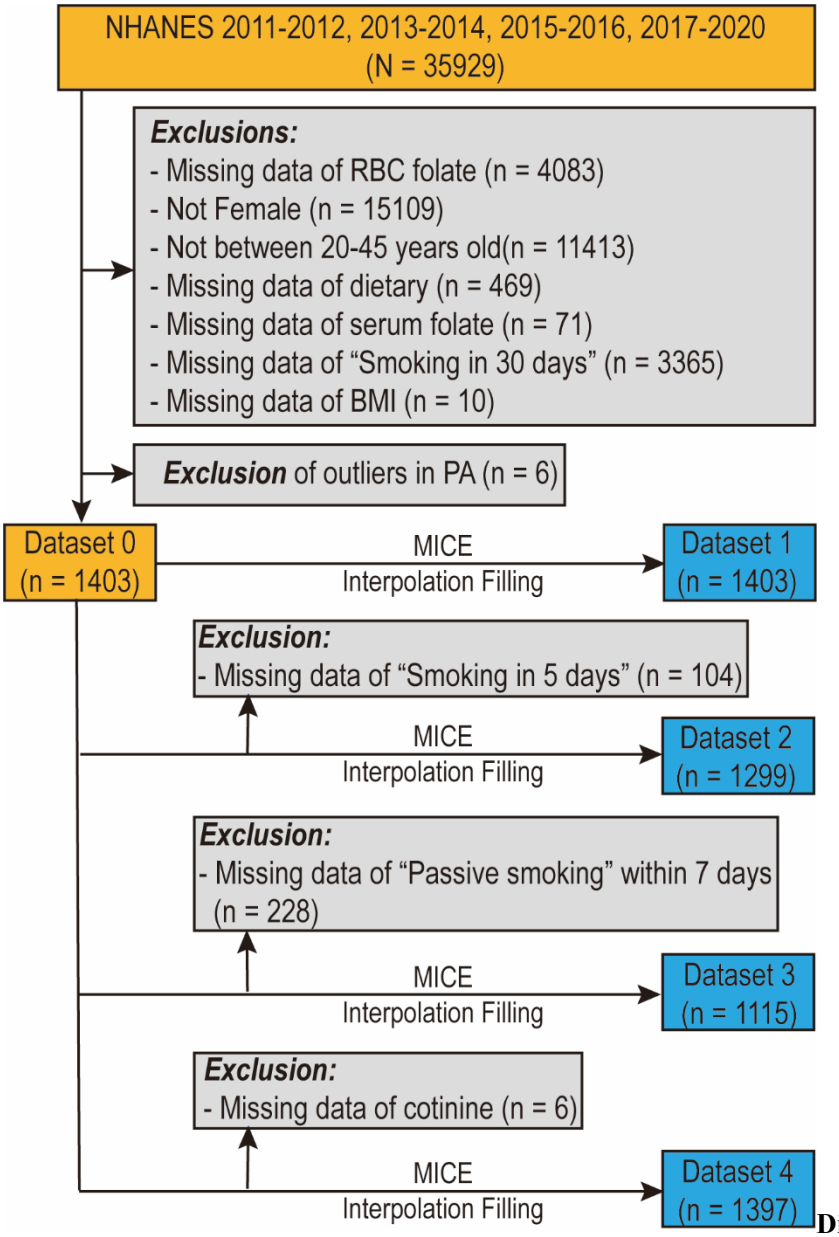


Dietary Factors and Physical Activity Resist Tobacco-exposure-induced Folate Deficiency in Childbearing-age Women

Peihao Wu[1, 2, 3], MS; Feng Wu[1, 2], MPH; Ziyi Zhang[1, 2, 3], MS; Yifan Zhang[1,2,3], MS; Balansama Marah[1, 2], MPH; Haonan Shi[1, 2, 3], MS; Qitong Yuan[1, 2], MB; Qinrou Chen[1, 2], MB; Yiran Zha[1, 2], MB; Xuan Jin[1, 2], MB; Lei Chen[1, 2], MB; Jing Wei[4, *], MD; Qiuqin Tang[5, *], MS; Wei Wu[1, 2, 3, *], PhD

[1] State Key Laboratory of Reproductive Medicine and Offspring Health, Center for Global Health,

Nanjing Medical University, Nanjing, China

[2] Key Laboratory of Modern Toxicology of Ministry of Education, School of Public Health, Nanjing Medical University, Nanjing, China

[3] Taizhou Clinical Medical College, Nanjing Medical University, Taizhou, China

[4] Department of Obstetrics, The Affiliated Taizhou People's Hospital to Nanjing Medical University, Taizhou, China.

[5] Department of Obstetrics, Women's Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, Nanjing, China

[*] Corresponding author. Wei Wu, State Key Laboratory of Reproductive Medicine and Offspring Health, Center for Global Health, School of Public Health, Nanjing Medical University, 101 Longmian Avenue, Nanjing 211166, China. Email: wwu@njmu.edu.cn. Qiuqin Tang, Department of Obstetrics, Women's Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, Nanjing, China. Email: tqq19871004@126.com. Jing Wei, Department of Obstetrics, The Affiliated Taizhou People's Hospital to Nanjing Medical University, Taizhou, China. Email: qlm_weijin@163.com.

Key Points

Question What moderating role do dietary factors and physical activity play in tobacco-exposure-induced folate deficiency?

Findings Active smoking causes folate deficiency, but passive smoking simply causes lower folate levels. Appropriate dietary factors or increased physical activity can be effective in reducing this risk, and related dietary factors were screened.

Meaning Provide guidance and advice on behavioral interventions for women who are preparing for or may become pregnant.

Abstract

IMPORTANCE Although reduced folate levels due to tobacco exposure have been reported, the association of tobacco exposure with the risk of folate insufficiency in women of gestational age has rarely been reported, and the moderating role of diet and physical activity in this process requires further analysis.

OBJECTIVE To analyze the correlations, causal associations, and dose-risk relationships between tobacco exposure and folate levels/folate deficiency, and to analyze the moderating role of dietary factors and physical activity.

DESIGN, SETTING, AND PARTICIPANTS The data for the study was obtained from NHANES (https://www.cdc.gov/nchs/nhanes/). We developed detailed exclusion criteria and obtained relevant data for women aged 20-45 years.

MAIN OUTCOMES AND MEASURES Folate levels, including erythrocyte folate and serum folate. Folate deficiency, defined in this study as <400 ng/mL, is the prevention of neural tube malformations in the offspring. Microbiological assay was employed to measure folate levels in whole blood, while serum folate was determined using LC-MS/MS coupled with tandem mass spectrometry analysis. Subsequently, the data obtained from these measurements were utilized to calculate red blood cell folate levels.

RESULTS Association analyses and two-sample Mendelian randomization suggest that smoking causes folate deficiency. Dietary factors and physical activity can counteract folate deficiency from tobacco exposure. Women of advanced age are suspected sensitizers.

CONCLUSIONS AND RELEVANCE Families preparing for pregnancy should stay away from tobacco exposure, and where it is difficult to eliminate this exposure, it can be prevented through

diet and physical activity.

1. Introduction

The “Global Tobacco Use Trends Report 2000-2030,” released by the World Health Organization, estimates that there are currently 1.25 billion adult smokers worldwide. Each year, smoking directly contributes to nearly 7 million deaths globally, while second-hand smoke exposure among non-tobacco users accounts for about 1.3 million deaths. Smoking presents significant risks to human systems, including the reproductive, respiratory, cardiovascular, immune, and digestive systems, leading to long-term irreversible effects on health[1,2]. Although many women have never smoked, they still have a high chance of being exposed to secondhand smoke, especially if their partner smokes[3]. Smoking prevalence in America has been informed to be steadily declining, but 17.3% of childbearing-age women and 6.8% of pregnant women in the 2009-2013 survey were still active smokers, and about 35% of these pregnant women were unavoidably exposed to environmental tobacco smoke[4]. Tobacco exposure prior to and during pregnancy can lead to notable alterations in various maternal organs and systems. These changes, in turn, elevate the likelihood of experiencing complications during pregnancy and unfavorable outcomes, which include without limiting, gestational diabetes, placental abruption, fetal growth restriction, and a variety of birth defects[5–7].

Folate, also referred to as vitamin B9, plays a vital role in numerous essential biological processes across various organisms due to its water-soluble nature. Sufficient folate not only promotes normal fetal growth and development, significantly reducing the occurrence of neural tube defects, but also provides protection against other specific congenital abnormalities[8,9]. Folate, an essential nutrient, cannot be synthesized by mammalian cells and therefore needs to be obtained from external sources such as food and gut microbiota[10]. To meet the needs of both the

mother and the fetus, pregnant women typically require additional folate supplementation during pregnancy. Folic acid deficiency has been associated with an increased risk of cardiovascular disease, various types of cancer, and neurocognitive disorders[11]. The World Health Organization defines a red blood cell folate level below 400 ng/mL or 906 nmol/L as the optimal threshold for preventing neural tube defects (NTDs).

Recently, an increasing number of researchers have emphasized the impact of tobacco exposure on folate levels, suggesting a correlation between tobacco exposure and reduced folate levels[12,13], but few have proposed behavioral interventions other than smoking cessation. Physical activity (PA) and appropriate dietary structure can promote human growth and development, improve human health, and reduce the risk of many chronic diseases[14,15]. Considering that many women of childbearing age are unable to avoid or are unconsciously exposed to tobacco smoke, NHANES data was used to figure out the association between tobacco exposure and folate levels in childbearing-age women and to explore the potential moderating role of diet and PA on tobacco-induced folate deficiency in this population. In doing so, we aim to provide guidance and recommendations for behavioral interventions that are easily accessible and feasible for those preparing for pregnancy pregnant or being pregnant.

2. Materials and methods

2.1 Study design and participants

NHANES (https://www.cdc.gov/nchs/nhanes/) is a cross-sectional survey under the auspices of the CDC in the US, which is updated every two years and selects representative samples from across the country, including demographic data, dietary data, laboratory test data, and questionnaire data, which were applied to the analyses in this study[16]. The four “2011-2012”,

"2013-2014", "2015-2016", "2017-2020 pre-pandemic" parts were combined and analyzed. The variables that were accommodated included demographics, smoking exposure, secondhand smoke exposure, exercise, 24h review diet, red blood cell (RBC) folate, and serum folate. RBC folate was the primary outcome; lack of RBC folate (n = 4083) was excluded, and the study population was set at women aged 20-45 years, an age group that is typically fertile and in need of childbearing, and failure to meet sex (n = 15109) and age (n = 11413) was excluded. Missing dietary data (n = 469), serum folate (n = 71), 30-day smoking status (n = 3365), and body mass index (BMI) (n = 10) were excluded. The presence of outliers in physical activity (PA) (n = 6) was excluded. Finally, 1403 women aged 20-45 years were included to form Dataset 0 (n = 1403). Using the multiple imputation by chained equations (MICE) method, Dataset 0 was filled in to form Dataset 1 (n = 1403), in addition to this, Dataset 0 was further screened for variables based on differences in the content of the analysis by excluding samples with smoking status within 5 days, passive smoking, and missing samples of cotinine, respectively, and MICE resampling was also performed to form Dataset 2 (n = 1299), Dataset 3 (n = 1115), and Dataset 4 (n = 1397), respectively (Fig. 1). Dataset 3 was excluded more than Dataset 0 because no secondhand smoke exposure data were included in "2011-2012".

2.2 Measurements of folate level

In the NHANES survey, total folate measurements provide information about an individual's folate status, with serum folate serving as a short-term indicator and red blood cell folate serving as a long-term one. The microbiological assay was employed to measure folate levels in whole blood, while serum folate was determined using isotope-dilution high-performance liquid chromatography coupled with tandem mass spectrometry analysis (LC-MS/MS)[17]. Subsequently,

the data obtained from these measurements were utilized to calculate RBC folate levels, which were maintained the same way from 2011 to 2020.

2.3 Tobacco exposure

Exposures of "smoking in 30 days", "smoking in 5 days", and "passive smoking" were defined according to different tobacco exposure statuses. The first two were designed to analyze long-term and short-term exposures, while the NHANES definition of passive smoking is secondhand smoke exposure within 7 days, which is a short-term, low-dose exposure. In addition, we assumed that the serum cotinine dose was set at the relative level of tobacco exposure for the dose-risk assessment. Cotinine, the primary metabolite of nicotine found in body fluids, is commonly regarded as the preferred marker to assess active smoking and indicate exposure to secondhand smoke[18].

2.4 Dietary factors

Dietary data were based on the total nutrient intakes of NHANES dietary interview on the first day. Dietary fiber, total fat, total saturated fatty acids, total monounsaturated fatty acids, total polyunsaturated fatty acid, cholesterol, vitamin E, vitamin A, food folate, iron, and zinc variables were chosen to be included for analysis, and the specific distributions are shown in Table 1.

2.5 Physical activity

Metabolic Equivalent (MET) refers to the amount of oxygen consumption required to sustain resting metabolism. It is commonly used as an indicator to compare the relative energy metabolism levels during various activities, based on the energy consumed while at rest or sitting[19]. PA is collected through the NHANES Physical Activity Questionnaire (PAQ), which assigns different MET values[20] to different types of PA (Table S1), and the formula for calculating

PA is: $PA\ (MET \cdot h/wk) = MET \times weekly\ frequency\ (d/wk) \times daily\ time\ (h/d)$[21,22]. PA is defined as low, median, or high according to its tertile.

2.6 Covariates

Age, race (Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, Non-Hispanic Asian, Other Race-Including Multi-Racial), education (< 9th grade, 9-11th grade, 12th grade, University/College, College graduate or above), income (included a ratio of family income to poverty), alcohol consumption, and BMI variables were collected as confounders for inclusion in the analysis. In multivariate models assessing the effects of smoking, passive smoking is taken into account and adjusted. These models correct for smoking status over a 30-day period.

2.7 Statistical analysis

Data were downloaded from the NHANES database for "2011-2012", "2013-2014", "2015-2016", and "2017-2020 pre-pandemic". Continuous variables are expressed through mean and standard deviation as "mean (s.d.)", categorical variables are expressed as "n (%)", and the description of the original data Dataset 0 is presented in Table 1. Analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were used to reveal the association between tobacco exposure and folate levels (serum folate as well as erythrocyte folate), with ANCOVA adjusting for confounders which are depicted above. Logistic regression and multiple logistic regression were employed to investigate the association between tobacco exposure and the risk of folate insufficiency. In multiple logistic regression, adjustments were made for potential confounding factors. Two-sample mendelian randomization analysis was performed between "Current tobacco smoking" (id: ukb-b-223) and "Folate" (id: ukb-b-11349) to resolve the causal association between tobacco exposure and body folate levels[23,24]. Additionally, different PA groups and BMI

groups[25] (< 25 kg/m^2, 25-30 kg/m^2, ≥ 30 kg/m^2) were analyzed using multivariate logistic regression, and the role of dietary factors in different exposure groups was analyzed using generalized additive model (GAM). The formula of the GAM[26] is $log\left(\frac{p(X)}{1-p(X)}\right) = \beta_0 + f_1X_1 + f_1X_1 + \cdots + f_pX_p$. Finally, to look for suspected sensitive populations, these participants were categorized into "young adulthood", "middle age", and "advanced age" according to 20-25, 25-35, and 35-45, and the risk of folate insufficiency at different cotinine levels was fitted using the restricted cubic spline (RCS) model[27,28] for all-age and different age groups of participants. All statistical analyses were performed using R-4.3.2, and all reported probabilities (*P* values) were two-sided, with $P < 0.05$ considered statistically significant.

3. Results

3.1 General characteristics of the participants

The general characteristics of the participants are vividly depicted in Table S1. A total of 1403 female participants of childbearing age were included for analysis. There was no difference in the distribution of outcome folate deficiency between data from different years ($P = 0.553$), which suggests that it is reasonable to analyze folate deficiency by combining these parts of the data, and also to increase the sample size. Pearson's chi-square test and Spearman's rank sum test showed that "Smoking in 30 days" ($P < 0.001$), "Smoking in 5 days" ($P < 0.001$), "Passive smoking" ($P = 0.032$) and cotinine ($P < 0.001$) were correlated with deficiency. Among dietary factors, dietary fiber ($P < 0.001$), vitamin E ($P < 0.001$), vitamin A ($P = 0.001$), dietary folate ($P < 0.001$), iron ($P < 0.001$), and zinc ($P = 0.025$) intake were associated with folate deficiency. Correlations were also present for age ($P = 0.008$), race ($P < 0.001$), education level ($P = 0.001$), and income status ($P < 0.001$), which were also included as part of our correction factors. PA ($P =$

0.621) itself was not associated with folate deficiency, but after triple categorical grouping, the association between the PA group ($P = 0.005$) and folate deficiency was surprisingly found. The distribution of folic acid in the participants is shown in Table S2.

3.2 Relationship between tobacco exposure and body folate

3.2.1 Correlation analysis

Tobacco exposure was shown to be associated with RBC folate levels through ANOVA analyses ("Smoking in 30 days": $P = 5.27\times10^{-22}$, "Smoking in 5 days": $P = 5.48\times10^{-20}$, "Passive smoking": $P = 0.040$) and ANCOVA ("Smoking in 30 days": $P = 3.32\times10^{-23}$, "Smoking in 5 days": $P = 9.81\times10^{-21}$, "Passive smoking": $P = 0.031$; Table 2), and this correlation actually suggests that tobacco exposure can tend to reduce RBC folate levels. Folate deficiency was defined as a binary variable in this study using RBC folate level < 400 ng/mL, used to logistic regression and multivariate logistic regression analyses, which revealed that tobacco exposure increases the risk of folate deficiency (Table 3). However, after correction for covariates, the OR (1.28, CI: 0.94-1.74, $P = 0.121$) for passive smoking was no longer significant, implying that exposure to passive smoking affects erythrocyte folate levels but not sufficiently to cause the development of folate deficiency. The effect of tobacco exposure on serum folate is supplemented in Table 4, where exposure to "smoking in 5 days" had the most significant effect on serum folate.

3.2.2 Two-sample mendelian randomization (MR) analysis

A two-sample mendelian randomization (TSMR) method was used to demonstrate the causal relationship between tobacco exposure and folate levels. Significant results (Fig. 2-A, Fig. 2-B) were observed in the "IVW", "IVW-FE" and "IVW-MRE" methods, and current tobacco smoking leads to the reduction of folate levels. MR leave-one-out sensitivity analysis showed that the

exclusion of SNPs one by one does not cause a remarkable change in the conclusions (Fig. 2-C). The funnel plot also shows the reliability of these 3 methods (Fig. 2-D).

3.3 Effects of dietary factors on blood folate deficiency by tobacco-exposed status

The GAM model was used to model folate deficiency with each nutrient level separately in tobacco-exposed or non-exposed groups. Dietary folate supplementation significantly reduced the risk of folate insufficiency ("smoking in 30 days": *P* for nonlinear = 0.012, "smoking in 5 days": *P* for nonlinear = 0.035) in tobacco-exposed populations, and the results suggest that dietary folate supplementation should be at least higher than 250 mcg per day for planned or potential pregnancies (Fig. 3-A). Dietary fiber ("smoking in 30 days": *P* for nonlinear = 0.006, "smoking in 5 days": *P* for nonlinear = 0.037, "passive smoking": *P* for nonlinear = 0.038) and iron ("smoking in 30 days": *P* for nonlinear = 0.009, "smoking in 5 days": *P* for nonlinear = 0.042, "passive smoking": *P* for nonlinear = 0.033) intake were proved to be successful in diminishing the risk of folate deficiency in tobacco-exposed populations (Fig. 3-B). Vitamin E intake also reduced the risk of folate deficiency ("smoking in 30 days": *P* for nonlinear = 0.014, "smoking in 5 days": *P* for nonlinear = 0.038) in tobacco-exposed populations. Interestingly, in our analysis of zinc intake, we found that zinc intake in tobacco-exposed populations reduced the risk of folate deficiency ("smoking in 30 days": *P* for nonlinear = 0.047, "smoking in 5 days": *P* for nonlinear = 0.049), but that zinc intake and the risk of folate insufficiency in non-exposed populations were in a U-shaped curve, suggesting that there is a complex interaction between zinc intake and tobacco exposure.

3.4 Regulation of PA and BMI on tobacco exposure and blood folate deficiency

In the analysis of passive smoking exposure, no positive results were found after analyzing

each subgroup for PA and BMI (Fig. 4). For PA (Fig. 4-A), the risk of folate deficiency due to tobacco exposure was attenuated as the PA group intensified. For BMI (Fig. 4-B), the risk of folate deficiency was found to aggravate with increasing BMI group in the "smoking in 30 days" exposure. Interestingly in the "smoking in 5 days" exposure, the risk of folate deficiency at low BMI was no longer statistically significant.

3.5 Dose-risk assessment of cotinine as an internal exposure dose in different age groups

Serum cotinine doses were used to characterize levels of tobacco exposure. In the whole population, the risk of folate deficiency increased with increasing serum cotinine (Fig. 5-A). Participants, split into "young adulthood", "middle age", and "advanced age" according to 20-25, 25-35, and 35-40, were analyzed. The result found that women of advanced age had a higher risk of folate deficiency at the same level of cotinine exposure (Fig. 5-B).

4. Discussion

The negative correlation between tobacco exposure and folate levels in childbearing-age women was explored based on NHANES data, and the beneficial effects of PA and appropriate diet on folate levels who exposed to smoking were analyzed. Several studies and discussions have explored the relationship between tobacco exposure and folate levels in the body. Ulvik et al. found that smoking decreased the circulating concentration of folate[29]. A systematic review that included 28 studies indicated that pregnant women exposed to smoking had significantly lower levels of folate compared to those not exposed to smoking[13]. Furthermore, we utilized the two-sample Mendelian randomization (TSMR) method[30], which provided evidence supporting a causal relationship in the general population, thus supporting the same in reproductive-age women to some extent. The reduction in folate levels due to tobacco exposure may result from a number

of mechanisms. Some components of tobacco smoke, such as nitrites, nitrogen oxides, cyanates and isocyanates, can increase oxidative stress and interact with folate, leading to inactivation of folic acid and consequently lower folate levels[31]. Furthermore, nicotine stimulates the body's sympathetic nervous system[32], leading to an increase in basal metabolic rate, which makes smokers require more of certain nutrients in order to achieve the same biochemical parameters as non-smokers[33]. This need is already high during pregnancy, which leads to a significant increase in the risk of folic acid deficiency in pregnant women who smoke. It has also been hypothesized that differences in dietary habits and folic acid supplementation compliance between smokers and non-smokers may potentially bring about the differences in folate levels[34].

It is well established that active smoking leads to lower folate levels, whereas the impact of passive smoking on folate levels has not been definitively established. We found that passive smoking affects erythrocyte folate levels, but not enough to cause the development of folate deficiency. Similarly, Prasodjo et al. evaluated the relationship between serum whole blood folate and cotinine at 16 weeks of gestation in women and found that folate levels were reduced in passive smokers (limit of detection ~ 3 ng /mL) relative to unexposed individuals (below the limit of detection), but the difference was not statistically significant[35]. However, within a cross-sectional study based on data from the third National Health and Nutrition Examination Survey (NHANES III), serum cotinine concentrations <15 ng/mL were defined as exposure to tobacco smoke, and 1.5 times greater risk of folate deficiency was found in populations with high levels of exposure to environmental tobacco smoke (0.4-15 ng/mL) than low levels of exposure (0.05-0.1 ng/mL)[36]. Differences in findings may be due to inconsistencies in the methods used to evaluate environmental tobacco smoke exposure in existing studies. In our study, questionnaires

were used to assess secondhand smoke exposure within 7 days, while a subset of studies evaluated passive smoking exposure by serum or plasma cotinine. Although the exposure dose from passive smoking is lower than from active smoking, side-stream smoke from environmental tobacco smoke contains higher concentrations of toxins, making passive smokers potentially more at risk than active smokers[37]. Therefore, it is important to pay sufficient attention to the impact of tobacco exposure on folate levels in reproductive-age women and actively implement effective intervention strategies.

Apart from tobacco exposure, factors such as alcohol consumption, diet, age, BMI, medication intake, health status, and level of physical activity can have varying types and degrees of impact on folate levels in the human body[38–40]. In this study, we found that increasing dietary folate intake significantly reduces the risk of folate deficiency in individuals exposed to tobacco. The results indicate that the recommended dietary folate intake for women planning or potentially planning pregnancy should be higher than 250 micrograms, which aligns with the guidelines suggested by the Chinese National Health Commission (400-600 micrograms) and the National Institutes of Health in the United States (400-800 micrograms). Additionally, we discovered that intake of vitamin E, iron, and dietary fiber can also lower the risk of folate deficiency in the population exposed to tobacco. In recent years, researches on the association between intake of other nutrients and folate levels in humans have been fewer, and a study by Christos et al. showed that intake of dietary fiber, calcium, magnesium, folate, and vitamins A, B1, B6, C and E were positively in association with serum folate[41], which is somewhat different from our findings. This may be due to the fact that dietary data in both the present study and the former were obtained using the 24-hour dietary recall method, and despite the continuous improvement of dietary data

collection methods by NHANES, there is still a recall bias in the data obtained by self-reporting[16]. Future relevant studies could incorporate anthropometric measurements, biomarkers, or the use of food recording methods to assess diet to reduce bias or error. According to a study carried out by Tajima et al., they discovered a negative correlation between the intake of certain nutrients, such as dietary fiber, soluble and insoluble cellulose, and dietary folate, and serum homocysteine concentrations[42]. Another study conducted by Bermejo et al. pointed out that an increased consumption of fruits and vegetables played a role in lower levels of plasma homocysteine[43]. This also validates the results of the present study on the other hand, as B vitamins (especially folic acid) have a direct relationship with homocysteine metabolism, and folic acid levels were negatively correlated with homocysteine levels[13]. The above studies show that there is indeed a correlation between folate levels and certain dietary micronutrients, which recommends that childbearing-age women consume fruits, vegetables, legumes, and mushrooms (which are the main sources of dietary folate, dietary fiber, iron, and vitamins) for reducing the hazards of folate deficiency in tobacco-exposed populations[44].

Exercise is known to be beneficial to physical health. Using subgroup analyses we found that increasing physical activity levels also reduces the risk of folate deficiency in tobacco-exposed women of childbearing age. It is rather unfortunate that the relationship between PA and folate levels has been less well studied and is still controversial. A cross-sectional study encompassing an extensive evaluation of B vitamin (folate, vitamin B6, and vitamin B12) profiles in highly active and sedentary women yielded no substantial disparities in B vitamin levels between the two cohorts[45]. A study of athletes found that plasma folate was significantly higher in the highly trained group[46]. The difference in results may be due to the fact that these experiments, including

our study, did not control for dietary conditions. People who perform physical activities of a certain intensity will consume more energy and nutrients, which may lead to changes in folate levels. In conclusion, most of the current relevant studies are limited to the general population and athletes, and there are few studies on women of reproductive age. Taking into account the results of this study, in order to keep folate levels at appropriate levels, women of childbearing age may be able to engage in appropriate physical activities while consuming sufficient dietary folate.

Finally, but equally important, our analysis based on different age groups has found that it is more probable for advanced-age women to be at risk of folate deficiency, who may be a sensitive population requiring special attention. This potential association could be attributed to the decline in estrogen production in perimenopausal women[47]. This decline directly triggers receptors distributed along the brain-gut axis, which in turn affects gut motility and gastrointestinal sensitivity[48]. This ultimately affects the digestion and absorption of folate in the gut. Therefore, as estrogen levels decline during the perimenopausal period, the metabolism and utilization of folate in the body may be affected. Additionally, this may also be related to the use of contraceptive medications around the perimenopausal period, as it has been shown that women taking oral contraceptives have higher rates of nicotine and cotinine metabolism compared to non-users[49]. Lastly, advanced-age women who have been long-term smokers may have accumulated higher levels of nicotine in their bodies, which can have a greater impact on folate levels. Long-term smoking can disrupt 1-carbon metabolism, leading to folate depletion or metabolic imbalances[50], thereby increasing the risk of folate deficiency.

5. Conclusions

These findings have significant implications for public health, highlighting the need for

targeted interventions and awareness campaigns to address the impact of tobacco exposure on the nutritional status of women in their childbearing years, particularly regarding folate deficiency. Passive smoking affects red blood cell folate levels, but insufficient to cause folate deficiency. Appropriate physical activity, as well as dietary intake of dietary folate, dietary fiber, and iron all reduced the risk of folate deficiency in tobacco-exposed childbearing-age women. Women preparing for or during pregnancy should stay away from tobacco exposure, and where avoidance is difficult, folate reduction can be ameliorated by supplementing with foods containing folate, dietary fiber, and iron (fruits, vegetables, legumes, mushrooms, etc.) and by engaging in appropriate physical activity. Folate deficiency in advanced-age women may warrant more attention.

Data availability

Data are all accessible through the NHANES website.

Authors' roles

P. W. is responsible for study designing, modeling and evaluation. P. W., F. W., Y. Z. and B. M. wrote the first draft, key revisions and figure formations were undertaken by Z. Z. and W. W. Others have contributed to data collection and cleansing. All authors read and approved the final manuscript.

Funding

This work was supported by the National Key Project of Research and Development Program (2022YFC2702902), the National Natural Science Foundation of China (82273662, 81971405), the Jiangsu Natural Science Foundation (BK20221307), Major Program of Wuxi Medical Center, Nanjing Medical University (WMCM202306).

Conflict of interest statement

The authors declare no conflict of interest.

References

1. Benowitz NL, Liakoni E. Tobacco use disorder and cardiovascular health. *Addiction*. 2022;117(4):1128-1138. doi:10.1111/add.15703

2. Practice Committee of the American Society for Reproductive Medicine. Electronic address: asrm@asrm.org, Practice Committee of the American Society for Reproductive Medicine. Smoking and infertility: a committee opinion. *Fertil Steril*. 2018;110(4):611-618. doi:10.1016/j.fertnstert.2018.06.016

3. Yi C, Yeh CC, Wang PH. Voluntary and involuntary smoking during pregnancy. *J Chin Med Assoc*. 2017;80(12):745-746. doi:10.1016/j.jcma.2017.04.006

4. Mazurek JM, England LJ. Cigarette smoking among working women of reproductive age-united states, 2009-2013. *Nicotine Tob Res*. 2016;18(5):894-899. doi:10.1093/ntr/ntv292

5. Masalin S, Kautiainen H, Gissler M, Pennanen P, Eriksson JG, Laine MK. Impact of smoking on gestational diabetes mellitus and offspring birthweight in primiparous women. *Acta Obstet Gynecol Scand*. 2020;99(12):1632-1639. doi:10.1111/aogs.13924

6. Mortensen JT, Thulstrup AM, Larsen H, Møller M, Sørensen HT. Smoking, sex of the offspring, and risk of placental abruption, placenta previa, and preeclampsia: a population-based cohort study. *Acta Obstet Gynecol Scand*. 2001;80(10):894-898. doi:10.1034/j.1600-0412.2001.801005.x

7. Salihu HM, Wilson RE. Epidemiology of prenatal smoking and perinatal outcomes. *Early Hum Dev*. 2007;83(11):713-720. doi:10.1016/j.earlhumdev.2007.08.002

8. US Preventive Services Task Force, Barry MJ, Nicholson WK, et al. Folic Acid Supplementation to Prevent Neural Tube Defects: US Preventive Services Task Force Reaffirmation Recommendation Statement. *JAMA*. 2023;330(5):454-459. doi:10.1001/jama.2023.12876

9. Czeizel AE, Dudás I, Vereczkey A, Bánhidy F. Folate deficiency and folic acid supplementation: the prevention of neural-tube defects and congenital heart defects. *Nutrients*. 2013;5(11):4760-4775. doi:10.3390/nu5114760

10. Engevik MA, Morra CN, Röth D, et al. Microbial metabolic capacity for intestinal folate production and modulation of host folate receptors. *Front Microbiol*. 2019;10:2305. doi:10.3389/fmicb.2019.02305

11. Shulpekova Y, Nechaev V, Kardasheva S, et al. The concept of folic acid in health and disease. *Molecules*. 2021;26(12). doi:10.3390/molecules26123731

12. Li M, Chen X, Zhang Y, et al. RBC folate and serum folate, vitamin B-12, and homocysteine in chinese couples prepregnancy in the shanghai preconception cohort. *J Nutr*. 2022;152(6):1496-1506. doi:10.1093/jn/nxac050

13. Tuenter A, Bautista Nino PK, Vitezova A, et al. Folate, vitamin B12, and homocysteine in smoking-exposed pregnant women: A systematic review. *Matern Child Nutr*. 2019;15(1):e12675. doi:10.1111/mcn.12675

14. Joubert LM, Manore MM. Exercise, nutrition, and homocysteine. *Int J Sport Nutr Exerc Metab*. 2006;16(4):341-361. doi:10.1123/ijsnem.16.4.341

15. Piercy KL, Troiano RP, Ballard RM, et al. The physical activity guidelines for americans. *Jama*. 2018;320(19):2020-2028. doi:10.1001/jama.2018.14854

16. Ahluwalia N, Dwyer J, Terry A, Moshfegh A, Johnson C. Update on NHANES dietary data: Focus on collection, release, analytical considerations, and uses to inform public policy. *Adv Nutr*. 2016;7(1):121-134. doi:10.3945/an.115.009258

17. Yetley EA, Pfeiffer CM, Phinney KW, et al. Biomarkers of folate status in NHANES: a roundtable summary. *Am J Clin Nutr*. 2011;94(1):303S-312S. doi:10.3945/ajcn.111.013011

18. Benowitz NL. Cotinine as a biomarker of environmental tobacco smoke exposure. *Epidemiol Rev*. 1996;18(2):188-204. doi:10.1093/oxfordjournals.epirev.a017925

19. Mendes M de A, da Silva I, Ramires V, et al. Metabolic equivalent of task (METs) thresholds as an indicator of physical activity intensity. *PLoS One*. 2018;13(7):e0200701. doi:10.1371/journal.pone.0200701

20. Ainsworth BE, Haskell WL, Herrmann SD, et al. 2011 Compendium of Physical Activities: a second update of codes and MET values. *Med Sci Sports Exerc*. 2011;43(8):1575-1581. doi:10.1249/MSS.0b013e31821ece12

21. Chen L, Cai M, Li H, et al. Risk/benefit tradeoff of habitual physical activity and air pollution on chronic pulmonary obstructive disease: findings from a large prospective cohort study. *BMC Med*. 2022;20(1):70. doi:10.1186/s12916-022-02274-8

22. Ran J, Zhang Y, Han L, et al. The joint association of physical activity and fine particulate matter exposure with incident dementia in elderly Hong Kong residents. *Environ Int*. 2021;156:106645. doi:10.1016/j.envint.2021.106645

23. Davey Smith G, Ebrahim S, Lewis S, Hansell AL, Palmer LJ, Burton PR. Genetic epidemiology and public health: hope, hype, and future prospects. *Lancet*. 2005;366(9495):1484-1498. doi:10.1016/S0140-6736(05)67601-5

24. Holmes MV, Ala-Korpela M, Smith GD. Mendelian randomization in cardiometabolic disease: challenges in evaluating causality. *Nat Rev Cardiol*. 2017;14(10):577-590. doi:10.1038/nrcardio.2017.78

25. Flegal KM, Kit BK, Orpana H, Graubard BI. Association of All-Cause Mortality With Overweight and Obesity Using Standard Body Mass Index Categories. *JAMA*. 2013;309(1):71-82. doi:10.1001/jama.2012.113905

26. Gould CF, Bejarano ML, Kioumourtzoglou MA, et al. Widespread Clean Cooking Fuel Scale-Up and under-5 Lower Respiratory Infection Mortality: An Ecological Analysis in Ecuador, 1990-2019. *Environ Health Perspect*. 2023;131(3):37017. doi:10.1289/EHP11016

27. Tan Y, Fu Y, Yao H, et al. Relationship between phthalates exposures and hyperuricemia in U.S. general population, a multi-cycle study of NHANES 2007-2016. *Sci Total Environ*. 2023;859(Pt 1):160208. doi:10.1016/j.scitotenv.2022.160208

28. Harrell FE, Lee KL, Pollock BG. Regression models in clinical studies: determining relationships between predictors and response. *J Natl Cancer Inst*. 1988;80(15):1198-1202. doi:10.1093/jnci/80.15.1198

29. Ulvik A, Ebbing M, Hustad S, et al. Long- and short-term effects of tobacco smoking on circulating concentrations of B vitamins. *Clin Chem*. 2010;56(5):755-763. doi:10.1373/clinchem.2009.137513

30. Sekula P, Del Greco MF, Pattaro C, Köttgen A. Mendelian randomization as an approach to assess causality using observational data. *J Am Soc Nephrol*. 2016;27(11):3253-3265. doi:10.1681/asn.2016010098

31. Northrop-Clewes CA, Thurnham DI. Monitoring micronutrients in cigarette smokers. *Clin Chim Acta*. 2007;377(1-2):14-38. doi:10.1016/j.cca.2006.08.028

32. Jones CA, Wallace MJ, Bandaru P, Woodbury ED, Mohler PJ, Wold LE. E-cigarettes and arrhythmogenesis: a comprehensive review of pre-clinical studies and their clinical implications. *Cardiovasc Res*. 2023;119(12):2157-2164. doi:10.1093/cvr/cvad113

33. Picciano MF. Pregnancy and lactation: physiological adjustments, nutritional requirements and the role of dietary supplements. *J Nutr*. 2003;133(6):1997s-2002s. doi:10.1093/jn/133.6.1997S

34. Oncel MY, Ozdemir R, Erdeve O, Dilmen U. Influence of maternal cigarette smoking during pregnancy on neonatal serum folate levels. *Eur J Nutr*. 2012;51(3):385-387. doi:10.1007/s00394-011-0261-9

35. Prasodjo A, Pfeiffer CM, Fazili Z, et al. Serum cotinine and whole blood folate concentrations in pregnancy. *Ann Epidemiol*. 2014;24(7):498-503.e1. doi:10.1016/j.annepidem.2014.04.004

36. Mannino DM, Mulinare J, Ford ES, Schwartz J. Tobacco smoke exposure and decreased serum and red blood cell folate levels: data from the Third National Health and Nutrition Examination Survey. *Nicotine Tob Res*. 2003;5(3):357-362. doi:10.1080/1462220031000094330

37. Raghuveer G, White DA, Hayman LL, et al. Cardiovascular consequences of childhood secondhand tobacco smoke exposure: Prevailing evidence, burden, and racial and socioeconomic disparities: a scientific statement from the american heart association. *Circulation*. 2016;134(16):e336-e359. doi:10.1161/cir.0000000000000443

38. Steane SE, Cuffe JSM, Moritz KM. The role of maternal choline, folate and one-carbon metabolism in mediating the impact of prenatal alcohol exposure on placental and fetal development. *J Physiol*. 2023;601(6):1061-1075. doi:10.1113/jp283556

39. Kreusler P, Vogel M, Willenberg A, et al. Folate and cobalamin serum levels in healthy children and adolescents and their association with age, sex, BMI and socioeconomic status. *Nutrients*. 2021;13(2). doi:10.3390/nu13020546

40. Sebastiani G, Borrás-Novell C, Casanova MA, et al. The effects of alcohol and drugs of abuse on maternal nutritional profile during pregnancy. *Nutrients*. 2018;10(8). doi:10.3390/nu10081008

41. Hatzis CM, Bertsias GK, Linardakis M, Scott JM, Kafatos AG. Dietary and other lifestyle correlates of serum folate concentrations in a healthy adult population in Crete, Greece: a cross-sectional study. *Nutr J*. 2006;5:5. doi:10.1186/1475-2891-5-5

42. Tajima A, Kubo Y, Horiguchi S, Shoji K, Kawabata T. Relationship between serum homocysteine concentration and dietary factors in young japanese women. *Nutrients*. 2023;15(22). doi:10.3390/nu15224740

43. Bermejo LM, Aparicio A, Andrés P, López-Sobaler AM, Ortega RM. The influence of fruit and vegetable intake on the nutritional status and plasma homocysteine levels of institutionalised elderly people. *Public Health Nutr*. 2007;10(3):266-272. doi:10.1017/s1368980007246580

44. López-González L, Becerra-Tomás N, Babio N, et al. Variety in fruits and vegetables, diet quality and lifestyle in an older adult mediterranean population. *Clin Nutr*. 2021;40(4):1510-1518. doi:10.1016/j.clnu.2021.02.024

45. Woolf K, Hahn NL, Christensen MM, Carlson-Phillips A, Hansen CM. Nutrition assessment of b-vitamins in highly active and sedentary women. *Nutrients*. 2017;9(4). doi:10.3390/nu9040329

46. König D, Bissé E, Deibert P, Müller HM, Wieland H, Berg A. Influence of training volume and acute physical exercise on the homocysteine levels in endurance-trained men: interactions with plasma folate and vitamin B12. *Ann Nutr Metab*. 2003;47(3-4):114-118. doi:10.1159/000070032

47. Santoro N, Roeca C, Peters BA, Neal-Perry G. The menopause transition: Signs, symptoms, and management options. *J Clin Endocrinol Metab*. 2021;106(1):1-15. doi:10.1210/clinem/dgaa764

48. Jiang Y, Greenwood-Van Meerveld B, Johnson AC, Travagli RA. Role of estrogen and stress on the brain-gut axis. *Am J Physiol Gastrointest Liver Physiol*. 2019;317(2):G203-g209. doi:10.1152/ajpgi.00144.2019

49. Allen AM, Weinberger AH, Wetherill RR, Howe CL, McKee SA. Oral contraceptives and

cigarette smoking: a review of the literature and future directions. *Nicotine Tob Res*. 2019;21(5):592-601. doi:10.1093/ntr/ntx258

50. Steegers-Theunissen RPM, Twigt J, Pestinger V, Sinclair KD. The periconceptional period, reproduction and long-term health of offspring: the importance of one-carbon metabolism. *Hum Reprod Update*. 2013;19(6):640-655. doi:10.1093/humupd/dmt041

Figure legends

Figure 1. Flow Chart for participants recruitment of this study, NHANES 2011-2020.

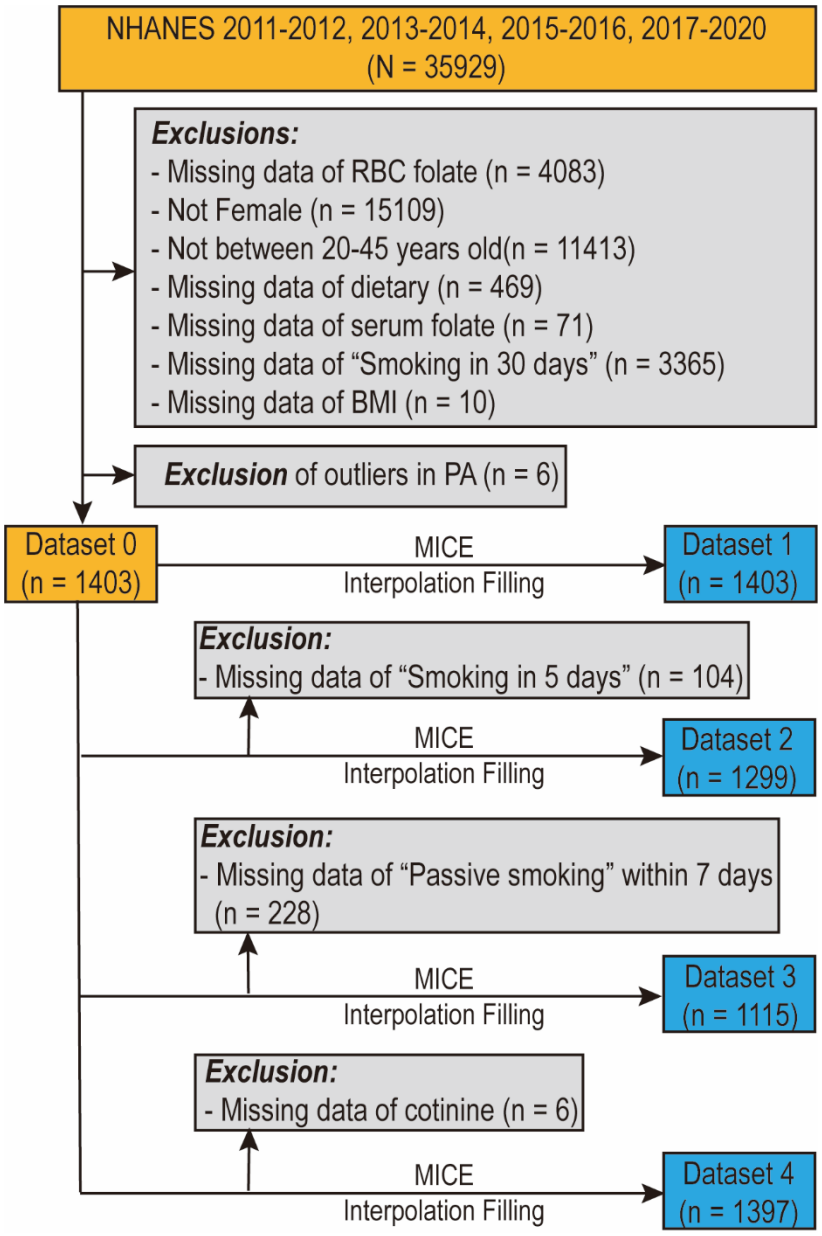
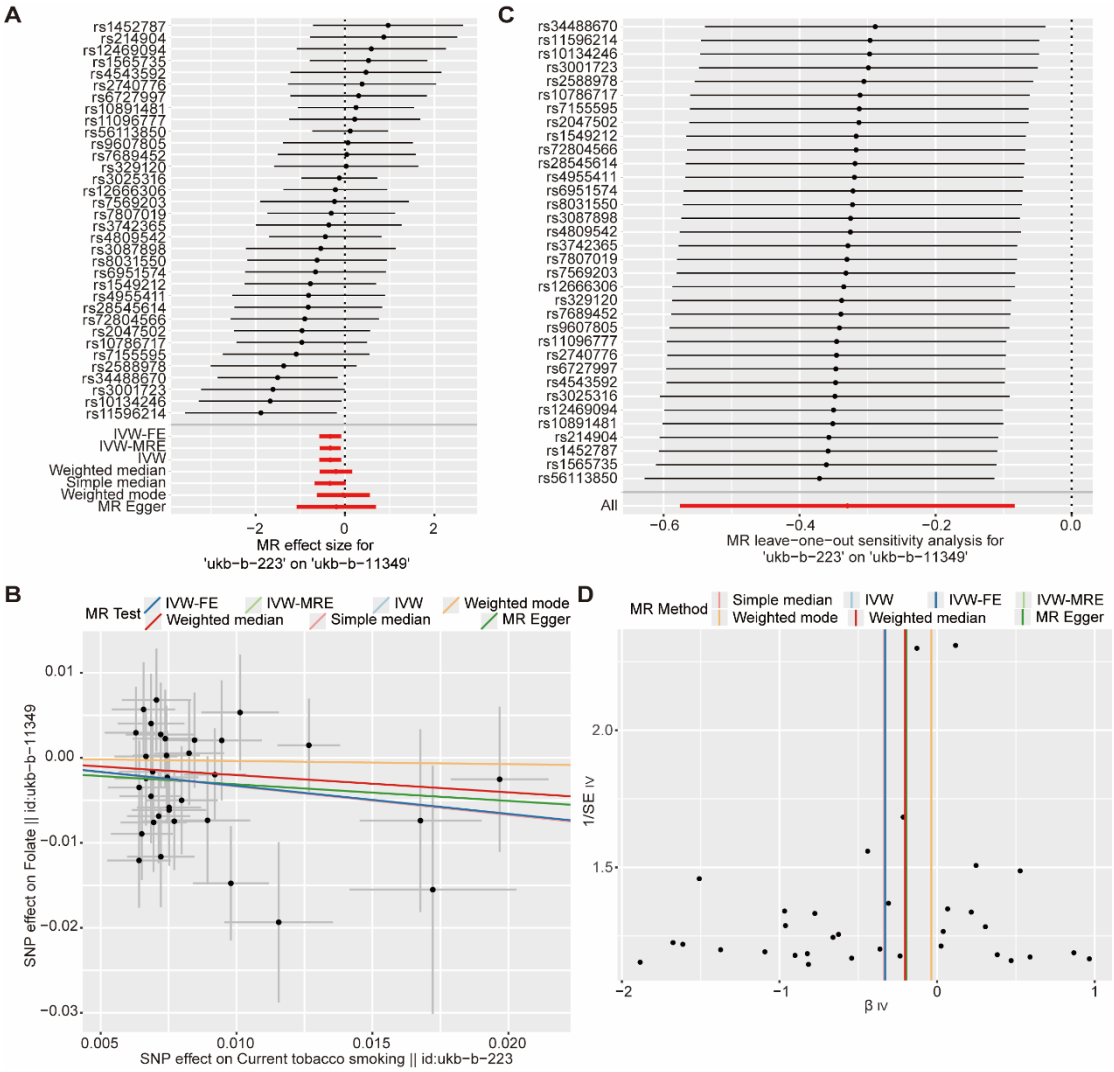


Figure 2. Two sample mendelian randomization analysis of the effects of tobacco exposure on folate.


A
rs1452787
rs214904
rs12469094
rs1565735
rs4543592
rs2740776
rs6727997
rs10891481
rs11096777
rs56113850
rs9607805
rs7689452
rs329120
rs3025316
rs12666306
rs7569203
rs7807019
rs3742365
rs4809542
rs3087898
rs8031550
rs6951574
rs1549212
rs4955411
rs28545614
rs72804566
rs2047502
rs10786717
rs7155595
rs2588978
rs34488670
rs3001723
rs10134246
rs11596214
IVW-FE
IVW-MRE
IVW
Weighted median
Simple median
Weighted mode
MR Egger
−2
0
2
MR effect size for
'ukb−b−223' on 'ukb−b−11349'
C
rs34488670
rs11596214
rs10134246
rs3001723
rs2588978
rs10786717
rs7155595
rs2047502
rs1549212
rs72804566
rs28545614
rs4955411
rs6951574
rs8031550
rs3087898
rs4809542
rs3742365
rs7807019
rs7569203
rs12666306
rs329120
rs7689452
rs9607805
rs11096777
rs2740776
rs6727997
rs4543592
rs3025316
rs12469094
rs10891481
rs214904
rs1452787
rs1565735
rs56113850
All
−0.6
−0.4
−0.2
0.0
MR leave−one−out sensitivity analysis for
'ukb−b−223' on 'ukb−b−11349'
B
MR Test
IVW-FE
IVW-MRE
IVW
Weighted mode
Weighted median
Simple median
MR Egger
0.01
0.00
−0.01
−0.02
−0.03
SNP effect on Folate || id:ukb−b−11349
0.005
0.010
0.015
0.020
SNP effect on Current tobacco smoking || id:ukb−b−223
D
MR Method
Simple median
IVW
IVW-FE
IVW-MRE
Weighted mode
Weighted median
MR Egger
2.0
1.5
1/SE IV
−2
−1
0
1
β IV

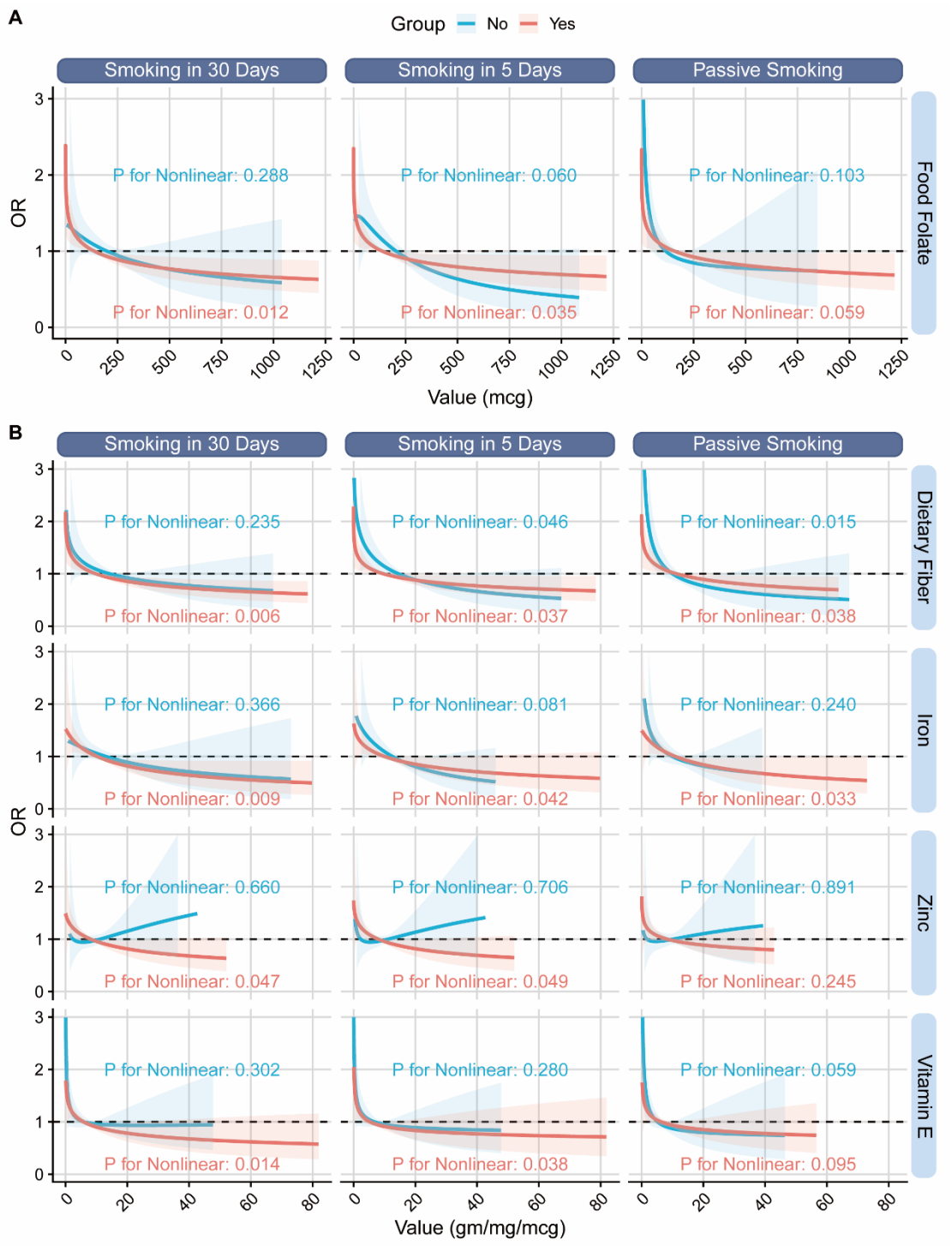


Figure 3. The odds ratio of folate deficiency with different types of tobacco exposure by dietary factor, NHANES 2011-2020. OR: odds ratio; age, race, income, alcohol consumption, BMI, and passive smoking are adjusted for "Smoking in 30 Days" and "Smoking in 5 Days"; age, race, income, alcohol consumption, BMI, and "Smoking in 30 Days" are adjusted for "Passive Smoking".

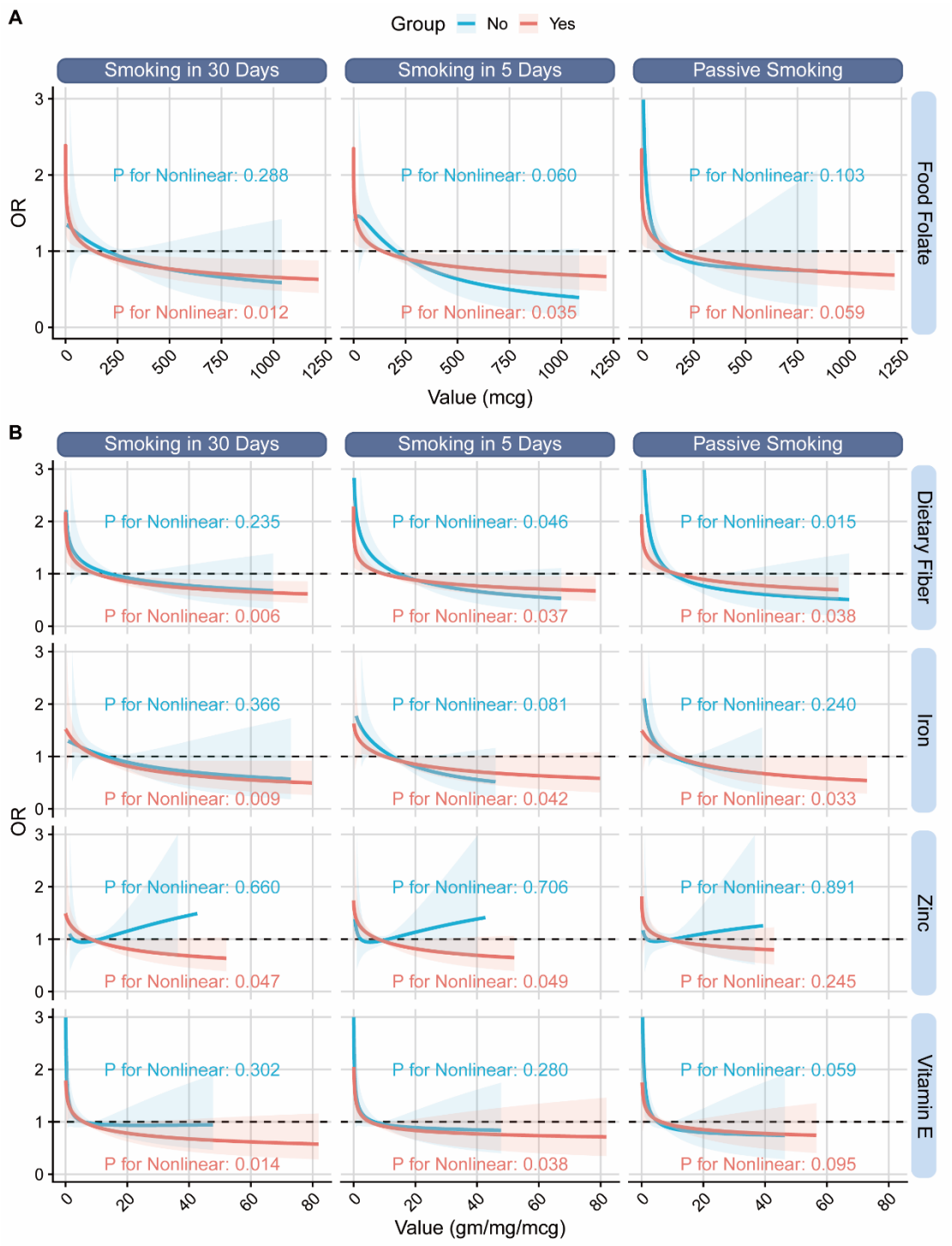


Figure 4. The odds ratio of folate deficiency with different types of tobacco exposure by PA/BMI group, NHANES 2011-2020. OR: odds ratio; age, race, income, alcohol consumption, BMI and passive smoking are adjusted for "Smoking in 30 Days" and "Smoking in 5 Days"; age, race, income, alcohol consumption, BMI, and "Smoking in 30 Days" are adjusted for "Passive Smoking". PA is defined as low, medium, or high according to its tertile. BMI groups: < 25 kg/m2,

25-30 kg/m2, ≥ 30 kg/m2.

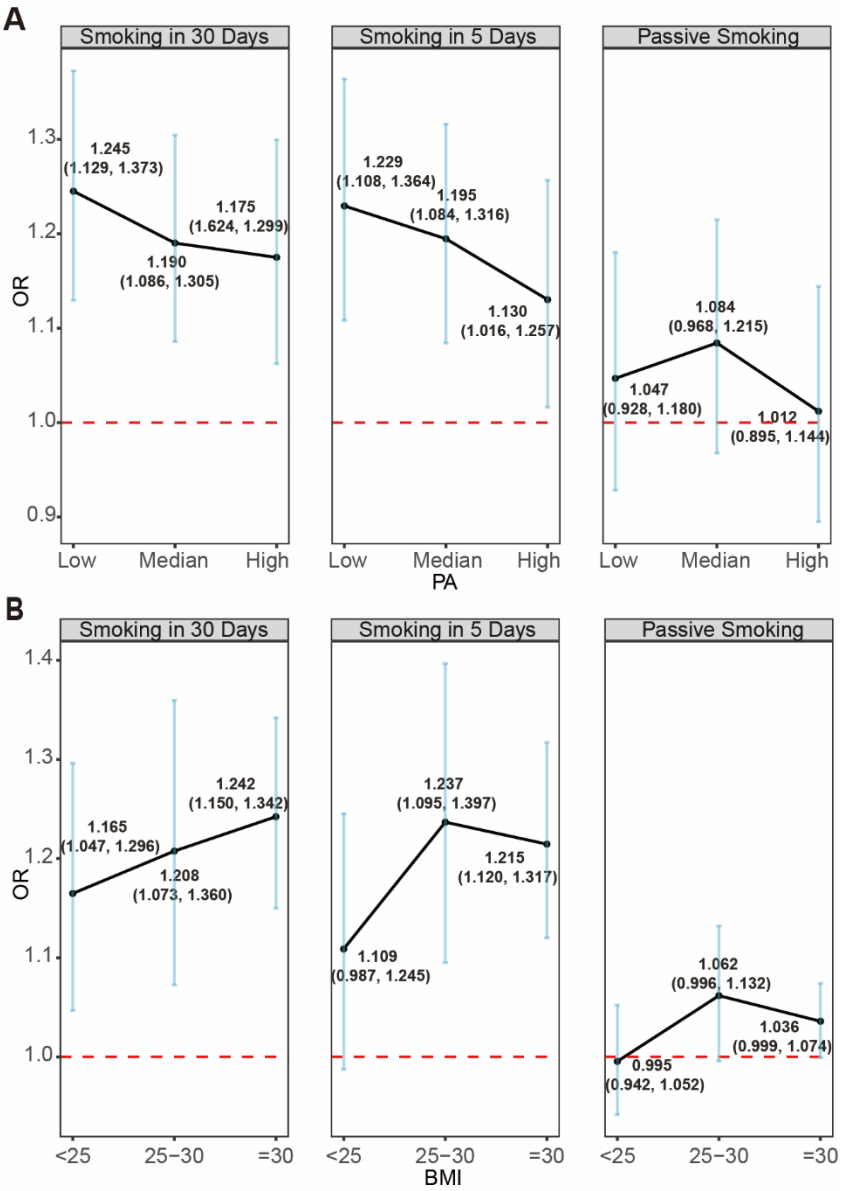


Figure 5. The odds ratio of folate deficiency with cotinine exposure by age group, NHANES 2011-2020. OR: odds ratio; Age groups: young adulthood, middle age, and advanced age.

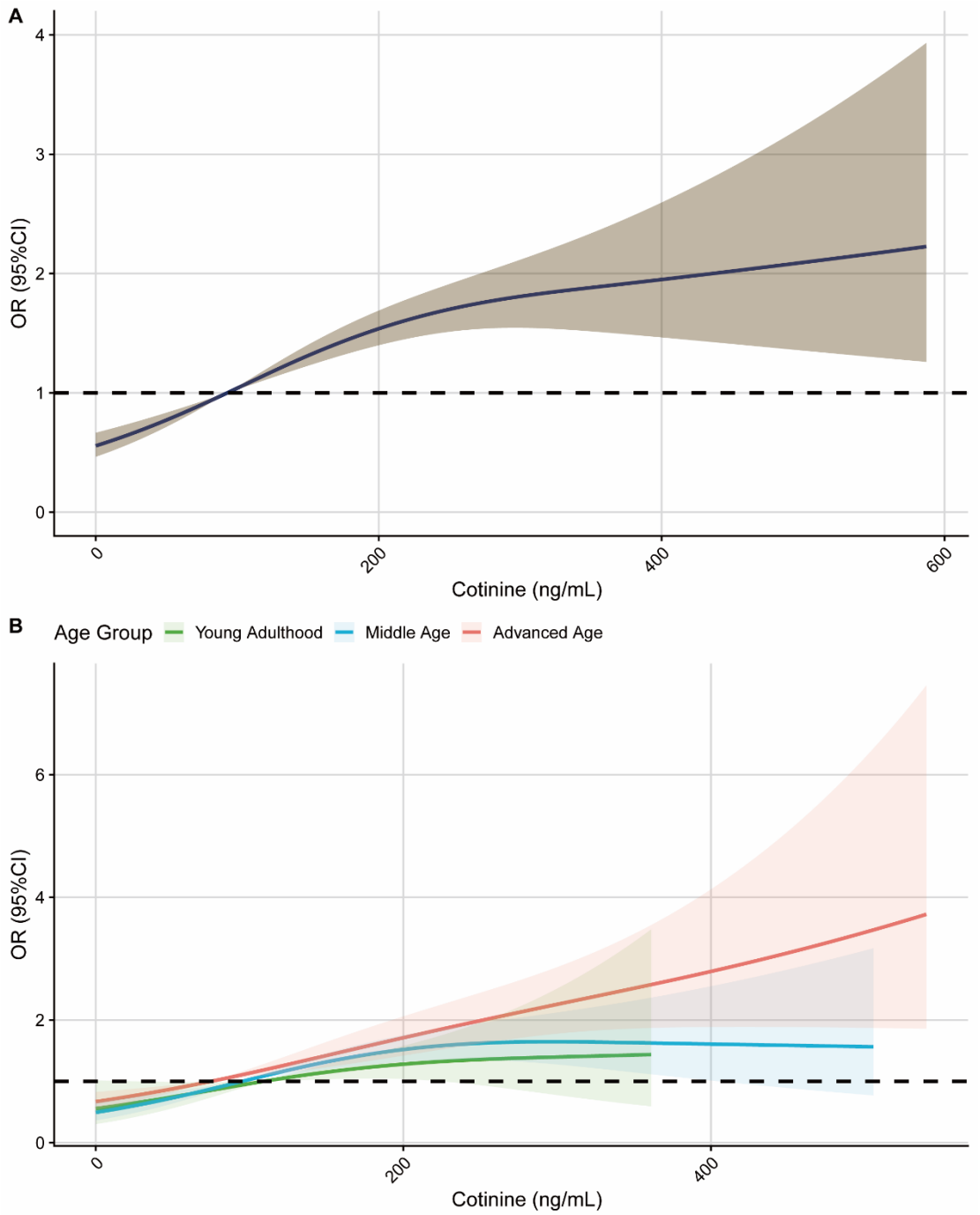

A
OR (95%CI)
0
1
2
3
4
0
200
400
600
Cotinine (ng/mL)
B
Age Group
Young Adulthood
Middle Age
Advanced Age
OR (95%CI)
0
2
4
6
0
200
400
Cotinine (ng/mL)

Table 1. Participant characteristics of "Dataset 0" by folate deficiency, NHANES 2011-2020.

Characteristics	All (N = 1403)	Non-deficiency (n = 810)	Deficiency (n = 593)	*P* value[b]
Year, (%):				0.553
2011-2012	288 (20.53)	159 (19.63)	129 (21.75)	
2013-2014	356 (25.37)	211 (26.05)	145 (24.45)	
2015-2016	294 (20.96)	177 (21.85)	117 (19.73)	
1017-2020	465 (33.14)	263 (32.47)	202 (34.06)	
Smoking in 30 days, (%):				< 0.001
No	535 (38.13)	384 (47.41)	151 (25.46)	
Yes	868 (61.87)	426 (52.59)	442 (74.54)	
Smoking in 5 days, (%):				< 0.001
No	481 (37.03)	346 (46.07)	135 (24.64)	
Yes	818 (62.97)	405 (53.93)	413 (75.36)	
Passive smoking, (%):				0.032
No	253 (22.69)	163 (25.04)	90 (19.40)	
Yes	862 (77.31)	488 (74.96)	374 (80.60)	
Cotinine, ng/mL, mean (s.d.)	134.61 (149.02)	105.06 (136.65)	174.69 (155.71)	< 0.001
Dietary fiber, gm, mean (s.d.)	13.70 (9.27)	15.01 (9.63)	11.92 (8.43)	< 0.001
Total fat, gm, mean (s.d.)	80.99 (47.18)	81.62 (44.06)	80.13 (51.15)	0.568
Total saturated fatty acids, gm, mean (s.d.)	26.36 (16.54)	26.52 (15.43)	26.16 (17.96)	0.695
Total monounsaturated fatty acids, gm, mean (s.d.)	27.80 (17.19)	28.05 (16.69)	27.46 (17.85)	0.531
Total polyunsaturated fatty acids, gm, mean (s.d.)	19.21 (13.78)	19.41 (12.98)	18.95 (14.81)	0.545
Cholesterol, gm, mean (s.d.)	264.27 (218.23)	264.04 (214.83)	264.58 (222.98)	0.963
Vitamin E, as alpha-tocopherol (mg), mean (s.d.)	8.11 (6.69)	8.71 (7.13)	7.29 (5.94)	< 0.001
Vitamin A, RAE (mcg), mean (s.d.)	525.08 (584.49)	566.94 (625.65)	467.91 (518.14)	0.001
Food folate, (mcg), mean (s.d.)	188.37 (136.40)	205.22 (143.16)	165.34 (123.01)	< 0.001
Iron, (mcg), mean (s.d.)	12.26 (7.44)	13.05 (7.48)	11.18 (7.25)	< 0.001
Zinc, (mcg), mean (s.d.)	9.59 (5.58)	9.88 (5.42)	9.19 (5.77)	0.025
Age, years old, Mean (s.d.)	33.46 (7.16)	33.90 (7.01)	32.87 (7.32)	0.008
Race, (%):				< 0.001
Mexican American	143 (10.19)	84 (10.37)	59 (9.95)	

Other Hispanic	109 (7.77)	64 (7.90)	45 (7.59)	
Non-Hispanic White	711 (50.68)	463 (57.16)	248 (41.82)	
Non-Hispanic Black	299 (21.31)	113 (13.95)	186 (31.37)	
Non-Hispanic Asian	53 (3.78)	32 (3.95)	21 (3.54)	
Other Race-Including Multi-Racial	88 (6.27)	54 (6.67)	34 (5.73)	
Education level, (%):				0.001
Less than 9th grade	43 (3.07)	23 (2.84)	20 (3.38)	
9-11th grade	219 (15.62)	114 (14.07)	105 (17.74)	
High school graduate	345 (24.61)	193 (23.83)	152 (25.68)	
Some college or AA degree	571 (40.73)	323 (39.88)	248 (41.89)	
College graduate or above	224 (15.98)	157 (19.38)	67 (11.32)	
Income, a ratio of family income to poverty guidelines, mean (s.d.)	1.92 (1.49)	2.10 (1.54)	1.67 (1.38)	< 0.001
Alcohol consumption, ever have 4/5 or more drinks every day, (%):				0.075
Yes	230 (18.27)	121 (16.55)	109 (20.64)	
No	1029 (81.73)	610 (83.45)	419 (79.36)	
BMI, mean (s.d.)	30.67 (8.64)	30.96 (8.60)	30.28 (8.68)	0.145
PA, MET-h/week, mean (s.d.)	69.98 (106.51)	68.78 (105.86)	71.63 (107.46)	0.621
PA Group, (%):				0.005
Low physical activity	466 (33.21)	250 (30.86)	216 (36.42)	
Median intensity physical activity	476 (33.93)	303 (37.41)	173 (29.17)	
High intensity physical activity	461 (32.86)	257 (31.73)	204 (34.40)	

[a]Passive smoking data were not collected by NHANES during 2011-2012.

[b]Categorical variables were tested using the Pearson chi-square test and continuous variables were tested using the Spearman rank sum test.

Table 2. Effects of tobacco exposure on erythrocyte folate.

Variables	Models	F value[c]	*P* values
Smoking in 30 days	ANOVA	96.17***	5.27×10^{-22}
	ANCOVA[a]	102.08***	3.32×10^{-23}

Smoking in 5 days	ANOVA	86.6***	5.48×10^{-20}
	ANCOVA[a]	90.27***	9.81×10^{-21}
Passive smoking	ANOVA	4.21*	0.040
	ANCOVA[b]	4.64*	0.031

[a] Adjusted for age, race, education, income drinking, BMI, and passive smoking.

[b] Adjusted for age, race, education, income drinking, BMI, and smoking in 30 days.

[c] *$P < 0.05$; *** $P < 0.001$.

Table 3. Risk of folate insufficiency from tobacco exposure.

Variables	Models	OR (95% CI)[d]	*P* values
Smoking in 30 days	LR[a]	2.64 (2.10, 3.32)***	1.61×10^{-16}
	MLR[b]	2.33 (1.81, 3.00)***	4.60×10^{-11}
Smoking in 5 days	LR	2.61 (2.05, 3.33)***	6.41×10^{-15}
	MLR[b]	2.18 (1.67, 2.85)***	8.62×10^{-09}
Passive smoking	LR	1.39 (1.04, 1.86)*	0.027
	MLR[c]	1.28 (0.94, 1.74)	0.121

[a] LR: logical regression;

[b] MLR: multivariate logistic regression; adjusted for age, race, education, income drinking, BMI, and passive smoking.

[c] Adjusted for age, race, education, income drinking, BMI, and smoking in 30 days.

[d] *$P < 0.05$; *** $P < 0.001$

Table 4. Effects of tobacco exposure on serum folate composition.

Models	Parameters	Smoking in 30 Days		Smoking in 5 days		Passive smoking	
		F values	*P* values[a]	F values	*P* values[a]	F values	*P* values[a]
ANOVA	Folic acid	3.97	0.108	5.81*	0.028	10.88**	0.007
	MeFox	1.19	0.321	3.29	0.081	0.11	0.739
	THF	2.84	0.161	4.63*	0.044	7.43*	0.017
	5-formylTHF	0.33	0.567	0.91	0.339	0.41	0.610
	5,10-methenylTHF	1.43	0.321	6.53*	0.025	7.22*	0.017
	5-methylTHF	35.34***	< 0.001	35.02***	< 0.001	1.57	0.295
	tFOL	36.63***	< 0.001	37.47***	< 0.001	3.22	0.128
ANCOVA[b]	Folic acid	4.00	0.106	5.84*	0.028	10.90**	0.007
	MeFox	1.24	0.311	3.33	0.079	0.11	0.738
	THF	2.85	0.160	4.76*	0.041	7.60*	0.016
	5-formylTHF	0.33	0.568	0.92	0.339	0.41	0.611
	5,10-methenylTHF	1.46	0.311	6.67*	0.023	7.36*	0.016
	5-methylTHF	35.85***	< 0.001	35.46***	< 0.001	1.61	0.286
	tFOL	37.22***	< 0.001	38.00***	< 0.001	3.32	0.120

[a] Benjamini & Hochberg method was used for the adjustment of P-values; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

[b] Adjusted for covariates: (1) “Smoking in 30 days”: age, race, education, income, alcohol consumption, BMI and passive smoking; (2) “Smoking in 5 days”: age, race, education, income, alcohol consumption, BMI and passive smoking; (3) “Passive smoking”: age, race, education, income, alcohol consumption, BMI, and smoking in 30 days.