Effect of Folic Acid Fortification of Foods on Folate Intake in Female Smokers With Cervical Dysplasia

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OBJECTIVE: We investigated the effect of folic acid fortification of enriched cereal grains on folate intake in women of predominantly childbearing age at high risk for cervical cancer.

METHODS: Subjects in this cross-sectional study were 77 women randomized between November 1999 and December 2000 in the Women's Intervention to Stay Healthy (WISH), a clinical trial evaluating the effect of a tobacco control intervention on the progression of cervical dysplasia. All subjects were cigarette smokers, had a previously abnormal Papanicolaou test, and were positive for high-risk human papillomavirus at entry. Dietary intake was assessed with food-frequency questionnaires completed at the baseline visit for WISH. The effect of folic acid fortification on folate intake was assessed by using pre- and postfortification folate databases to estimate folate intake.

RESULTS: Mean folate intake assessed with the postfortification database was 63% higher than intake assessed with the prefortification database: 417 versus 256 μg/d of dietary folate equivalents ($P < 0.0001$). The proportion of subjects below the estimated average requirement for folate was smaller after fortification than before fortification: 40.3% versus 75.3% ($P < 0.0001$). Several foods, including white bread, cheese dishes, spaghetti, and rice, became major sources of folate as a result of fortification.

CONCLUSIONS: Folic acid fortification resulted in an increased intake of folate in these subjects. However, even with fortification, folate intake in a large proportion of these women remained below recommended levels. These results should be considered before decisions regarding future levels of folic acid fortification are made. *Nutrition* 2004;20:409–414. ©Elsevier Inc. 2004

KEY WORDS: cervix neoplasms, diet, folic acid, folic acid deficiency, neural tube defects, nutrition policy, nutrition status, women's health

INTRODUCTION

The potential role of folate in the prevention of cervical cancer has been equivocal. Whereas some studies have demonstrated an inverse association between plasma or red cell folate and cervical cancer risk,[1,2] others have not.[3,4] Many of these studies have methodologic limitations, including a lack of information on high-risk human papillomavirus (HR-HPV) infection, a risk factor for cervical cancer.[5] Low red blood cell folate has been shown to enhance the association between HR-HPV and cervical dysplasia.[6] Folate deficiency may increase the risk of cervical cancer in individuals infected with HR-HPV through multiple pathways: by causing megaloblastic changes in the cervicovaginal epithelium[7]; by reducing immunocompetence[8]; by promoting the integration of HR-HPV into cervical cells, thereby introducing chromosomal instability in the affected cells[9]; and by causing alterations in global DNA methylation.[10] A recent study has shown that higher circulating concentrations of folate are independently associated with a significantly lower likelihood of a woman being infected with HR-HPV, a lower likelihood of having a persistent HR-HPV infection, a greater likelihood of clearing HR-HPV, and a lower likelihood of developing high-grade cervical dysplasia.[11] Smoking has been identified as a risk factor for cervical cancer and may act in part through its deleterious effects on folate status.[12,13]

The neural tube defects (NTDs) spina bifida and anencephaly also have been associated in epidemiologic studies with a low intake of folate.[14–16] Clinical trials have shown that folic acid supplementation prevents many NTDs.[17–19] To reduce their risk of giving birth to children with NTDs, in 1992 the US Public Health Service recommended women of childbearing age consume 400 μg/d of folic acid.[20] Surveys have shown that most young women are not meeting this goal. Data from national surveys showed that 85% of females 14 to 18 y of age consumed less than 400 μg/d.[21,22] In addition, a survey in 1998 found that only 29% of US women were taking a multivitamin containing folic acid on a daily basis.[23] To help women achieve the recommended intake and reduce their risk of having a pregnancy complicated by NTDs, in 1996 the US Food and Drug Administration (FDA) recommended fortifying foods with folic acid.[24] Fortification of all enriched cereal grains was authorized by the FDA in March 1996 and mandated beginning January 1, 1998.[24]

Folic acid–fortified foods include enriched bread, rolls, and buns; enriched and self-rising flour; enriched corn grits, corn meals, and farina; enriched rice; enriched macaroni products; and enriched noodle products. Cereal grain products were chosen as vehicles for folic acid fortification because these products are

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commonly consumed in significant amounts by more than 90% of women of childbearing age and have a long history of being successful vehicles for improving nutrition.[25] It was hypothesized that fortification of cereal grains would increase folate intake in young women without requiring significant changes in eating patterns.[24] After considering higher and lower levels of fortification, the FDA settled on 140 μg of folic acid per 100 g of cereal grains. This level of fortification was determined to be the maximum level that would significantly increase intake in the target population but be safe for all segments of the population by providing daily intakes that would remain within the recommended safe upper limit of 1 mg/d, thereby minimizing adverse effects such as masking of anemia in persons with vitamin B12 deficiency.[24] At this level of fortification, it was estimated that women of childbearing age would consume an additional 80 to 100 μg/d of folic acid[26] and that NTDs in the United States would be reduced by 50%.[24] In addition, the percentage of females 12 to 49 years of age with folate intakes at least 400 μg/d was projected to increase from 10% to 20%, and the contribution of cereal grains to total folate intake was projected to increase from 37% to 53%.[27]

Large surveys have shown that folic acid fortification results in improved folate status. Data from the fifth and sixth examinations of the Framingham Offspring Study, National Health and Nutrition Exercise Survey (NHANES) 1999 and NHANES III (1988 to 1994), and Kaiser Permanente's Southern California Endocrinology Laboratory have documented increases of 48% to 157% in plasma folate concentrations and 38% to 74% in red blood cell folate concentrations as a result of fortification.[28–32] Caudill et al.[33] examined folate status in healthy, non-pregnant Southern California women ages 18 to 45 years after folic acid fortification. Serum and red blood cell folate concentrations in socioeconomically advantaged and disadvantaged women greatly exceeded acceptable concentrations. These data made it clear that fortification was reaching at least some segments of lower-income, minority women of childbearing age.[34] Folic acid fortification apparently has had the intended effect of preventing NTDs. Using birth certificate data, an analysis by the National Center for Health Statistics determined that there was a 23% decline in NTDs between 1996 and 2001.[35]

Despite the apparent improvements in folate status and the reduction in the prevalence of NTDs that have occurred since the initiation of folic acid fortification, little has been reported on the specific changes in folate intake that have occurred as the result of fortification. A recent study has estimated that fortification has resulted in increases in folate intake ranging from 215 to 240 μg/d for the US population as a whole.[36] However, there are few reports in the literature using pre- and postfortification folate databases to analyze the direct effect of fortification in specific populations.

In this study, we compared folate intake by using pre- and postfortification folate databases to assess the effects of folic acid fortification in women of childbearing age at high risk for cervical cancer. We investigated the effect of fortification on mean folate intake, report on changes in the proportion of the study population meeting the recommended intake as the result of fortification, and identify the foods that have been most responsible for increased folate intake.

MATERIALS AND METHODS

Subjects

Subjects were women participating in the Women's Intervention to Stay Healthy (WISH), a 5-y randomized clinical trial evaluating the effect of a theory-based tobacco control intervention on the progression of cervical dysplasia. Subjects were recruited from a population of women at risk for cervical cancer who presented to the Colposcopy Clinic at the University of Alabama at Birmingham for diagnostic evaluation of abnormal Papanicolaou tests (atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion). All subjects were at least 16 y of age; were cigarette smokers; were positive for HR-HPV; and had no previous history of cervical or other lower genital cancer, infection with the human immunodeficiency virus, or other immunosuppressive conditions. Between November 1999 and December 2000, 97 subjects were randomly assigned to an immediate or a delayed tobacco control condition.

Dietary Assessment

At the baseline visit for WISH, dietary assessment was conducted in all subjects. Dietary intake over the previous 3-mo period was assessed with the Block 98.2 semiquantitative food-frequency questionnaire (FFQ). This FFQ was developed by Gladys Block at the National Cancer Institute and has been continually updated. The latest revision is based on dietary data from NHANES III, incorporating foods important in the US diet in the 1990s and nutrient content changes resulting from recently mandated food fortification, including folic acid fortification.[37] Previous versions of this questionnaire have been validated in multiple studies.[38–41] Completed FFQs were analyzed by Block Dietary Data Systems (Berkeley, CA, USA). To assess the effect of folic acid fortification on folate intake in these subjects, FFQs were analyzed with two different folate databases: a prefortification database that incorporated the folate content of foods before the initiation of fortification in 1998, and a postfortification database that reflected the folate content of foods after the implementation of folic acid fortification.

Data Analysis

Eight subjects who were enrolled in WISH before the initiation of dietary assessment were not included in the analyses. Twelve other subjects with reported energy intakes that were considered to be excessively high or low (>5,000 or <600 kcal/d) also were excluded, resulting in a final sample size of 77.

All data on folate intake are presented as dietary folate equivalents (DFEs) to take into account that folic acid added to foods during fortification is 85% bioavailable, whereas folate naturally occurring in food is only about 50% bioavailable.[42] Therefore, for a mixture of folic acid and food folate, DFEs are calculated as follows:

$$\mu\text{g of DFEs provided} = \mu\text{g of food folate} + (1.7 \times \mu\text{g of folic acid})^{42}$$

Mean daily dietary folate intake in addition to minimum and maximum intakes was estimated with the pre- and postfortification folate databases. The proportions of subjects with dietary folate intakes below the estimated average requirement (EAR) for folate (320 μg/d of DFEs), below the recommended dietary allowance (RDA) for folate (400 μg/d of DFEs), and above the upper limit (UL) for folate (1,000 μg/d) were determined using each database.[42] The major dietary sources of folate were identified with the pre- and postfortification databases. Results are presented as means $\pm$ standard deviations and as medians (ranges) for continuous data. Categorical variables are presented as frequencies and percentages. Rankings of major dietary sources are presented as mean daily intake and percentage of total intake. Differences between prefortification and postfortification folate intakes were compared with the *t* test for paired data. The proportions of subjects below the EAR and RDA for folate using the prefortification and postfortification databases were compared with McNemar's test for matched pairs. For analyses of the relations of age, race, education, smoking duration, cigarettes smoked per day at baseline, and age at smoking initiation with folate intakes from the pre- and postfortification databases, a linear regression was performed, with

TABLE I.

DEMOGRAPHIC CHARACTERISTICS OF SUBJECTS

Characteristic	*n* (%)
Total	77 (100)
Age (y)	
19	12 (15.6)
20–29	37 (48.0)
30–39	22 (28.6)
40–49	3 (3.9)
≥50	3 (3.9)
Race	
White	68 (88.3)
Black	9 (11.7)
Education (y)	
<12	22 (28.6)
12	37 (48.0)
>12	18 (23.4)
Smoking*	
Cigarettes/d	18.5 ± 11.9
Duration (y)	12.3 ± 8.3
Age (y) at initiation	15.6 ± 3.3

* Values are mean ± standard deviation.

folate intake as the dependent variable, as was logistic regression, with folate intake as a dichotomous variable (0 = below cutpoint of EAR or RDA and 1 = above cutpoint of EAR or RDA). For all analyses, $P < 0.05$ was considered statistically significant.

RESULTS

Subjects ranged from 16 to 54 y of age, with 92% younger than 40 y (Table I). Approximately 88% of subjects were white, and the majority had at least a high school education. Subjects smoked on average just under one pack of cigarettes per day. The effects of folic acid fortification are presented in Table II. Mean dietary folate intake using the postfortification database was 63% higher than intake using the prefortification database: 417 versus 256 μg/d of DFEs ($P < 0.0001$). Minimum and maximum dietary folate intakes also were substantially higher using the postfortification database compared with intakes using the prefortification database: 100 versus 39 μg/d of DFEs and 1243 versus 631 μg/d of DFEs, respectively. The proportion of subjects with dietary folate intakes below the EAR for folate was significantly smaller using the postfortification database compared with the proportion using the prefortification database: 40.3% versus 75.3% ($P < 0.0001$), as was the proportion of subjects with dietary folate intakes below the RDA for folate: 58.4% versus 87.0% ($P < 0.0001$). Only one subject exceeded the upper limit for folate using the postfortification database, whereas none exceeded the upper limit using the prefortification database.

TABLE II.

PARAMETERS OF FOLATE INTAKE COMPARING POST- AND PREFORTIFICATION FOLATE DATABASES

Variable	Postfortification	Prefortification	*P*
Mean intake ± SD (μg/d of DFEs)	417 ± 197	256 ± 127	<0.0001
Quartiles of intake (μg/d of DFEs)			
25%	272	168	
50% (median)	362	217	
75%	531	315	
Minimum intake (μg/d of DFEs)	100	39	
Maximum intake (μg/d of DFEs)	1243	631	
Number (%) below EAR (320 μg/d of DFEs)	31 (40.3)	58 (75.3)	<0.0001
Number (%) below RDA (400 μg/d of DFEs)	45 (58.4)	67 (87.0)	<0.0001

DFEs, dietary folate equivalents; EAR, estimated average requirement; RDA, recommended dietary allowance; SD, standard deviation.

No significant relations were found between age, race, education, cigarettes smoked per day at baseline, or smoking duration and folate intake as a continuous or dichotomous variable using the pre- or postfortification databases. Advancing age at smoking initiation was associated with a slightly greater likelihood of meeting the EAR for folate using the prefortification database (odds ratio, 1.19; 95% confidence interval, 1.01 to 1.40).

Major dietary sources of folate using the postfortification database and their estimated contribution to folate intake using the prefortification folate database are presented in Table III. Cereal (excluding fiber or fortified cereal), a food not mandated to be fortified, was the greatest single source of dietary folate using the pre- and postfortification databases. However, nine of the next 11 greatest sources of folate using the postfortification database were insignificant sources of folate using the prefortification database. For example, white bread ascended from the 13th highest source to the 2nd highest source as the result of fortification, contributing 24.9 μg/d of DFEs using the postfortification database, but only 5.6 μg/d of DFEs using the prefortification database. Likewise, cheese dishes (such as macaroni and cheese) provided only 1.4 μg/d of DFEs and were the 41st greatest source of dietary folic acid using the prefortification database, but provided 22.2 μg/d of DFEs and were the 3rd highest source of folate using the postfortification database. Other foods that were relatively insignificant sources of folate prefortification, but became important sources of folate postfortification, included spaghetti (which moved from the 20th to the 5th leading source); rice and dishes with rice (from 83rd to 6th); biscuits and muffins (from 39th to 7th); pasta salad and other pasta dishes (from 51st to 9th); pizza (from 23rd to 10th); cooked cereal and grits (from 66th to 11th); cornbread and hush puppies (from 54th to 12th); and bagels, English muffins, and buns (from 27th to 15th).

Several non-fortified foods that were important sources of folate using the postfortification database were also major sources using the prefortification database. Besides cereal (excluding fiber or fortified), these included tea or iced tea, which was the fourth leading source of folate using the postfortification database, providing 22.1 μg/d of DFEs; orange juice and grapefruit juice; french fries and other fried potatoes; liver and liverwurst; and baked beans, blackeye peas, and pintos. Although the absolute amounts of folate contributed by these foods were the same using both databases, their rankings as folate sources decreased as a result of fortification due to greater contributions to total folate intake by fortified foods.

DISCUSSION

Folic acid fortification of cereal grains resulted in higher folate intake than would have occurred in the absence of fortification in women at high risk for cervical cancer. Fortification resulted in a mean folate intake that was 63% higher than that which would have occurred without fortification. In addition, fortification resulted in increases in minimum and maximum folate intakes and

TABLE III.

EFFECT OF FORTIFICATION ON THE MAJOR DIETARY SOURCES OF FOLATE

Food	Postfortification			Prefortification		
	Mean intake (μg/d of DFEs)	% Total intake	Ranking as folate source	Mean intake (μg/d of DFEs)	% Total intake	Ranking as folate source
Cereal, excluding fiber or fortified	27.3	6.5	1	25.8	10.1	1
White bread, French, Italian, etc.	24.9	6.0	2	5.6	2.2	13
Cheese dish (e.g., macaroni and cheese)	22.2	5.3	3	1.4	0.6	41
Tea or iced tea (not herbal tea)	22.1	5.3	4	22.1	8.6	2
Spaghetti with tomato sauce + meat	16.1	3.9	5	3.8	1.5	20
Rice or dishes with rice	14.3	3.4	6	0.3	0.1	83
Biscuits, muffins	13.7	3.3	7	1.4	0.6	39
Orange juice, grapefruit juice	13.4	3.2	8	13.4	5.2	3
Pasta salad, other pasta dish	12.4	3.0	9	1.0	0.4	51
Pizza	12.2	2.9	10	3.2	1.3	23
Cooked cereal or grits	11.6	2.8	11	0.6	0.2	66
Cornbread or hush puppies	11.5	2.8	12	0.8	0.3	54
French fries, fried potatoes	10.9	2.6	13	10.9	4.2	4
Liver, liverwurst	10.8	2.6	14	10.8	4.2	5
Bagels, English muffins, buns	10.6	2.5	15	2.6	1.0	27
Baked beans, blackeye peas, pintos	10.1	2.4	16	10.1	4.0	6
Salty snacks (chips, popcorn)	8.8	2.1	17	8.8	3.4	7
Green salad	8.1	1.9	18	8.1	3.2	8
Breakfast or diet shakes, Ensure	7.9	1.9	19	4.9	1.9	15
Doughnuts, pastry	7.3	1.7	20	0.9	0.4	52
Product 19, Total, Just Right	6.8	1.6	21	7.2	2.8	10
Crackers	6.3	1.5	22	1.4	0.6	40
Raisin Bran cereal	6.2	1.5	23	7.8	3.1	9
Eggs or egg biscuits	6.0	1.4	24	6.0	2.3	11
Pancakes, waffles, Pop Tarts	5.9	1.4	25	1.2	0.5	47
Peanuts, other nuts and seeds	5.9	1.4	25	5.9	2.3	12

DFE, dietary folate equivalent.

significant reductions in the proportions of subjects with intakes below the EAR and RDA for folate. At the same time, fortification resulted in only one subject exceeding the upper limit for folate.

The subjects included in this study were, for the most part, the intended target of folic acid fortification efforts: women of childbearing age. The effectiveness of fortification efforts has been assessed through different means. Several studies have documented increases in plasma and red blood cell folate concentrations among reproductive-age women in the United States as a result of folic acid fortification.[28–32] However, although some studies have projected the effects of folic acid fortification on folate intake[26,27] or have indirectly assessed the effect of fortification on folate intake,[36] few studies have directly studied the effect of fortification on folate intake by comparing intake in the same individuals using pre- and postfortification folate databases. These studies can immediately demonstrate the impact of fortification on folate intake in specific populations, determine which fortified foods have the greatest impact in increasing folate intake, and assess whether the foods selected for fortification and the level of fortification utilized were appropriate.

The average increase in daily folate intake in this study as a result of fortification (161 μg/d of DFEs) coincides with the increase in intake that was projected when fortification was initiated: 80 to 100 μg/d of folic acid or 136 to 170 μg/d of DFEs.[26] However, the increase in folate intake in this study was somewhat less than the increase of 215 to 240 μg/d recently estimated by Quinlivan and Gregory.[36] We found that folic acid fortification results in a higher percentage of subjects meeting the RDA for folate based on dietary intake, increasing from 13.0 to 41.6%. This exceeded estimates from a previous study that projected that the percentage of females 12 to 49 y of age with folate intake greater than 400 μg/d would increase from 10% to 20% as a result of fortification.[27]

The increase in folate intake of 215 to 240 μg/d predicted by Quinlivan and Gregory[36] was more than twice the level originally predicted when fortification was initiated. The researchers noted that, because of this finding, calls for higher levels of fortification should be carefully assessed. Nevertheless, the present results suggest that there are still segments of the population, most importantly, segments of the female population of childbearing years at high risk for cervical cancer, that are not benefitting fully from the current level of fortification. In our study, dietary folate intake in 40.3% of subjects remained below the EAR for folate, and intake in 58.4% of subjects remained below the RDA for folate despite fortification.

As expected, fortified foods became much more important sources of folate compared with their prefortification contributions to folate intake. Except for three non-fortified foods—cereal (excluding fiber or fortified, which had been voluntarily fortified with lower levels of folic acid for several years before 1998), tea or iced tea, and orange and grapefruit juices—none of the top 12 dietary sources of folate using the postfortification database were among the top 12 sources using the prefortification database. Examples included white bread, whose contribution to folate intake increased 4-fold as the result of fortification; cheese dishes such as macaroni and cheese, whose contribution to folate intake increased 15-fold as the result of fortification; and rice and dishes with rice, whose contribution to folate intake increased 47-fold as the result of fortification.

Tea, a non-fortified food, was a surprisingly important source of folate in this population using pre- and postfortification databases. The concentration of folate in tea is very low: 5 μg/100 g or approximately 12 μg per 8-oz glass.[43] However, tea was consumed frequently and in large quantities by study subjects. The mean daily consumption of tea was 425 mL, and tea was consumed an average of 3.7 times per week in these subjects, the third most commonly consumed food or beverage in these women. Data from the NHANES III 1999 to 2000 data release showed that tea is the 10th leading source of folate in white adults in the United States (T. Block, Block Dietary Data Systems, personal communication).

Using pre- and postfortification folate databases to estimate folate intake from the same FFQs is not without precedent. Folate intake data from the Block 98 FFQ and pre- and postfortification databases were used to assess the effect of fortification in current, previous, and never smokers in a recent study. Significantly higher folate intakes were seen in current and previous smokers using the postfortification database.[44] In a study conducted in Australia, dietary data were collected with FFQs from 1992 to 1994, before voluntary folic acid fortification. Using a postfortification database, these dietary data were then used to estimate what folate intake would have been in 1992 to 1994 had the current (1999) level of folic acid fortification been in effect at that time.[45]

The analysis of dietary data using two nutrient databases was not without challenges. Primary among these was that nutrient databases are refined and updated over time, resulting in different nutrient values for the same intake when using a recent, updated database as opposed to an older database. This was the case for cereal. Average intake using the prefortification database was 25.8 μg/d of DFEs, whereas intake using the postfortification database was 27.3 μg/d of DFEs. This difference was due to refinement of the folate nutrient database, not to differences in actual intake. In this study, these differences were uncommon and small when present (generally less than 5%).

Potential limitations to the use of the FFQ should be noted. As with any study including self-reported dietary data, imprecision and underestimation of intakes were possible. The decision to use an FFQ rather than other dietary assessment instruments in this study was based on several factors. The FFQ is structured, facilitating administration; it is scannable, ensuring accurate data entry; and, most important, it may be more representative of usual intake than a few days of food records or a 24-h dietary recall.[46] FFQs are designed to measure average long-term diet (the goal in this study) rather than to provide a precise estimate of short-term intake.[47] The use of FFQs to provide a measure of usual dietary intake by individuals in epidemiologic studies of diet and health relations has expanded markedly over the past several years.[48] The Block questionnaire has been validated and used in a variety of populations.

We assessed not only the proportion of subjects with folate intakes below the RDA but also the proportion of those with intakes below the EAR. Although the RDA typically has been used to assess the adequacy of vitamin (including folate) intake in a group, this is probably inappropriate.[49] Because the RDA is an intake level that meets the nutrient requirement in 97% to 98% of all individuals, using the percentage of the group with intakes below the RDA to assess the prevalence of inadequacy would result in a serious overestimation of this prevalence.[50] The EAR is an intake level that meets the nutrient requirement in 50% of all individuals.[42] A more appropriate way to assess adequacy of folate intake in a group is the EAR cutpoint method.[49] Using this method, the number of individuals in the group that have usual folate intakes below the EAR is counted. This proportion is an estimate of the proportion of individuals in the group with inadequate intakes.[49] Using this method, although some individuals with usual folate intakes below the EAR of 320 μg/d of DFEs will meet their individual (lower-than-average) requirements, they will be counterbalanced by a similar number of individuals with intakes above the EAR but below their individual (higher-than-average) requirements. Thus, the proportion of individuals below the EAR reflects the proportion that does not meet their requirements.[50,51]

This study showed that folic acid fortification of the US food supply resulted in a significantly higher mean intake of folate in women of predominantly childbearing age. However, even with fortification, folate intake in a large proportion of these women remained below recommended levels. These results and those of similar studies in other populations should be considered before decisions regarding future levels of folic acid fortification are made.

SUMMARY

The effect of folic acid fortification on folate intake was assessed in 77 women of childbearing age using pre- and postfortification folate databases. Fortification resulted in an increased intake of folate. However, even with fortification, folate intake in a large proportion of these women remained below recommended levels.

REFERENCES

1. VanEenwyk J, Davis FG, Colman N. Folate, vitamin C, and cervical intraepithelial neoplasia. Cancer Epidemiol Biomarkers Prev 1992;1:119
2. Orr JW, Wilson K, Bodiford C, et al. Nutritional status of patients with untreated cervical cancer. II. Vitamin assessment. Am J Obstet Gynecol 1985;151:632
3. Alberg AJ, Selhub J, Shah KV. The risk of cervical cancer in relation to serum concentrations of folate, vitamin B_{12}, and homocysteine. Cancer Epidemiol Biomarkers Prev 2000;9:761
4. Potischman N, Brinton LA, Laiming VA. A case-control study of folate levels and invasive cervical cancer. Cancer Res 1991;51:4785
5. Bosch FX, Manos MM, Munoz N, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International Biological Study on Cervical Cancer (IBSCC) Study Group. J Natl Cancer Inst 1995;87:796
6. Butterworth CE, Hatch KD, Macaluso M, et al. Folate deficiency and cervical dysplasia. JAMA 1992;267:528
7. Whitehead N, Reyner F, Lindenbaum J. Megaloblastic changes in the cervical epithelium: association with oral contraceptive therapy and reversal with folic acid. JAMA 1973;226:1421
8. Dhur A, Galan P, Hercberg S. Folate status and the immune system. Prog Food Nutr Sci 1991;15:43
9. Butterworth CE. Effect of folate on cervical cancer. Synergism among risk factors. Ann NY Acad Sci 1992;668:293
10. Kim YI, Giuliano A, Hatch KD, et al. Global DNA hypomethylation increases progressively in cervical dysplasia and carcinoma. Cancer 1994;74:893
11. Piyathilake CJ, Henao OL, Meleth S, Partridge E, Heimburger D. Folate is associated with the acquisition, persistence and clearance of high-risk (HR) human papillomavirus (HPV). FASEB J 2003;17:A373
12. Dastur DK, Quadros EV, Wadia NH, Desai MM, Bharucha EP. Effect of vegetarianism and smoking on vitamin B12, thiocyanate, and folate levels in the blood of normal subjects. Br Med J 1972;3:260
13. Piyathilake CJ, Macaluso M, Hine RJ, Richards EW, Krumdieck CL. Local and systemic effects of cigarette smoking on folate and vitamin B-12. Am J Clin Nutr 1994;60:559
14. Werler MM, Shapiro S, Mitchell AA. Periconceptional folic acid exposure and risk of occurrent neural tube defects. JAMA 1993;269:1257
15. Mulinare J, Cordero JF, Erickson JD, Berry RJ. Periconceptional use of multivitamins and the occurrence of neural tube defects. JAMA 1988;260:3141
16. Milunsky A, Jick H, Jick SS, et al. Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. JAMA 1989;262:2847
17. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. Lancet 1991;338:131
18. Vergel RG, Sanchez LR, Heredero BL, Rodriguez PL, Martinez AJ. Primary prevention of neural tube defects with folic acid supplementation: Cuban experience. Prenat Diagn 1990;10:149
19. Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med 1992;327:1832
20. Centers for Disease Control. Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. MMWR 1992;41(RR-14), 601.

21. National Center for Health Statistics. *Plan and operation of the Third National Health and Nutrition Examination Survey, 1988–94. Vital and health statistics: series I: programs and collection procedures, no. 32.* DHHS Publication PHS 94-1308; GPO no. 017-022-01260-0. Hyattsville, MD: National Center for Health Statistics, 1994
22. US Department of Agriculture. *Continuing Survey of Food Intakes by Individuals (CSFII). Nationwide food consumption survey*. Washington, DC: US Department of Agriculture, Human Nutrition Information Service, 1986
23. Centers for Disease Control and Prevention. Knowledge and use of folic acid by women of childbearing age—United States, 1995 and 1998. MMWR 1999;48:325
24. Department of Health and Human Services. Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid. Fed Reg 1996;61:8781
25. US Department of Agriculture. *Nationwide food consumption survey, continuing survey of food intakes by individuals: women 19–50 years old and their children 1–5 years, 1 day, 1985.* Report no. 85-1. Hyattsville, MD: US Department of Agriculture, 1985
26. Department of Health and Human Services. Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid. Fed Reg 1993;58:53305
27. Cook A, Friday J, Ho J, Perloff B. Folate fortification and projected average folate intakes of Americans. FASEB J 1998;12:A226
28. Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. N Engl J Med 1999;340:1449
29. Choumenkovitch SF, Jacques PF, Nadeau MR, et al. Folic acid fortification increases red blood cell folate concentrations in the Framingham Study. J Nutr 2001;131:3277
30. Centers for Disease Control and Prevention. Folate status in women of childbearing age—United States, 1999. MMWR 2000;49:962
31. Lawrence JM, Petitti DB, Watkins M, Umekubo MA. Trends in serum folate after food fortification. Lancet 1999;354:915
32. Lawrence JM, Chiu V, Petitti DB. Fortification of foods with folic acid. N Engl J Med 2000;343:970
33. Caudill MA, Le T, Moonie SA, Esfahani ST, Cogger EA. Folate status in women of childbearing age residing in Southern California after folic acid fortification. J Am Coll Nutr 2001;20:129
34. Rader JI. Folic acid fortification, folate status and plasma homocysteine. J Nutr 2002;132:2466S
35. Mathews TJ, Honein MA, Erickson JD. Spina bifida and anencephaly Prevalence—United States, 1991–2002. MMWR 2002;51(RR-13):9
36. Quinlivan EP, Gregory JF. Effect of food fortification on folic acid intake in the United States. Am J Clin Nutr 2003;77:221
37. Berkeley Nutrition Services. Available at: www.nutritionquest.com
38. Block G, Woods M, Potosky A, Clifford C. Validation of a self-administered diet history questionnaire using multiple diet records. J Clin Epidemiol 1990;43:1327
39. Block G, Thompson FE, Hartman AM, Larkin FA, Guire KE. Comparison of two dietary questionnaires validated against multiple dietary records collected during a 1-year period. J Am Diet Assoc 1992;92:686
40. Mares-Perlman JA, Klein BEK, Klein R, et al. A diet history questionnaire ranks nutrient intakes in middle-aged and older men and women similarly to multiple food records. J Nutr 1993;123:489
41. Sobell J, Block G, Koslowe P, Tobin J, Andres R. Validation of a retrospective questionnaire assessing diet 10–15 years ago. Am J Epidemiol 1989;130:173
42. Institute of Medicine. *Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline*. Washington, DC: National Academy Press, 1998
43. USDA National Nutrient Database for Standard Reference. Available at: www.nal.usda.gov/fnic/cgi-bin/nut_search.pl?tea
44. Ford R, Crutchley TM, Heimburger DC, Piyathilake CJ. The effect of folic acid fortification on folate intake among smokers and non-smokers. FASEB J 2002;16:A269
45. Flood VM, Webb KL, Smith W, et al. Folate fortification: potential impact on folate intake in an older population. Eur J Clin Nutr 2001;55:793
46. Block G. Human dietary assessment: methods and issues. Prev Med 1989;18:653
47. Rimm EB, Giovannucci EL, Stampfer MJ, et al. Reproducibility and validity of an expanded self administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol 1992;135:1114
48. Willett WC. Future directions in the development of food-frequency questionnaires. Am J Clin Nutr 1994;59(suppl):171S
49. Barr SI, Murphy SP, Poos MI. Interpreting and using the Dietary Reference Intakes in dietary assessment of individuals and groups. J Am Diet Assoc 2002;102:780
50. Institute of Medicine. *Dietary reference intakes: applications in dietary assessment*. Washington, DC: National Academy Press, 2000
51. Carriquiry AL. *Assessing the prevalence of nutrient inadequacy.* Public Health Nutr 1999;2:23