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Tobacco smoke exposure and decreased serum and red blood cell folate levels: data from the Third National Health and Nutrition Examination Survey

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The aim of this cross-sectional study was to determine the effects of smoke exposure on serum and red blood cell folate levels. Data collected as part of the Third National Health and Nutrition Examination Survey were analyzed. Serum and red blood cell folate levels were measured in active smokers and nonsmokers with high, moderate, and low exposure to environmental tobacco smoke. After adjusting for dietary intake of folate and other covariates, we found that both smokers and nonsmokers with high smoke exposure had lower red blood cell folate levels than did nonsmokers with low smoke exposure (-86 nmol/l [95% confidence interval, CI , -101 to -71 nmol/l] for smokers; -50 nmol/l [95% CI -69 to -31 nmol/l] for nonsmokers with high smoke exposure, compared with nonsmokers with low smoke exposure). Similarly, after adjustment of dietary intake of folate and other covariates, the log serum folate level also was decreased ($-0.29 \log \text{ nmol/l}$ [95% CI -0.33 to $-0.25 \log \text{ nmol/l}$] for smokers; $-0.16 \log \text{ nmol/l}$ [95% CI -0.20 to $-0.12 \log \text{ nmol/l}$] for nonsmokers with high smoke exposure, compared with nonsmokers with low smoke exposure). Tobacco smoke exposure is associated with decreased folate levels, which may be a mechanism for some of the health effects of active and passive smoking.

Introduction

Passive exposure to tobacco smoke is a risk factor for the development and exacerbation of respiratory and cardiovascular disease among children and adults (California Environmental Protection Agency, 1997). One of the proposed mechanisms for this finding is that the oxidative stress of smoke results in disturbances in the lung's antioxidant defenses (Howard, Ota, Briggs, Hampton, & Pritsos, 1998).

Folate is an essential vitamin. Folate levels have been hypothesized as important in the pathophysiology of many diseases, including neural tube defects in neonates (Butterworth & Bendich, 1996), breast cancer and colorectal cancer in adults (Kim, 1999; Langman & Boyle, 1998), and atherosclerotic disease in adults (Klor, Hauenschild, Holbach, Schnell-Kretschmer, & Stroh, 1997). Active cigarette smokers have lower folate levels in their serum, red blood cells, and respiratory tract (Giles, Kittner, Croft, Anda, Casper, & Ford, 1998; Heimbürger, 1992; Piyathilake, Macaluso, Hine, Richards, & Krumdieck, 1994), and these low levels may be an important factor in the morbidity and mortality associated with tobacco smoking. The extent to which passive smoking decreases folate levels has not been examined.

The present study analyzed data from the Third National Health and Nutrition Examination Survey (NHANES III) to determine the effects of both active and passive exposure to tobacco smoke, defined using serum cotinine levels, on serum and red blood cell levels of folate.

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Methods

Study population

NHANES III was conducted from 1988 through 1994 by the National Center for Health Statistics (NCHS, 1994). NHANES III was approved by the NCHS Institutional Review Board. In this survey a stratified multistage clustered probability design was used to select a representative sample of the civilian, non-institutionalized U.S. population. Survey participants completed extensive questionnaires in their homes and received a comprehensive physical examination, including blood testing, at a specially equipped mobile examination center.

Subjects and demographics

For our study, we limited the analysis to adults aged 17 years and older who underwent an examination and from whom serum cotinine, serum folate, and red blood cell folate levels were obtained. In addition, we further limited the analytic data set to subjects for whom dietary and vitamin or supplement data were available.

Variable definition

The race or ethnicity of the participants was classified as non-Hispanic white, non-Hispanic black, Mexican American, or "other" and was determined by self-report on the questionnaire. Socioeconomic status (SES) was determined to be "low" if the reference adult in the family (one of the persons who owned the home or paid the rent) had a 12th-grade education or less or if the poverty index for the family (determined on the basis of family income and number of people living in the home) was <1 . We used three strata age—17 to <50 , 50 to <70 , and 70 or older—to present data in tables but used age as a continuous variable in our models. Alcohol intake was determined by summing the reported consumption of beer, wine, or liquor in the previous month and classifying subjects as having 0, 1–30, or >30 drinks in the previous month.

Cotinine levels and active and passive smoking status

Serum cotinine levels were determined using high-performance liquid chromatography atmospheric-pressure chemical ionization tandem mass spectrometry, as is described elsewhere (Pirkle, Flegal, Bernert, Brody, Etzel, & Maurer, 1996). We defined smokers as any subjects with a cotinine level >15 ng/ml. We stratified the remaining subjects into three groups (with approximately one-third of the remaining sample in each group) on the basis of cotinine levels

of 0.050 to <0.1 ng/ml (0.050 is the limit of detection; subjects with no detectable cotinine were included in this group), 0.1 to <0.4 ng/ml, and 0.4 to <15 ng/ml. In addition, subjects were asked whether they were exposed to tobacco smoke in either their home or their workplace and whether they had used cigarettes or any other nicotine-containing product in the 5 days prior to the examination.

Folate levels and mean corpuscular volume (MCV)

Serum folate levels and red blood cell folate levels were measured in NHANES III using a commercially available radioassay (Quanti-phase I and Quanti-phase II; Bio-Rad Laboratories, Hercules, CA). A detailed discussion of the methods used to measure folate concentrations in this survey has been published elsewhere (Raiten & Fisher, 1995).

Folate intake

Average daily intake of folate was estimated using data from a single 24-hr food recall, which was administered in-person to participants attending the medical examination (NCHS, 1994). These data were obtained using the Dietary Data Collection System, a microcomputer-based interview system developed by the University of Minnesota Nutrition Coordinating Center (Ford & Ballew, 1998). Subjects also were asked about their intake of vitamins and supplements in the previous 30 days. These data were then analyzed to estimate the daily intake of folate in micrograms through vitamins or supplements. Total folate intake was determined by summing the estimated dietary intake and the estimated vitamin and supplement intake.

Analysis

We calculated all estimates using the sampling weights to represent adults aged 17 years or older in the United States. For analyses, we used SAS, Version 6, and SUDAAN, Release 7.5. Serum folate levels and estimated daily folate intake using the 24-hr recall were skewed and had to be log-transformed prior to analysis. Using linear regression, we modeled factors predicting red blood cell and log serum folate levels, including cotinine levels (using three nonsmoking groups and one smoking group), age, race/ethnicity, SES, estimated daily folate intake (using the log 24-hr folate estimate), estimated folate vitamin intake (using three categories: 0, ≤ 400 , and >400 μ g daily), and alcohol intake. We evaluated each model for evidence of effect modification and confounding. Because of a relation between cotinine level and folate intake, the data were stratified by quartile of total folate intake (sum of the daily intake and vitamin intake) and

analyzed separately in each quartile. The same covariates noted above were used in the logistic regression models to predict low serum or red blood cell folate levels or a high MCV, using clinically significant levels of 340 nmol/l for red blood cell folate, 6.8 nmol/l for serum folate, and 100 fl for MCV (Snow, 1999). We used S-Plus (MathSoft Inc., Seattle, WA) to graphically display the continuous relation between covariate-adjusted red blood cell folate levels and cotinine levels in nonsmokers by using loess smoothing within a generalized additive model and determined the confidence limits of the prediction using the following formulas: If $f = Sy$, where f is the predicted effect, y is the data, and S the smoothing matrix, then $\text{cov}(f) = SS's^2$, where $s^2 = \text{var}(y)$. The square root of the diagonal elements is the pointwise standard error of the prediction. The confidence limits are plus or minus 1.96 of these standard errors. All confidence intervals (CI) reported in this paper are 95%.

Results

A total of 20,050 adults participated in the questionnaire component of NHANES III. Of these subjects, 1,888 did not participate in the examination, 1,600 did not have serum cotinine levels obtained, 819 did not have dietary data available, and 179 did not have serum or red blood cell folate levels obtained, leaving 15,564 subjects (78% of adult participants) available for analysis. The 2,598 subjects who participated in the examination but were excluded from this study

were similar to the 15,564 subjects included in the study with regard to SES, sex, smoking status, and reported smoke exposure ($p > .05$ for each) but were more likely to be age 70 years or older (12.9% vs. 10.4%, $p < .01$) or black (16.5% vs. 10.6%, $p < .01$).

The demographics of the study population are shown in Table 1. Older subjects were less likely to have high exposure to environmental tobacco smoke or to be current smokers than younger subjects ($p < .001$). Among all subjects with no reported tobacco or nicotine use in the previous 5 days, 65.7% of subjects in the high exposure to passive smoke category reported tobacco smoke exposure at home or work, whereas only 9.0% of subjects with the lowest cotinine levels reported this exposure ($p < .001$). All indicators of folate intake (geometric mean daily folate intake from 24-hr recall, geometric mean total daily folate intake, and proportion with folate-containing vitamin intake) decreased with increasing smoke exposure, and both indicators of folate status (geometric mean serum folate and mean red blood cell folate) also decreased with increasing smoke exposure ($p < .01$ for each comparison).

In the linear regression models, both active and passive smoking were associated with decreased red blood cell folate levels. The r^2 value for the adjusted model, which included the cotinine category, age, sex, race/ethnicity, SES, log of the daily estimated folate intake, vitamin usage, and alcohol intake, was 0.31 (Table 2). Similarly, both active and passive smoke exposure were associated with decreased log serum folate levels, with 0.36 as the r^2 value for the adjusted

Table 1. Covariates of age, race, sex, socioeconomic status (SES), and mean or geometric mean levels of serum folate, red blood cell (RBC) folate, daily folate intake, daily fruit and vegetable intake, total daily folate intake, and proportion with folate-containing vitamin intake

Category	Covariates and outcomes by smoke exposure category			
	Smokers (<i>n</i> = 4,508)	High exposure to passive smoke (<i>n</i> = 3,279)	Moderate exposure to passive smoke (<i>n</i> = 3,983)	Low exposure to passive smoke (<i>n</i> = 3,794)
Cotinine levels	≥ 15 ng/ml	0.4 to < 15 ng/ml	0.1 to < 0.4 ng/ml	< 0.1 ng/ml
Age (years)				
17 to < 50	73.8% (1.1)	71.9% (1.2)	64.3% (1.2)	56.9% (1.9)
50 to < 70	20.6% (1.0)	21.1% (1.0)	24.6% (1.0)	24.4% (1.3)
≥ 70	5.6% (0.4)	7.0% (0.6)	11.1% (0.7)	18.7% (1.4)
Race/ethnicity				
White	78.7% (1.3)	68.8% (1.8)	76.2% (1.6)	79.9% (1.8)
Black	12.3% (0.8)	16.1% (0.9)	9.5% (0.7)	5.1% (0.6)
Mexican American	3.3% (0.3)	5.6% (0.5)	5.7% (0.6)	6.9% (0.7)
Other	5.7% (0.8)	9.5% (1.1)	8.6% (1.2)	8.1% (1.4)
Male	56.9% (0.8)	52.1% (1.2)	45.7% (1.1)	34.7% (1.1)
Low SES	71.9% (1.6)	64.6% (2.1)	51.6% (1.9)	45.3% (2.2)
≥ 30 Drinks of alcohol per month	14.3% (0.8)	11.2% (1.0)	5.8% (0.6)	7.8% (1.3)
Geometric mean serum folate	9.8 nmol/l (0.3)	11.3 nmol/l (0.5)	13.1 nmol/l (0.4)	16.0 nmol/l (0.5)
Mean RBC folate	379 nmol/l (5.6)	415 nmol/l (7.4)	474 nmol/l (7.3)	532 nmol/l (8.6)
Geometric mean daily folate intake	204 μg (4.1)	216 μg (6.6)	227 μg (4.8)	236 μg (6.9)
Proportion with folate-containing vitamin intake	24% (1.2)	26% (1.1)	32% (1.4)	37% (1.0)
Geometric mean total daily folate intake	260 μg (5.1)	278 μg (9.1)	309 μg (5.2)	346 μg (8.2)

The data are stratified into smokers and three groups of passive smokers, on the basis of serum cotinine levels, and list the weighted proportion of each covariate or the weighted mean or geometric mean value. The standard error of each estimate is given in parentheses. From the Third National Health and Nutrition Examination Survey, 1988–1994.

Table 2. Results of linear regression models determining the effect of active and passive smoke exposure on red blood cell folate levels and log serum folate levels

Regression models predicting folate levels				
	Smokers (<i>n</i> =4,508)	High exposure to passive smoke (<i>n</i> =3,279)	Moderate exposure to passive smoke (<i>n</i> =3,983)	Low exposure to passive smoke (<i>n</i> =3,794)
Red blood cell folate mean decrease in nmol/l (95% confidence interval)				
Unadjusted	-154 (-168 to -140)	-117 (-127 to -97)	-58 (-66 to -40)	Referent
Adjusted ^a	-86 (-101 to -71)	-50 (-69 to -31)	-25 (-40 to -10)	Referent
Log serum folate mean decrease in log nmol/l (95% confidence interval)				
Unadjusted	-0.49 (-0.53 to -0.45)	-0.35 (-0.39 to -0.31)	-0.20 (-0.25 to -0.15)	Referent
Adjusted ^a	-0.29 (-0.33 to -0.25)	-0.16 (-0.20 to -0.12)	-0.10 (-0.14 to -0.06)	Referent

From the Third National Health and Nutrition Examination Survey, 1988–1994.

^aAdjusted for age, sex, race/ethnicity, socioeconomic status, log daily folate intake from 24-h recall, vitamin use, and alcohol use.

model. Figure 1 depicts the covariate-adjusted red blood cell folate levels plotted against the serum cotinine levels for a subset of 11,056 nonsmoking participants with cotinine levels <15 ng/ml.

In our adjusted linear regression models stratified by total daily folate intake quartiles, passive smoke exposure was associated with significantly decreased red blood cell folate levels in each stratum. The estimates, which compared the highest passive smoke category with the lowest category, were as follows (by increasing folate intake quartile): -42 nmol/l (*CI* -66 to -18), -31 nmol/l (*CI* -56 to -6), -37 nmol/l (*CI* -68 to -6), and -70 nmol/l (*CI* -90 to -50).

Serum folate levels <6.8 nmol/l were found in 19.3% of the study population, red blood cell folate levels <340 nmol/l were found in 38.6% of the population, and MCVs of 100 fl or greater were found in 2.4% of the population. After adjustment for

covariates, subjects with high exposure to passive smoke were more likely to have decreased red blood cell folate levels (odds ratio 1.5, *CI* 1.3–1.9) and serum folate levels (odds ratio=2.0, *CI* 1.6–2.6) but not an increased MCV (odds ratio 1.4, *CI* 0.8–2.4) (Table 3).

Discussion

We found, in a nationally representative study of the U.S. adult population, that exposure to passive smoke is associated with decreases in both serum and red blood cell folate levels. In our study, among subjects with heavy exposure to passive smoke these decrements were about 60% of those seen in active cigarette smokers. These differences were not due completely to differences in folate intake from diet or supplements.

Folate is an essential B vitamin. It is naturally found in leafy green vegetables, fruits, orange juice, whole grains, and organ meats and is important in DNA synthesis and repair. Serum levels of folate reflect recent intake, whereas red blood cell levels reflect long-term intake and tend to be more stable (Snow, 1999). Overall, we found that red blood cell folate levels in current smokers were 20% lower than those in our entire group of nonsmokers. This decrement is similar to the 18%, 16%, and 30% reported in other studies (Cafolla et al., 2000; Mansoor et al., 1997; Verhoef et al., 1997). The adjusted difference of mean red blood cell folate level of 10%, comparing subjects with high exposure to passive smoke with those with low exposure, can be compared with results of other studies. One study found that mean red blood cell folate levels were 10% lower in subjects with heart disease than in population control subjects (Verhoef et al.), and another found that folate supplementation increased mean red blood cell folate levels by 11% (Brouwer et al., 1999). In our analysis, subjects with high exposures to environmental tobacco smoke were more likely to have low levels of red blood cell and serum folate using clinical criteria, suggesting this mean difference could be clinically significant (Table 3).

Figure 1. Smoothed plot of covariate-adjusted red blood cell folate levels (y-axis) vs. serum cotinine levels in 11,056 nonsmoking U.S. adults. Covariates included in the analysis are age, sex, race/ethnicity, socioeconomic status, and the log of the daily total folate intake. The middle line represents the estimate, and the bottom and top lines represent the 2.5% and 97.5% confidence intervals around the estimate. The vertical lines represent the cutpoints of the low, moderate, and high environmental tobacco smoke categories used in this analysis. From the Third National Health and Nutrition Examination Survey, 1988–1994.

Table 3. Results of logistic regression models determining the effect of active and passive smoke exposure on decreased serum and red blood cell folate levels and increased mean corpuscular volume (MCV)

Regression models predicting outcomes				
	Smokers (<i>n</i> =4,508)	High exposure to passive smoke (<i>n</i> =3,279)	Moderate exposure to passive smoke (<i>n</i> =3,983)	Low exposure to passive smoke (<i>n</i> =3,794)
Odds ratios of red blood cell folate level <340 nmol/l (95% confidence interval)				
Unadjusted	3.4 (2.9–3.9)	2.3 (1.9–2.7)	1.5 (1.3–1.8)	Referent
Adjusted ^a	2.4 (2.0–2.8)	1.5 (1.3–1.9)	1.3 (1.1–1.5)	Referent
Odds ratios of serum folate level <6.8 nmol/l (95% confidence interval)				
Unadjusted	4.3 (3.4–5.5)	2.9 (2.3–3.6)	1.8 (1.4–2.4)	Referent
Adjusted ^a	3.2 (2.4–4.1)	2.0 (1.6–2.6)	1.5 (1.1–2.0)	Referent
Odds ratios of MCV ≥100 fL (95% confidence interval)				
Unadjusted	3.2 (2.0–5.2)	0.9 (0.6–1.6)	1.1 (0.6–2.2)	Referent
Adjusted ^a	4.3 (2.5–7.4)	1.4 (0.8–2.4)	1.5 (0.8–2.9)	Referent

From the Third National Health and Nutrition Examination Survey, 1988–1994.

^aAdjusted for age, sex, race/ethnicity, socioeconomic status, log daily folate intake from 24-hr recall, vitamin use, and alcohol use.

Decreased folate intake has been shown to be associated with an increased risk of breast cancer (Zhang et al., 1999) and colon cancer (Giovannucci et al., 1998). Evidence from intervention trials has shown that consumption of folic acid before conception and during early pregnancy will prevent many but not all neural tube defects (Berry et al., 1999; MRC Vitamin Study Research Group, 1991). Our data provide evidence that decreased folate levels related to smoke exposure may be important in studies linking active or passive smoking and diseases such as breast cancer, colon cancer, and birth defects (Chao, Thun, Jacobs, Henley, Rodriguez, & Calle, 2000; Johnson, Hu, & Mao, 2000; Lieff, Olshan, Werler, Strauss, Smith, & Mitchell, 1999).

Recent analysis of a large Canadian database has shown that exposure to tobacco smoke was associated with an increased risk of breast cancer in premenopausal women (odds ratio 2.3, *CI* 1.2 to 4.6) (Johnson et al., 2000). Although colon cancer has been associated with active smoking only recently (Chao et al., 2000), previously it was associated with occupational exposures to soot and wood smoke, which contain constituents similar to those found in tobacco smoke (Gerhardsson de Verdier, Plato, Steineck, & Peters, 1992). New evidence suggests that genetic damage is not minimized until red blood cell folate levels are above 700 nmol/l (Fenech, 2001).

Passive exposure to tobacco smoke increases the risk of ischemic heart disease by 25%–30%, similar to the effect of smoking one cigarette daily (30% increment) (Law, Morris, & Wald, 1997). The precise mechanism for this risk is unknown, but several hypotheses include increased platelet aggregation, effects on vascular endothelium, and worsened exercise tolerance (Howard & Thun, 1999). Effects of tobacco smoke on folate, which previously was linked to heart disease (Morrison, Schaubel, Desmeules, & Wigle, 1996; Voutilainen, Lakka, Porkkala-Sarataho, Rissanen, Kaplan, & Salonen, 2000), provide another mechanism linking passive tobacco smoke exposure to heart disease.

Our analysis found that folate intake was lower among people with the highest passive smoke exposure. A similar finding was reported previously using a different database (Law et al., 1997).

There may be several explanations for our findings of decreased folate levels that persist after adjusting for dietary intake among people exposed to tobacco smoke. Misclassification of dietary intake, residual confounding, or differential absorption remain possible explanations. Other studies have demonstrated that localized deficiencies of folate can occur in aerodigestive tissue and are thought to be related to elevated folate turnover in response to rapid tissue proliferation or repair, or to inactivation or alteration of its function by external agents such as tobacco (Heimbürger, 1992; Piyathilake et al., 1992). Free radicals and oxidants, which cause DNA and membrane damage, are present in higher levels in the gas-phase portion of tobacco smoke than in the particulate-phase portion and may play a role in local decreases in folate levels (Niki, Minamisawa, Oikawa, & Komuro, 1993; Pryor & Stone, 1993).

Our analysis is subject to several limitations. Because it is based on a cross-sectional survey, we cannot say with certainty that tobacco smoke exposure caused lower folate levels. Serum cotinine reflects recent tobacco smoke exposure, which may or may not represent the subjects' typical exposure. Dietary recalls may not accurately nor completely reflect one's true intake. These potential limitations are lessened because our findings in passive smokers were similar to those seen in active smokers, there was evidence of a dose response across passive smoking strata, and the findings were consistent in different strata of folate intake.

In conclusion, our analysis of a representative sample of the U.S. population suggests lower serum and red blood cell folate levels among adults with passive exposure to tobacco smoke. This finding provides biological support for recent studies linking tobacco smoke exposure to heart disease and breast cancer and provides biological plausibility to examine

the role of tobacco smoke exposure in other folate-related diseases such as neural tube defects and colon cancer.

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