6) | 168(39.2) | | 530(40.7) | |
| Drinking | | | <0.001* | | <0.001* |
| Never | 2256(20.1) | 218(50.8) | | 655(50.3) | |
| Low to moderate | 5333(47.4) | 89(20.7) | | 270(20.7) | |
| Heavy | 3657(32.5) | 122(28.5) | | 378(29.0) | |
| Self-reported health | | | <0.001* | | <0.001* |
| Very good to excellent | 4164(37.1) | 83(19.4) | | 305(23.4) | |
| Good | 4545(40.4) | 162(37.7) | | 490(37.6) | |
| Poor to fair | 2537(22.5) | 184(42.9) | | 508(39.0) | |
| Waist, mean (SD), cm | 98.2(16.3) | 102.8(15.6) | <0.001* | 102.3(15.6) | <0.001* |
| HEI, mean (SD), | 51.11(11.8) | 52.18(11.0) | 0.084 | 52.20(11.1) | 0.001* |
| Diabetes | 1266(11.2) | 126(29.3) | <0.001* | 321(24.6) | <0.001* |
| Hypertension | 2000(17.7) | 177(41.2) | <0.001* | 490(37.6) | <0.001* |
| Nephropathy | 300(2.6) | 29(6.76) | <0.001* | 86(6.6) | <0.001* |
| PAHs, median (IQR), ug/mg Cr | | | | | |
| 1-PHE | 123(78–200) | 136(80–206) | 0.488 | 135(82–221) | <0.001* |
| 2-FLU | 204(127–489) | 209(132.2–502) | 0.696 | 208(128–513) | 0.275 |
| 3-FLU | 77(45–227) | 68.88(42–174) | 0.023* | 72(40–203) | 0.010* |
| 1-NAP | 1794(810–6235) | 2679(1069–8478) | <0.001* | 2746(1144–8814) | <0.001* |
| 2-NAP | 4300(2139–9193) | 3209(1702–7353) | <0.001* | 3154(1674–7834) | <0.001* |
| Folate biomarker, median (IQR) | | | | | |
| Folate in red blood cells, ng/mL | 406(291–556) | 438(291–667) | <0.001* | 427(295–657) | <0.001* |
| Folate in serum, ng/mL | 14(10−21) | 16(10–28) | <0.001* | 16(10–27) | <0.001* |
| Daily dietary folate equivalent intake, mcg | 455(319–652) | 404(292–581) | <0.001* | 416(298–607) | <0.001* |
| Daily folic acid supplementation intake (yes) | 1777(15.8) | 62(14.45) | 0.074 | 215(16.50) | 0.010* |

*P* value [a] Comparison between CVDs mortality and alive. *P* value [b] Comparison between all-cause mortality and alive. *P* values were accounted for the study sampling scheme, data clustering, and sample weights in NHANES to be nationally representative.

**Table 2**
Hazard ratios of CVDs mortality and all-cause mortality for folate and PAHs concentrations.[a]

| | Model 1[b] | Model 2[c] | Model 3[d] |
|---|---|---|---|
| **CVDs mortality** | | | |
| Folate in red blood cells (ng/mL)[e] | 1.00 (0.89,1.12) | 1.15 (0.87,1.51) | 1.06 (0.80,1.41) |
| Folate in serum (ng/mL)[e] | 0.88 (0.78,0.99) | 0.93 (0.83,1.05) | 0.96 (0.85,1.08) |
| Daily dietary folate equivalent intake (mcg)[f] | 0.73 (0.61,0.87) | 0.80 (0.67,0.96) | 0.84 (0.70,1.02) |
| Daily folic acid supplementation[f] | 0.83 (0.51,1.33) | 0.88 (0.55,1.40) | 0.96 (0.59,1.57) |
| 1-PHE (ug/mg Cr)[e] | 0.96 (0.84,1.11) | 0.97 (0.85,1.11) | 0.99 (0.86,1.13) |
| 2-FLU (ug/mg Cr)[e] | 1.32 (1.17,1.48) | 1.26 (1.12,1.41) | 1.24 (1.10,1.39) |
| 3-FLU (ug/mg Cr)[e] | 1.20 (1.07,1.34) | 1.17 (1.04,1.31) | 1.16 (1.04,1.29) |
| 1-NAP (ug/mg Cr)[e] | 1.07 (0.99,1.16) | 1.08 (1.00,1.16) | 1.07 (0.99,1.15) |
| 2-NAP (ug/mg Cr)[e] | 1.24 (1.10,1.39) | 1.17 (1.05,1.32) | 1.17 (1.04,1.31) |
| **All-cause mortality** | | | |
| Folate in red blood cells (ng/mL)[e] | 1.04 (0.89,1.22) | 1.12 (0.97,1.28) | 1.14 (0.99,1.32) |
| Folate in serum (ng/mL)[e] | 0.83 (0.72,0.95) | 0.92 (0.81,1.05) | 0.96 (0.83,1.10) |
| Daily dietary folate equivalent intake (mcg)[f] | 0.83 (0.74,0.92) | 0.90 (0.81,1.01) | 0.93 (0.83,1.05) |
| Daily folic acid supplementation[f] | 0.76 (0.60,0.98) | 0.84 (0.66,1.09) | 0.86 (0.67,1.11) |
| 1-PHE (ug/mg Cr)[e] | 1.05 (0.96,1.15) | 1.05 (0.97,1.14) | 1.06 (0.98,1.16) |
| 2-FLU (ug/mg Cr)[e] | 1.25 (1.17,1.34) | 1.20 (1.18,1.29) | 1.18 (1.10,1.27) |
| 3-FLU (ug/mg Cr)[e] | 1.18 (1.11,1.26) | 1.15 (1.08,1.22) | 1.13 (1.07,1.21) |
| 1-NAP (ug/mg Cr)[e] | 1.10 (1.05,1.15) | 1.10 (1.06,1.15) | 1.09 (1.04,1.14) |
| 2-NAP (ug/mg Cr)[e] | 1.17 (1.10,1.25) | 1.12 (1.05,1.19) | 1.11 (1.04,1.18) |

Abbreviation: 1-NAP, 1-Hydroxynapthalene; 2-NAP, 2-Hydroxynapthalene; 2-FLU, 2-Hydroxyfluorene; 3-FLU, 3-Hydroxyfluorene; 1-PHE, 1-Hydroxyphenanthrene; CVDs, cardiovascular diseases; PAHs, Polycyclic aromatic hydrocarbons.

[a] All estimates accounted for complex survey designs. Folate concentrations in red blood cells, serum, daily dietary folate equivalent intake and PAHs concentrations underwent natural logarithmic transformation.

[b] Model 1were adjusted for age, sex.

[c] Model 2were further adjusted for ethnicity, education, PIR, marital status, smoking, drinking, self-reported health, waist, HEI.

[d] Model 3were further adjusted for diabetes, hypertension, nephropathy.

[e] $N = 11,246$.

[f] $N = 10,601$.

reduction in urinary concentration of 1-PHE, a 6.66 % (−7.84 %,-5.49 %) reduction of 2-FLU, a 5.78 % (−6.77 %,-4.78 %) reduction of 3-FLU, and a 2.75 % (−3.48 %,-2.01 %) reduction of 1-NAP. A 2·7-fold increase in serum folate concentrations was associated with a 5.91 %(−7.68 %,-4.13 %) reduction in urinary concentration of 1-PHE, a 8.29 % (−9.45 %,-7.12 %) reduction of 2-FLU, a 7.82 %(−8.82 %,-6.82 %) reduction of 3-FLU, a 3.33 %(−4.15 %,-2.51 %) reduction of 1-NAP, and a 4.10 %(−5.26 %,-2.94 %) reduction of 2-NAP. Further, there were negative associations between daily dietary folate equivalent intake and 1-PHE, 2-FLU, 3-FLU, 1-NAP, and 2-NAP. Taking daily folic acid supplementation seem to negative associated with 3-FLU and 2-NAP (Table 3).

**Table 3**
Percentage change in urinary PAHs concentrations per 2·7-fold increase in folate biomarker concentrations.[a]

| | Model 1[b] | Model 2[c] | Model 3[d] |
|---|---|---|---|
| **Folate in red blood cells (ng/mL)[e]** | | | |
| 1-PHE (ug/mg Cr) | −4.(−6.19 %,-2.86 %) | −4.26 %(−5.90 %,-2.62 %) | −4.19 %(−5.80 %,-2.56 %) |
| 2-FLU (ug/mg Cr) | −7.52 %(−8.75 %,-6.29 %) | −6.71 %(−7.89 %,-5.52 %) | −6.66 %(−7.84 %,-5.49 %) |
| 3-FLU (ug/mg Cr) | −6.64 %(−7.68 %,-5.61 %) | −5.78 %(−6.77 %,-4.78 %) | −5.78 %(−6.77 %,-4.78 %) |
| 1-NAP (ug/mg Cr) | −3.39 %(−4.13 %,-2.65 %) | −2.76 %(−3.48 %,-2.03 %) | −2.75 %(−3.48 %,-2.01 %) |
| 2-NAP (ug/mg Cr) | −1.63 %(−2.77 %,-0.50 %) | −0.65 %(−1.74 %,0.44 %) | −0.57 %(−1.64 %,0.49 %) |
| **Folate in serum (ng/mL)[e]** | | | |
| 1-PHE (ug/mg Cr) | −6.19 %(−8.12 %,-4.26 %) | −6.08 %(−7.87 %,-4.29 %) | −5.91 %(−7.68 %,-4.13 %) |
| 2-FLU (ug/mg Cr) | −9.44 %(−10.66 %,-8.22 %) | −9.44 %(−10.66 %, 8.22 %) | −8.29 %(−9.45 %,-7.12 %) |
| 3-FLU (ug/mg Cr) | −8.41 %(−9.47 %,-7.35 %) | −8.41 %(−9.47 %,-7.35 %) | −7.82 %(−8.82 %,-6.82 %) |
| 1-NAP (ug/mg Cr) | −3.65 %(−4.49 %,-2.82 %) | −3.43 %(−4.25 %,-2.61 %) | −3.33 %(−4.15 %,-2.51 %) |
| 2-NAP (ug/mg Cr) | −5.97 %(−7.21 %,-4.74 %) | −4.38 %(−5.57 %,-3.20 %) | −4.10 %(−5.26 %,-2.94 %) |
| **Daily dietary folate equivalent intake (mcg)[f]** | | | |
| 1-PHE (ug/mg Cr) | −4.40 %(−7.74 %,-1.06 %) | −4.46 %(−7.78 %,-1.15 %) | −4.27 %(−7.61 %,-0.93 %) |
| 2-FLU (ug/mg Cr) | −22.16 %(−26.88 %,-17.44 %) | −18.00 %(−22.64 %,-13.36 %) | −17.30 %(−21.94 %,-12.65 %) |
| 3-FLU (ug/mg Cr) | −25.99 %(−31.64 %,-20.35 %) | −22.21 %(−27.81 %,-16.61 %) | −21.43 %(−27.01 %,-15.85 %) |
| 1-NAP (ug/mg Cr) | −18.01 %(−24.29 %,-11.73 %) | −15.46 %(−21.62 %,-9.30 %) | −14.83 %(−20.99 %,-8.68 %) |
| 2-NAP (ug/mg Cr) | −28.11 %(−32.71 %,-23.52 %) | −23.09 %(−27.47 %,-18.71 %) | −22.34 %(−26.68 %,-18.00 %) |
| **Daily folic acid supplementation[f]** | | | |
| 1-PHE (ug/mg Cr) | −4.94 %(−21.52 %,11.64 %) | −3.14 %(−20.29 %,14.01 %) | −2.85 %(−22.98 %,17.28 %) |
| 2-FLU (ug/mg Cr) | −23.34 %(−37.66 %,-9.02 %) | −15.92 %(−31.72 %,-0.12 %) | −14.73 %(−31.22 %,1.74 %) |
| 3-FLU (ug/mg Cr) | −26.72 %(−40.78 %,-12.65 %) | −19.42 %(−35.65 %,-3.19 %) | −18.36 %(−34.93 %,-1.79 %) |
| 1-NAP (ug/mg Cr) | −20.37 %(−41.70 %,0.94 %) | −16.25 %(−39.65 %,7.13 %) | −15.13 %(−40.53 %,10.26 %) |
| 2-NAP (ug/mg Cr) | −22.94 %(−34.10 %,-11.77 %) | −15.14 %(−27.98 %,-2.30 %) | −14.31 %(−27.55 %,-1.07 %) |

Abbreviation: 1-NAP, 1-Hydroxynapthalene; 2-NAP, 2-Hydroxynapthalene; 2-FLU, 2-Hydroxyfluorene; 3-FLU, 3-Hydroxyfluorene; 1-PHE, 1-Hydroxyphenanthrene; PAHs, Polycyclic aromatic hydrocarbons.

[a] All estimates accounted for complex survey designs. Folate concentrations in red blood cells, serum, daily dietary folate equivalent intake, and PAHs concentrations underwent natural logarithmic transformation. Folate were the independent variables and the individual urinary PAHs concentrations were the dependent variables.
[b] Model 1were adjusted for age, sex.
[c] Model 2were further adjusted for ethnicity, education, PIR, marital status, smoking, drinking, self-reported health, waist, HEI.
[d] Model 3were further adjusted for diabetes, hypertension, nephropathy.
[e] $N = 11,246$.
[f] $N = 10,601$.

### 3.3. PAHs and mortality by quartiles of folate

#### 3.3.1. Folate in red blood cells

Folate in red blood cells significantly modified the associations between PAHs and CVDs mortality. Within the lowest quartile of folate levels in red blood cells, there is a strong association of 2-FLU (HR, 1.33; 95 %CI, 1.05–1.67), 3-FLU (HR, 1.22; 95 %CI, 1.00–1.39), 1-NAP (HR, 1.27; 95 %CI, 1.05–1.54), and 2-NAP (HR, 1.33; 95 %CI, 1.05–1.69) with an elevated risk of CVDs mortality. As folate levels in red blood cells increase to the second quartile, the HRs decrease gradually. Notably, the effect of 1-NAP (HR, 1.11; 95 %CI, 0.95–1.29) on CVDs mortality is no longer statistically significant. The statistical significance of the impact of 2-FLU (HR, 1.09; 95 %CI, 0.74–1.61), 3-FLU (HR, 1.04; 95 %CI, 0.69–1.57), 1-NAP (HR, 0.96; 95 %CI, 0.74–1.25), and 2-NAP (HR, 1.01; 95 %CI, 0.72–1.42) on CVDs mortality was no longer observed in the third quartile groups. In the highest quartile, this association still no longer exists (HRs (95 % CI) <1). Significant interactions between folate levels in red blood cells and 2-FLU, 3-FLU, 1-NAP, and 2-NAP were also identified ($P$-interaction<0.05) (Fig. 1a). Additionally, we also observed that folate in red blood cells significantly mitigated the effect of 1-NAP on the association with all-cause mortality. Within the lowest quartile of folate levels in red blood cells, there is a strong association of 1-NAP (HR, 1.13; 95 %CI, 1.03–1. 24) and all-cause mortality. As folate levels in red blood cells increase, the statistical significance of the impact no longer observed in the third (HR, 1.09; 95 %CI, 0.99–1.20) and highest (HR, 1.05; 95 %CI, 0.96–1.14) quartile groups, all $P$-interaction<0.05 (Table 4).

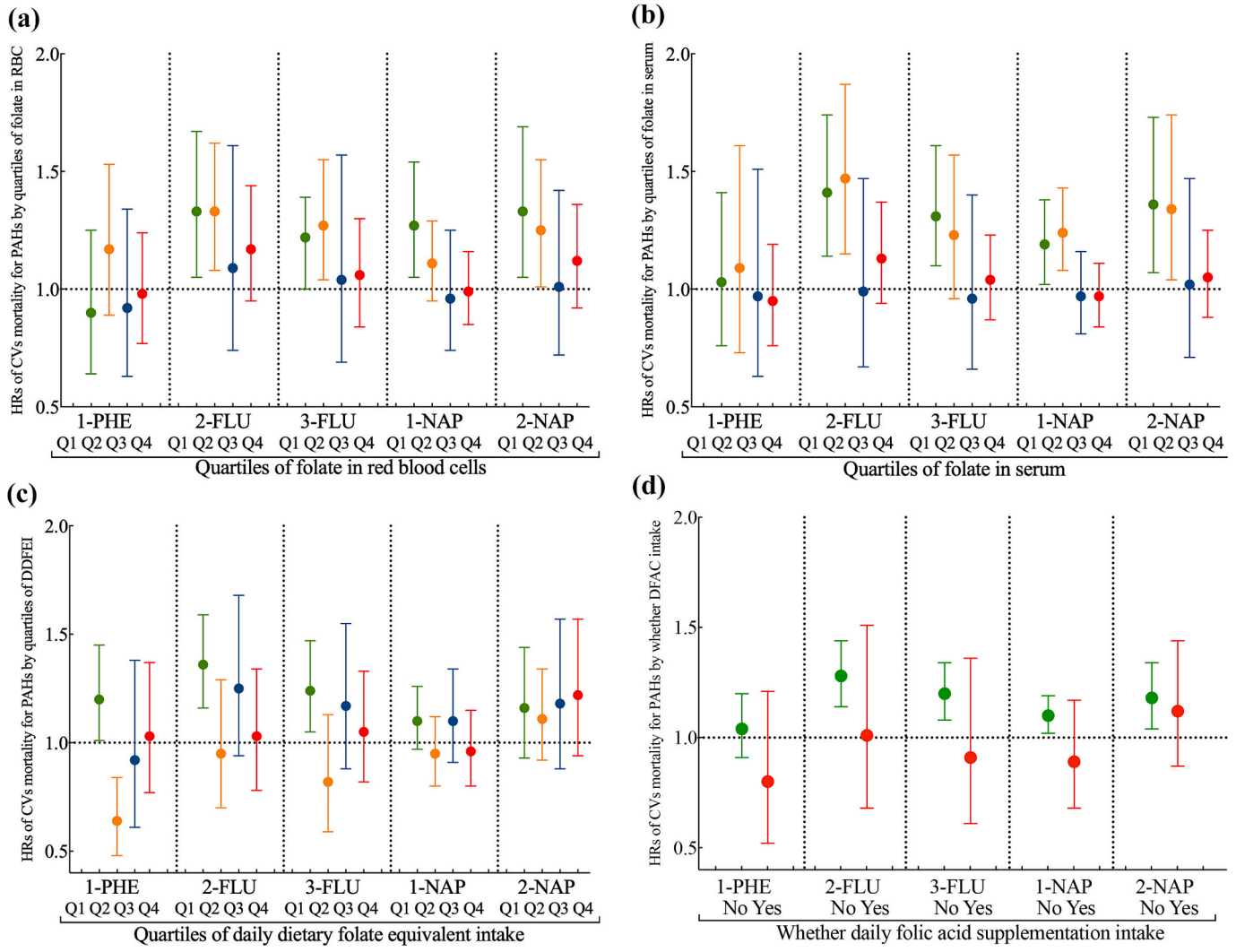


**Fig. 1.** The associations between PAHs and CVDs mortality quartile by (a) RBC, (b)serum, (c) DDFEI, and (d) DFAC. Abbreviation: 1-NAP, 1-Hydroxynapthalene; 2-NAP, 2-Hydroxynapthalene; 2-FLU, 2-Hydroxyfluorene; 3-FLU, 3-Hydroxyfluorene; 1-PHE, 1-Hydroxyphenanthrene; CVDs, cardiovascular diseases; PAHs, Polycyclic aromatic hydrocarbons; RBC, red blood cells; DDFEI, daily dietary folate equivalent intake; DFAC, daily folic acid supplementation; Q1, quartiles 1; Q2, quartiles 2; Q3, quartiles 3; Q4, quartiles 4. All estimates accounted for complex survey designs. Folate concentrations in red blood cells, serum, daily dietary folate equivalent intake, and PAHs concentrations underwent natural logarithmic transformation. Models were adjusted for age, sex, ethnicity, education, PIR, marital status, smoking, drinking, self-reported health, waist, HEI, diabetes, hypertension, nephropathy. (For interpretation of the references to colour in this figure legend, the reader could refer to the web version of this article.)

**Table 4**
Multivariable-adjusted hazard ratios of all-cause mortality for PAHs concentrations by quartiles of folate biomarker.[a]

| PAHs | Quartiles of folate | | | | *P*-interaction |
|---|---|---|---|---|---|
| | Quartiles 1 | Quartiles 2 | Quartiles 3 | Quartiles 4 | |
| **Folate in red blood cells**[b] | **<291, ng/mL** | **291–406, ng/mL** | **407–556, ng/mL** | **>556, ng/mL** | |
| 1-PHE (ug/mg Cr) | 1.03(0.84,1.26) | 1.09(0.94,1.27) | 1.10(0.90,1.33) | 1.06(0.93,1.22) | 0.015* |
| 2-FLU (ug/mg Cr) | 1.08(0.92,1.26) | 1.25(1.11,1.40) | 1.16(0.98,1.38) | 1.24(1.12,1.39) | <0.001* |
| 3-FLU (ug/mg Cr) | 1.03(0.91,1.17) | 1.21(1.08,1.35) | 1.15(0.99,1.33) | 1.19(1.07,1.31) | <0.001* |
| 1-NAP (ug/mg Cr) | 1.13(1.03,1.24) | 1.11(1.03,1.20) | 1.09(0.99,1.20) | 1.05(0.96,1.14) | <0.001* |
| 2-NAP (ug/mg Cr) | 1.08(0.93,1.25) | 1.16(1.04,1.30) | 1.13(0.97,1.32) | 1.08(0.97,1.21) | <0.001* |
| **Folate in serum**[b] | **<10, ng/mL** | **10–14, ng/mL** | **15–22, ng/mL** | **>22, ng/mL** | |
| 1-PHE (ug/mg Cr) | 1.12(0.92,1.37) | 0.99(0.93,1.20) | 1.14(0.92,1.41) | 1.05(0.93,1.19) | 0.860 |
| 2-FLU (ug/mg Cr) | 1.13(0.96,1.32) | 1.04(0.89,1.23) | 1.10(0.91,1.32) | 1.20(1.09,1.34) | 0.028* |
| 3-FLU (ug/mg Cr) | 1.20(1.06,1.37) | 1.09(0.95,1.24) | 1.07(0.88,1.29) | 1.14(1.03,1.26) | 0.021* |
| 1-NAP (ug/mg Cr) | 1.14(1.05,1.23) | 1.09(0.99,1.20) | 1.10(0.99,1.23) | 1.05(0.97,1.13) | <0.001* |
| 2-NAP (ug/mg Cr) | 1.21(1.04,1.41) | 1.04(0.89,1.23) | 1.15(0.97,1.36) | 1.06(0.96,1.18) | 0.568 |
| **Daily dietary folate equivalent intake**[c] | **<319, mcg** | **320–455, mcg** | **456–652, mcg** | **>652, mcg** | |
| 1-PHE (ug/mg Cr) | 1.17(1.05,1.31) | 0.96(0.78,1.18) | 1.03(0.85,1.24) | 1.07(0.84,1.36) | 0.616 |
| 2-FLU (ug/mg Cr) | 1.19(1.07,1.33) | 1.18(1.00,1.40) | 1.18(1.02,1.38) | 1.12(0.90,1.40) | <0.001* |
| 3-FLU (ug/mg Cr) | 1.15(1.03,1.28) | 1.10(0.94,1.28) | 1.16(1.02,1.32) | 1.08(0.89,1.32) | <0.001* |
| 1-NAP (ug/mg Cr) | 1.10(1.02,1.18) | 1.05(0.97,1.15) | 1.09(0.98,1.22) | 1.10(1.01,1.21) | 0.001* |
| 2-NAP (ug/mg Cr) | 1.10(0.98,1.24) | 1.10(0.98,1.23) | 1.03(0.87,1.20) | 1.18(0.98,1.42) | 0.082 |
| **Daily folic acid supplementation**[c] | **No** | **Yes** | | | |
| 1-PHE (ug/mg Cr) | 1.08(0.98,1.18) | 1.05(0.83,1.32) | – | – | 0.935 |
| 2-FLU (ug/mg Cr) | 1.18(1.09,1.28) | 1.27(0.96,1.68) | – | – | 0.599 |
| 3-FLU (ug/mg Cr) | 1.15(1.07,1.23) | 1.10(0.92,1.31) | – | – | 0.981 |
| 1-NAP (ug/mg Cr) | 1.11(1.06,1.16) | 1.03(0.91,1.18) | – | – | 0.273 |
| 2-NAP (ug/mg Cr) | 1.12(1.05,1.19) | 1.08(0.90,1.29) | – | – | 0.839 |

Abbreviation: 1-NAP, 1-Hydroxynapthalene; 2-NAP, 2-Hydroxynapthalene; 2-FLU, 2-Hydroxyfluorene; 3-FLU, 3-Hydroxyfluorene; 1-PHE, 1-Hydroxyphenanthrene; PAHs, Polycyclic aromatic hydrocarbons.

[a] All estimates accounted for complex survey designs. Folate concentrations in red blood cells, serum, dietary folate equivalent intake and PAHs concentrations underwent natural logarithmic transformation. Models were adjusted for age, sex, ethnicity, education, PIR, marital status, smoking, drinking, self-reported health, waist, HEI, diabetes, hypertension, nephropathy.

[b] N = 11,246.

[c] N = 10,601.

### 3.3.2. Folate in serum

In the lowest quartile of folate in serum, there is a significant association of 2-FLU (HR, 1.41; 95 %CI, 1.14–1.74), 3-FLU (HR, 1.13; 95 % CI, 1.10–1.61), 1-NAP (HR, 1.19; 95 %CI, 1.02–1.38), and 2-NAP (HR, 1.36; 95 %CI, 1.07–1.73) with an increased risk of CVDs mortality. As the serum folate levels increase to the second quartile, the HRs generally lower. Specifically, 3- FLU (HR, 1.23; 95 %CI, 0.96–1.57) no longer has a statistically significant impact on CVDs mortality. By the third quartiles, the statistical significance of the effects of 2-FLU (HR, 0.99; 95 %CI, 0.67–1.47), 3-FLU (HR, 0.96; 95 %CI, 0.66–1.40), 1-NAP (HR, 0.97; 95 %CI, 0.81–1.16), and 2-NAP (HR, 1.02; 95 %CI, 0.71–1.47) on CVDs mortality diminished. In the highest quartile, this association does not persist either [HRs (95 % CI) <1]. Significant interactions between folate levels in serum and 2-FLU, 3-FLU, 1-NAP, and 2-NAP were also found (*P*-interaction<0.05) (Fig. 1b). Furthermore, we found that folate in serum greatly reduced the impact of 1-NAP on the association with all-cause mortality (Table 4).

### 3.3.3. Daily dietary folate equivalent intake

In the lowest quartile of daily dietary folate equivalent intake, there is positive associations between 2-FLU (HR, 1.36; 95 %CI, 1.16–1.59) and an elevated risk of CVDs mortality. As levels increase to the second quartile (HR, 0.95; 95 %CI, 0.70–1.29), the association no longer exists. In the third (HR, 1.25; 95 %CI, 0.94–1.68) and highest quartiles (HR, 1.03; 95 %CI, 0.78–1.34), this association does not persist either (*P*-interaction = 0.038) (Fig. 1c). We also observed the mitigation effect of daily dietary folate equivalent intake on the association of 2-FLU with all-cause mortality (*P*-interaction<0.001) (Table 4).

### 3.3.4. Daily folic acid supplementation

Furthermore, 2-FLU (HR, 1.28; 95 %CI, 1.14–1.44), 3-FLU (HR, 1.20; 95 %CI, 1.08–1.34), 1-NAP (HR, 1.10; 95 %CI, 1.02–1.19), and 2-NAP (HR, 1.18; 95 %CI, 1.04–1.34) were found to be related to a higher risk of CVDs mortality in the no-folic acid supplementation group; whereas this association disappeared in the folic acid supplementation group, the HRs of 2-FLU (HR, 1.01; 95 %CI, 0.68–1.51), 3-FLU (HR, 0.91; 95 %CI, 0.61–1.36) 1-NAP (HR, 0.89; 95 %CI, 0.68–1.17) and 2-NAP (HR, 1.12; 95 %CI, 0.87–1.44), respectively. However, no interactions were found (Fig. 1d). Additionally, 2-FLU (HR, 1.18; 95 %CI, 1.09–1.28), 3-FLU (HR, 1.15; 95 %CI, 1.07–1.23), 1-NAP (HR, 1.11; 95 %CI, 1.06–1.16), and 2-NAP (HR, 1.12; 95 %CI, 1.05–1.19) also found to be associated with a higher risk of all-cause mortality in the no-folic acid supplementation group; whereas this association disappeared in the folic acid supplementation group, the HRs were 2-FLU (HR, 1.27; 95 % CI, 0.96–1.68), 3-FLU (HR, 1.10; 95 %CI, 0.92–1.31) 1-NAP (HR, 1.03; 95 %CI, 0.91–1.18) and 2-NAP (HR, 1.08; 95 %CI, 0.90–1.29), respectively. In spite of this, no interaction was observed (Table 4).

## 3.4. Sensitivity analyses

In this study, we performed several sensitivity analyses. First, we conducted stratified analysis by quartiles of folate concentrations in red blood cells, serum, daily dietary folate equivalent intake and whether daily folic acid supplementation used (categorical) to investigate associations between the sum of 5 urinary PAHs concentrations (continuous) and mortality in different subgroups (**table S2-S4**). Then, we excluded participants less than two years of follow-up (**table S5-S7**). Finally, we incorporated the survey cycle in the confounding adjustments to address

the evolution of technologies for assessing folate biomarkers and polycyclic aromatic hydrocarbon metabolites over the years (**table S8-S10**). All sensitivity analyses showed that the main results were robust.

## 4. Discussion

The results of this large, prospective study indicate that an increase in 1-NAP, 2-FLU, 3-FLU, and 2-NAP levels in the urine was associated with a higher risk of CVD mortality and all-cause mortality in the general population. But we did not find any association between mortality and folate levels in serum, red blood cells, daily dietary folate equivalent intake, or daily folic acid supplementation after adjusting for confounders. Nevertheless, we found a robust negative relationship between urinary PAHs and folate in serum, red blood cells, daily dietary folate equivalent intake and daily folic acid supplementation. Additionally, folate was found to effectively mitigate CVDs and all-cause mortality caused by PAHs. According to my knowledge, this is the first study to investigate the relationship between urinary PAHs and folate on their impact for health outcomes among the general population.

The relationship between folic acid intake/or blood folate concentrations and mortality has been examined in different populations with inconsistent results. Previous randomized controlled trials (RCTs) studies demonstrated that folic acid supplementation can lower blood pressure, blood glucose, and blood lipids levels, and reduce the risk of myocardial infarction in high-risk individuals [41–43]. However, in another randomized controlled trial study, among women at high cardiovascular risk, folic acid, vitamin B6, and vitamin B12 combined pills did not reduce a combined end point of total cardiac events after 7.3 years of treatment [44]. Longitudinal observational study found serum folate associated with higher risk of CVD mortality through diabetic patients [45] and among participants with rheumatoid arthritis [46], whereas hypertensives showed a U-shaped association with CVD mortality [47]. However, in the general population, there is an association between high folic acid intakes and elevated blood folate levels and negative health outcomes [48]. However, in this study, we did not find any association between the mortality and folate levels. These inconsistent outcomes may be partly due to different population, different status of folate and health endpoint events. In addition, different metabolic conditions may have different effects. Validation of large samples in prospective studies is still required.

Few reports have been reported between PAHs and folate levels. One clinical investigation observed a noteworthy decrease in benzo[*a*]pyrene (BaP)-DNA adducts among female smokers with vitamin treatment ($n = 426$) [49]. Another study have found similar negative correlation between serum folate concentrations and higher PAH-DNA adduct levels at birth, but with smaller sample sizes ($n = 54$) [50]. In our investigation, a sizable sample size of 11,246 general population was observed. We found reliable and robust results for the relationship between folate levels in serum and red blood cells and reduced PAHs levels. It is important to acknowledge that daily dietary folate intake and daily folic acid supplementation can also impact the PAHs levels. These notable inverse relationships identified between folate and PAHs concentrations provide additional evidence for the existence of antagonistic kinetic interactions between folate and environmental chemical exposures.

To date, researchers have mainly focused on pregnant women when studying the health effects of PAHs and folate. A nested case-control study included 83 preterm births (PB) and 82 term births. After stratification by dietary nutrient intakes, high BaP-DNA adduct levels were associated with an increased risk of PB among women with low dietary nutrient intakes [51]. Nevertheless, this study examined folate through dietary food intake instead of using biomarkers in relation to PAHs concentrations. Folate biomarkers have significant implications for mechanistic experiments and intervention studies, compared with food frequency questionnaires, which provide a less objective and less accurate measurement of this specific nutrient. Our study explored the effects of PAHs and folate on health outcomes among general population. There is accumulating evidence that folate may reduce PAHs levels and mitigate PAHs-related health problems.

PAHs metabolites in urine are commonly used as stable biomarkers of PAHs exposure, reflecting PAHs burden in the body [52]. PAHs are mainly metabolized in the liver by the CYP450 enzyme system, producing reactive metabolites such as epoxides and benzo[*a*]pyrene diol epoxide (BPDE). These reactive metabolites are carcinogenic and genotoxic, binding with DNA, RNA, and proteins, leading to oxidative stress, DNA damage [53]. Folate levels in serum, red blood cells daily dietary folate intake and daily folic acid supplementation can fully reflect the folate status. Previous study found a sufficient maternal consumption of folate is advantageous for combating oxidative stress linked to PAHs exposure [54]. Folate is extensively involved in the one-carbon metabolism pathway, acting as a methyl donor in the body, participating in DNA synthesis, repair, and methylation processes [55]. Adequate folate levels help maintain DNA integrity and reduce the damage caused by PAHs. However, when the body is exposed to high concentrations of PAHs over time, folate may be depleted, affecting its normal functions and leading to the accumulation of DNA damage. Study found that folate have been observed to mitigate DNA damage associated with exposure to PAHs by stimulating the activity of detoxifying enzymes [15,16]. Meanwhile, folate plays an important role in preventing BaP-induced cyclinD1 protein expression, that may assist cells in resuming their normal cell cycle [56]. Therefore, it is biologically plausible that folate may attenuate the adverse effects of PAHs exposure on health outcomes.

We also have several limitations to this study. First, the measurement was based on a single spot, so long-term PAHs concentrations may be impacted. However, prior investigations indicated that PAHs are stable markers of multi-pathway environmental exposure [52]. Then, the concentrations of PAHs and folate were measured at baseline, their temporality cannot be determined.

## 5. Conclusion

In this prospective study, we found a robust negative relationship between urinary PAHs and folate. Additionally, folate could effectively mitigate CVDs mortality and all-cause mortality caused by PAHs, although we did not observe a direct reduction in mortality attributable to folate.

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## CRediT authorship contribution statement

**Siyu Duan:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Data curation, Conceptualization. **Kairong Wang:** Writing – review & editing, Visualization, Resources, Conceptualization. **Chenming Gu:** Writing – review & editing, Formal analysis. **Junmin Zhu:** Writing – review & editing. **Yafei Wu:** Funding acquisition, Writing – review & editing, Conceptualization. **Ya Fang:** Funding acquisition, Writing – review & editing, Conceptualization

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.enceco.2024.10.009.

## Data availability

The datasets used for these analyses are publicly available (https://www.cdc.gov/nchs/nhanes/index.htm). The code will be provided with reasonable request.

## References

[1] S.K. Samanta, O.V. Singh, R.K. Jain, Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation, Trends Biotechnol. 20 (6) (2002) 243–248, https://doi.org/10.1016/s0167-7799(02)01943-1.
[2] WHO Guidelines for Indoor Air Quality: Selected Pollutants, World Health Organization, Geneva, 2010. http://www.ncbi.nlm.nih.gov/books/NBK138705/ (Accessed March 27, 2024).
[3] M.A. Mallah, L. Changxing, M.A. Mallah, et al., Polycyclic aromatic hydrocarbon and its effects on human health: an overeview, Chemosphere 296 (2022) 133948, https://doi.org/10.1016/j.chemosphere.2022.133948.
[4] Y. Ge, S. Wu, K. Yan, Concentrations, influencing factors, risk assessment methods, health hazards and analyses of polycyclic aromatic hydrocarbons in dairies: a review, Crit. Rev. Food Sci. Nutr. 63 (23) (2023) 6168–6181, https://doi.org/10.1080/10408398.2022.2028717.
[5] O. Alshaarawy, H.A. Elbaz, M.E. Andrew, The association of urinary polycyclic aromatic hydrocarbon biomarkers and cardiovascular disease in the US population, Environ. Int. 89-90 (2016) 174–178, https://doi.org/10.1016/j.envint.2016.02.006.
[6] L. Cao, D. Wang, C. Zhu, et al., Polycyclic aromatic hydrocarbon exposure and atherosclerotic cardiovascular disease risk in urban adults: the mediating role of oxidatively damaged DNA, Environ. Pollut. 265 (Pt A) (2020) 114860, https://doi.org/10.1016/j.envpol.2020.114860.
[7] M.A. Mallah, L. Changxing, M.A. Mallah, et al., Association of urinary polycyclic aromatic hydrocarbon metabolites and cardiovascular disease among US population: a cross-sectional study, Environ. Res. 209 (2022) 112775, https://doi.org/10.1016/j.envres.2022.112775.
[8] S. Duan, Y. Wu, J. Zhu, et al., Development of interpretable machine learning models associated with environmental chemicals to predict all-cause and specific-cause mortality: a longitudinal study based on NHANES, Ecotoxicol. Environ. Saf. 270 (2024) 115864, https://doi.org/10.1016/j.ecoenv.2023.115864.
[9] M.P. Lr F, Nutrition and mutagenesis, Annu. Rev. Nutr. (2008) 28, https://doi.org/10.1146/annurev.nutr.28.061807.155449.
[10] B. Hennig, A.S. Ettinger, R.J. Jandacek, et al., Using nutrition for intervention and prevention against environmental chemical toxicity and associated diseases, Environ. Health Perspect. 115 (4) (2007) 493–495, https://doi.org/10.1289/ehp.9549.
[11] B. Hennig, L. Ormsbee, C.J. McClain, et al., Nutrition can modulate the toxicity of environmental pollutants: implications in risk assessment and human health, Environ. Health Perspect. 120 (6) (2012) 771–774, https://doi.org/10.1289/ehp.1104712.
[12] K. Kordas, B. Lönnerdal, R.J. Stoltzfus, Interactions between nutrition and environmental exposures: effects on health outcomes in women and children, J. Nutr. 137 (12) (2007) 2794–2797, https://doi.org/10.1093/jn/137.12.2794.
[13] H. McNulty, M. Ward, L. Hoey, C.F. Hughes, K. Pentieva, Addressing optimal folate and related B-vitamin status through the lifecycle: health impacts and challenges, Proc. Nutr. Soc. 78 (3) (2019) 449–462, https://doi.org/10.1017/S0029665119000661.
[14] M.A. Kane, S. Waxman, Role of folate binding proteins in folate metabolism, Lab. Investig. 60 (6) (1989) 737–746.
[15] A.P. Wolterbeek, R. Roggeband, C.J. van Moorsel, et al., Vitamin a and beta-carotene influence the level of benzo[a]pyrene-induced DNA adducts and DNA-repair activities in hamster tracheal epithelium in organ culture, Cancer Lett. 91 (2) (1995) 205–214, https://doi.org/10.1016/0304-3835(95)03740-n.
[16] T. Duarte-Salles, M.A. Mendez, E. Morales, et al., Dietary benzo(a)pyrene and fetal growth: effect modification by vitamin C intake and glutathione S-transferase P1 polymorphism, Environ. Int. 45 (2012) 1–8, https://doi.org/10.1016/j.envint.2012.04.002.
[17] W. P, C. Y, W. L, et al., The intervention mechanism of folic acid for benzo(a) pyrene toxic effects in vitro and in vivo, Eur. J. Cancer Prev. 28 (4) (2019), https://doi.org/10.1097/CEJ.0000000000000461.
[18] M.V. Gamble, X. Liu, V. Slavkovich, et al., Folic acid supplementation lowers blood arsenic, Am. J. Clin. Nutr. 86 (4) (2007) 1202–1209, https://doi.org/10.1093/ajcn/86.4.1202.
[19] B.A. Peters, M.N. Hall, X. Liu, et al., Folic acid and creatine as therapeutic approaches to lower blood arsenic: a randomized controlled trial, Environ. Health Perspect. 123 (12) (2015) 1294–1301, https://doi.org/10.1289/ehp.1409396.
[20] L. Mínguez-Alarcón, A.J. Gaskins, Y.H. Chiu, et al., Dietary folate intake and modification of the association of urinary bisphenol A concentrations with in vitro fertilization outcomes among women from a fertility clinic, Reprod. Toxicol. 65 (2016) 104–112, https://doi.org/10.1016/j.reprotox.2016.07.012.
[21] S. Bölte, S. Girdler, P.B. Marschik, The contribution of environmental exposure to the etiology of autism spectrum disorder, Cell. Mol. Life Sci. 76 (7) (2019) 1275–1297, https://doi.org/10.1007/s00018-018-2988-4.
[22] D.C. Dolinoy, D. Huang, R.L. Jirtle, Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development, Proc. Natl. Acad. Sci. USA 104 (32) (2007) 13056–13061, https://doi.org/10.1073/pnas.0703739104.
[23] S. Singh, S.S.L. Li, Epigenetic effects of environmental chemicals bisphenol a and phthalates, Int. J. Mol. Sci. 13 (8) (2012) 10143–10153, https://doi.org/10.3390/ijms130810143.
[24] N. Zhao, W. Wu, S. Cui, et al., Effects of benzo[a]pyrene-DNA adducts, dietary vitamins, folate, and carotene intakes on preterm birth: a nested case-control study from the birth cohort in China, Environ. Health 21 (1) (2022) 48, https://doi.org/10.1186/s12940-022-00859-7.
[25] NCHS, 404 Error - Resource Not Available. https://www.cdc.gov/nchs/404.htm, 2019. Published June 1. Accessed March 27, 2024.
[26] M.M. Zarou, A. Vazquez, Helgason G. Vignir, Folate metabolism: a re-emerging therapeutic target in haematological cancers, Leukemia 35 (6) (2021) 1539–1551, https://doi.org/10.1038/s41375-021-01189-2.
[27] C.M. Ulrich, Nutrigenetics in cancer research–folate metabolism and colorectal cancer, J. Nutr. 135 (11) (2005) 2698–2702, https://doi.org/10.1093/jn/135.11.2698.
[28] A.B. Chapman, W.T. Abraham, S. Zamudio, et al., Temporal relationships between hormonal and hemodynamic changes in early human pregnancy, Kidney Int. 54 (6) (1998) 2056–2063, https://doi.org/10.1046/j.1523-1755.1998.00217.x.
[29] Y. Wang, L. Meng, E.N. Pittman, et al., Quantification of urinary mono-hydroxylated metabolites of polycyclic aromatic hydrocarbons by on-line solid phase extraction-high performance liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 409 (4) (2017) 931–937, https://doi.org/10.1007/s00216-016-9933-x.
[30] NHANES, Folate - RBC & Serum, and Vitamin B12 Data Documentation, Codebook, and Frequencies. https://wwwn.cdc.gov/Nchs/Nhanes/2003-2004/L06NB_C.htm, 2003-2004. Accessed April 17, 2024.
[31] NHANES, Folate - RBC & Serum Data Documentation, Codebook, and Frequencies. https://wwwn.cdc.gov/Nchs/Nhanes/2005-2006/FOLATE_D.htm, 2005-2006. Accessed April 17, 2024.
[32] FOLFMS_E. https://wwwn.cdc.gov/Nchs/Nhanes/2007-2008/FOLFMS_E.htm, 2024. Accessed April 17, 2024.
[33] FOLATE_F. https://wwwn.cdc.gov/Nchs/Nhanes/2009-2010/FOLATE_F.htm, 2024. Accessed April 17, 2024.
[34] FOLFMS_I. https://wwwn.cdc.gov/Nchs/Nhanes/2015-2016/FOLFMS_I.htm, 2024. Accessed April 18, 2024.
[35] FOLFMS_H. https://wwwn.cdc.gov/Nchs/Nhanes/2013-2014/FOLFMS_H.htm, 2024. Accessed April 18, 2024.
[36] FOLFMS_G. https://wwwn.cdc.gov/Nchs/Nhanes/2011-2012/FOLFMS_G.htm, 2024. Accessed April 18, 2024.
[37] FOLATE_I. https://wwwn.cdc.gov/Nchs/Nhanes/2015-2016/FOLATE_I.htm, 2024. Accessed April 18, 2024.
[38] FOLATE_H. https://wwwn.cdc.gov/Nchs/Nhanes/2013-2014/FOLATE_H.htm, 2024. Accessed April 18, 2024.
[39] FOLATE_G. https://wwwn.cdc.gov/Nchs/Nhanes/2011-2012/FOLATE_G.htm, 2024. Accessed April 18, 2024.
[40] Developing the Healthy Eating Index (HEI) | EGRP/DCCPS/NCI/NIH. https://epi.grants.cancer.gov/hei/developing.html#f1b, 2024. Accessed April 17, 2024.
[41] S.U. Khan, M.U. Khan, H. Riaz, et al., Effects of nutritional supplements and dietary interventions on cardiovascular outcomes: an umbrella review and evidence map, Ann. Intern. Med. 171 (3) (2019) 190–198, https://doi.org/10.7326/M19-0341.
[42] D.J.A. Jenkins, J.D. Spence, E.L. Giovannucci, et al., Supplemental vitamins and minerals for CVD prevention and treatment, J. Am. Coll. Cardiol. 71 (22) (2018) 2570–2584, https://doi.org/10.1016/j.jacc.2018.04.020.
[43] D.J.A. Jenkins, J.D. Spence, E.L. Giovannucci, et al., Supplemental vitamins and minerals for cardiovascular disease prevention and treatment: JACC focus seminar, J. Am. Coll. Cardiol. 77 (4) (2021) 423–436, https://doi.org/10.1016/j.jacc.2020.09.619.
[44] C.M. Albert, N.R. Cook, J.M. Gaziano, et al., Effect of folic acid and B vitamins on risk of cardiovascular events and total mortality among women at high risk for cardiovascular disease: a randomized trial, JAMA 299 (17) (2008) 2027–2036, https://doi.org/10.1001/jama.299.17.2027.
[45] Y. Liu, T. Geng, Z. Wan, et al., Associations of serum folate and vitamin B12 levels with cardiovascular disease mortality among patients with type 2 diabetes, JAMA Netw. Open 5 (1) (2022) e2146124, https://doi.org/10.1001/jamanetworkopen.2021.46124.
[46] K. Sonawane, Y. Zhu, W. Chan, D. Aguilar, A.A. Deshmukh, M.E. Suarez-Almazor, Association of serum folate levels with cardiovascular mortality among adults with rheumatoid arthritis, JAMA Netw. Open 3 (2) (2020) e200100, https://doi.org/10.1001/jamanetworkopen.2020.0100.
[47] S. Nkemjika, E. Ifebi, L.T. Cowan, et al., Association between serum folate and cardiovascular deaths among adults with hypertension, Eur. J. Clin. Nutr. 74 (6) (2020) 970–978, https://doi.org/10.1038/s41430-019-0533-7.

[48] C.M. Vanderwall, C.C. Tangney, M.J. Kwasny, K.A. Gustashaw, Examination of circulating folate levels as a reflection of folate intakes among older adult supplement users and nonusers in the National Health and nutrition examination survey 2003-2004, J. Acad. Nutr. Diet. 112 (2) (2012) 285–290, https://doi.org/10.1016/j.jada.2011.10.001.
[49] L.A. Mooney, A.M. Madsen, D. Tang, et al., Antioxidant vitamin supplementation reduces benzo(a)pyrene-DNA adducts and potential cancer risk in female smokers, Cancer Epidemiol. Biomarkers Prev. 14 (1) (2005) 237–242.
[50] T. Dodd-Butera, P.J.E. Quintana, M. Ramirez-Zetina, et al., Placental biomarkers of PAH exposure and glutathione-S-transferase biotransformation enzymes in an obstetric population from Tijuana, Baja California, Mexico, Environ. Res. 152 (2017) 360–368, https://doi.org/10.1016/j.envres.2016.04.019.
[51] N. Zhao, W. Wu, S. Cui, et al., Effects of benzo[a]pyrene-DNA adducts, dietary vitamins, folate, and carotene intakes on preterm birth: a nested case–control study from the birth cohort in China, Environ. Health 21 (1) (2022) 48, https://doi.org/10.1186/s12940-022-00859-7.
[52] H. Niu, Y. Cai, F. Wei, S. Mou, G. Jiang, Hydroxyl polycyclic aromatic hydrocarbons in human urine as biomarkers of exposure to PAHs, Prog. Chem. 18 (10) (2006) 1381–1390, https://doi.org/10.1007/s11095-006-9014-5.
[53] D. Kuang, W. Zhang, Q. Deng, et al., Dose-response relationships of polycyclic aromatic hydrocarbons exposure and oxidative damage to DNA and lipid in coke oven workers, Environ. Sci. Technol. 47 (13) (2013) 7446–7456, https://doi.org/10.1021/es401639x.
[54] H. Kim, J.Y. Hwang, E.H. Ha, et al., Fruit and vegetable intake influences the association between exposure to polycyclic aromatic hydrocarbons and a marker of oxidative stress in pregnant women, Eur. J. Clin. Nutr. 65 (10) (2011) 1118–1125, https://doi.org/10.1038/ejcn.2011.77.
[55] J. Selhub, Homocysteine metabolism, Annu. Rev. Nutr. 19 (1999) 217–246, https://doi.org/10.1146/annurev.nutr.19.1.217.
[56] P. Wang, Y. Chen, L. Wang, et al., The intervention mechanism of folic acid for benzo(a)pyrene toxic effects in vitro and in vivo, Eur. J. Cancer Prev. 28 (4) (2019) 355, https://doi.org/10.1097/CEJ.0000000000000461.