Folate, Vitamin C, and Cervical Intraepithelial Neoplasia

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Abstract
A case-control study was designed to assess the relationship between cervical intraepithelial neoplasia (CIN) and folate in serum, red blood cells, and diet. The association between CIN and dietary vitamin C was also investigated. Cases were selected from women with biopsy-confirmed CIN. Controls were age-, race-, and clinic-matched women with normal cervical (Pap) smears. Study participants completed self-administered food frequency (n = 100 matched pairs) and health (n = 102 matched pairs) questionnaires. Fasting venous blood samples were collected for serum (n = 98 matched pairs) and red cell (n = 68 matched pairs) folate assays. Conditional logistic regression models were used to estimate crude odds ratios and odds ratios adjusted for smoking, income, number of sexual partners, frequency of cervical smear, use of spermicidal contraceptive agents, history of genital warts, and Quetelet index. Dietary intake variables were adjusted for total energy intake prior to logistic regression. A protective effect of red cell folate was evident with adjusted odds ratios (95% confidence intervals) of 0.1 (0.0–0.4), 0.6 (0.2–2.0), and 0.5 (0.2–1.9) for those in quartiles 4 (highest), 3, and 2 compared to quartile 1 (lowest). Supporting evidence for the protective effect of folate was provided by inverse associations between CIN and folate in both serum and diet. An inverse association was also found between CIN and dietary vitamin C with adjusted odds ratios (95% confidence intervals) of 0.2 (0.0–0.7), 0.6 (0.2–1.6), and 0.6 (0.2–1.8) for those in quartiles 4, 3, and 2, respectively, compared to quartile 1. These findings support dietary recommendations, such as those of the American Cancer Society, the National Cancer Institute, and the U.S. Dietary Guidelines, which allow for adequate intake of folate and vitamin C, both of which are found in good quantity in fruits and vegetables. Increased consumption of legumes and whole grains is also in accord with current dietary recommendations, and both of these types of foods are good sources of folates.

Introduction
Folic acid, a B-group vitamin, is necessary for normal cell replication, and cells grown in folate-deficient media manifest chromosomal abnormalities which correspond to those found in many types of tumor cells (1). In relation to cervical cancer, it has been noted that folate deficiency can lead to cervical cellular changes which resemble neoplastic change (2) and that preneoplastic cervical cellular changes among users of oral contraceptives regress with folate supplementation (3). Three case-control studies of folate consumption and cervical cancer (4–6) have found little evidence of an association between folate intake and disease.

This investigation assesses the relationship between folic acid and CIN. The premalignant condition was chosen to attenuate physiological changes which may ensue from rather than foreshadow disease. It was hypothesized that higher levels of serum and red cell folate and higher dietary intake of folate would be associated with a reduction in disease. Because folates and vitamin C are found in many of the same foods and because vitamin C protects folates from oxidative cleavage, dietary intake of vitamin C was also assessed.

Materials and Methods
Details of case and control selection, serum and dietary measurement procedures, measurement of confounding variables, and statistical analysis have been previously documented (7, 8). These topics are reviewed below.

Case and Control Selection. Participants were recruited from clinics at Cook County Hospital and University of Illinois Hospital between April 1987 and June 1989. Cases (n = 102) were selected from women aged 18 to 49 years with biopsy-confirmed CIN I, II, or III. Age- and race-matched controls were selected from women who attended the same clinics as the cases and whose Pap smears showed no abnormality of a severity greater than or equal to benign atypia. Women who had been pregnant or lactating within the past year were excluded from the study because of the potential for folate depletion under these circumstances. In this population, pregnant and postpartum women may also be at increased risk for a diagnosis of CIN, since pregnancy brings women into the clinic where cervical smears are obtained as part of prenatal care. Women with epilepsy or sickle cell anemia were also excluded, since these conditions are associated with low blood folate and with bringing women into the

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The abbreviations used are: CIN, cervical intraepithelial neoplasia; OR, crude odds ratio; ORa, adjusted odds ratios; CI, confidence interval.
medical system where they are likely to have cervical smears. Women with diabetes were excluded due to the requirement for a 10-h fast. All eligible women were requested to participate in the study. Of 166 eligible cases, 102 were enrolled, yielding a participation rate of approximately 61%. To enroll an equal number of controls, 195 eligible women were approached, giving a participation rate of 52%.

Measurement of Exposure. The food frequency portion of the Health Habits and History Questionnaire of the National Cancer Institute, Division of Cancer Prevention and Control, version 2.1, was used to assess dietary intake of folate and vitamin C (9). Participants were asked to complete this questionnaire prior to the clinic visit. The conversion of foods on the food frequency questionnaire to nutrients was accomplished via the microcomputer software version 2.2, August 1989, provided by the National Cancer Institute, Division of Cancer Prevention and Control (9). The measure of dietary vitamin C obtained from this procedure includes vitamin C from both food and vitamin supplements. There is no provision for including supplemental folates in the dietary folate measure. Adjustment for total energy intake using the regression procedure of Willett and Stampfer (10) was used to control for over- and underreporting of dietary intake. Two participants failed to adequately complete the food frequency questionnaire, resulting in 100 matched pairs available for analysis.

Fasting venous blood samples were collected for radioassay of serum and red cell folate. Red cell hemolysates were prepared on site by the method of Gutcho (11). Serum was also aliquoted on site, and all blood samples were stored at -70°C until shipment to the laboratory on dry ice every 6 to 10 weeks. Assays for serum and red cell folate were conducted by modification of the methods of Waxman et al. (12) and Longo and Herbert (13), respectively, using Becton Dickinson Simultrac kits as reagents (14). For the folate assays, the intraassay coefficient of variation was 1.4-4.6%, and the interassay coefficient of variation was 3.8-8.2% for control samples at the limits of sensitivity of the assay. Low levels were associated with the highest coefficients of variation. All laboratory personnel were unaware of case or control status of the blood samples.

Failure to withdraw blood from four women resulted in serum measures for 98 matched pairs. Inadequate on-site preparation of the red cell hemolysates resulted in unreliable data from the first 28 cases and 14 controls, leading to the exclusion of 28 matched pairs from the final red cell folate analyses. Failure to collect a lavender-top tube from one woman and missing red cell data from one woman resulted in the exclusion of an additional two pairs, leaving 68 matched observations for the red cell folate analyses.

Because more cases were enrolled at the beginning of the study and more controls enrolled toward the end of the study period, there was a disparity in the allocation of case and control blood samples to the laboratory assay groups. To enable control for confounding due to between-run variability, two quality control samples of pooled blood were included in 11 of the 16 shipments. These 11 batches accounted for 88% of the serum and 100% of red cell samples included in the logistic models. Laboratory personnel were unaware of the inclusion of these samples.

Measurement of Confounders. Participants completed a self-administered questionnaire which asked about background, health and pregnancy history, smoking, and sexual behavior. Information from this questionnaire was used to assess independent contributors to risk of CIN in this sample and to control for confounders of the disease-exposure relationship. Confounders were defined as variables which have been reported as risk factors in previous studies and variables whose inclusion led to a change of more than 20% in the adjusted odds ratio for the nutrients of interest.

Statistical Analysis. OR, and OR, and 95% CIs were estimated using the MCSTRAT program (15), which performs an iterative conditional maximum-likelihood fit of a logistic regression model. Quartiles for the hematological measures and calorie-adjusted nutrient intake were defined from the distribution of the controls. Those in the lowest quartile (quartile 1) served as the comparison group. Adjusted models included independent contributors to risk in this sample, as well as potential confounders of the disease-exposure relationship. Tests of trend were achieved by entering quartiles of a given nutrient into the logistic model as different values of a single ordinal variable. Pearson product-moment correlation coefficients for the correlation between the natural log of the hematological measures and calorie-adjusted nutrient and food intake measures were generated using SAS procedures.

Confounding due to interassay variability was assessed by including a dichotomous variable in the logistic models. This variable was created by calculating the mean value for the quality control samples and characterizing assay groups according to whether their quality control samples were above or below the mean.

Results. The distribution of cases and controls on demographic and nondietary risk factors associated with CIN have been presented (7, 8). Table 1 shows the ORs and 95% CIs for the nonnutrient variables included in the multivariable conditional logistic regression models. Increased ORs were associated with current smoking status, more than one year between cervical smears, any use of contraceptive spermicidal foams or gels, and a self-reported history of genital warts. An inverse association was observed between odds of disease and monthly income bracket in $400 increments to $2000. Quartile of Quetelet index (kg/m2) and number of sexual partners were not independent contributors to risk after adjustment for the other variables. However, these variables were retained in the final model, because they were considered to be potential confounders of the disease-exposure relationship. Use of oral contraceptives and parity have been reported to relate to both folate status and risk for CIN. However, since these factors were not independent contributors to risk in this sample and their inclusion in the logistic models did not alter the adjusted estimates, they were not included in the final models. Excessive alcohol consumption is associated with lowered blood folates. Controlling for this variable did not alter the findings and it is not included in the adjusted models.

Table 2 shows the quartiles of serum and red cell folate. The number of cases and controls in each quartile and the percentage with deficiencies are also presented.
Table 1  Adjusted odds ratios* and 95% CIs for nonnutrient variables included in all adjusted logistic regression models

<table>
<thead>
<tr>
<th>Nonnutrient variable</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current smoker (yes versus no)</td>
<td>2.6</td>
<td>1.2-5.5</td>
</tr>
<tr>
<td>Income bracket (ordinal, monthly income in $400 increments to $2000)</td>
<td>0.5</td>
<td>0.3-0.8</td>
</tr>
<tr>
<td>Frequency of cervical smear (less often than annual versus annual)</td>
<td>3.3</td>
<td>1.3-8.2</td>
</tr>
<tr>
<td>Use of contraceptive spermicidal agents (ever used versus never used)</td>
<td>2.8</td>
<td>1.3-6.3</td>
</tr>
<tr>
<td>Self-reported history of genital warts (yes versus no)</td>
<td>3.4</td>
<td>1.1-10.8</td>
</tr>
<tr>
<td>Quetelet index (kg/m²) (ordinal by quartile)</td>
<td>0.7</td>
<td>0.5-1.0</td>
</tr>
<tr>
<td>Number of sexual partners (log, transformed)</td>
<td>1.0</td>
<td>0.6-1.7</td>
</tr>
</tbody>
</table>

* Odds ratio for each variable is adjusted for all of the other variables shown.

For comparison, the percentage of women from the Second National Health and Nutrition Evaluation Survey in the deficiency ranges for folate are also given (16). The women in this sample appear to manifest poorer folate status than anticipated from the findings of the Second National Health and Nutrition Evaluation Survey.

The ORs, ORAs, and 95% CIs by quartile of serum and red cell folate are presented in Table 3. A decrease in odds of CIN is observed for those in the highest quartile of serum folate relative to those in the lowest quartile. However, while the OR is smaller than the ORA, the statistical significance of the decreased OR is attenuated in the adjusted model. The pattern of decreasing ORs with increasing quartile of serum folate is also evident in both the crude and adjusted analyses. For dietary vitamin C, a statistically significant decrease in the odds of CIN is evident in both the crude and adjusted models for those in the highest quartile of intake relative to those in the lowest quartile.

Associations among the vitamin assays and dietary measures are shown in Table 5. Among the vitamin assays, the strongest correlation is between serum and red cell folate. Intakes of dietary vitamin C and dietary folate are highly correlated with each other, and both of these measures seem to be derived primarily from the intake of total fruit and citrus fruit. In contrast, the hematological folate measures are modestly correlated with vegetable intake.

Discussion

Issues of Bias. To assess possible bias due to differential participation rates of cases and controls, information on age, ethnic origin, zip code, and type of payment for medical services was collected for all women asked to participate in the study. Response rates were lower (P < 0.05) for controls than for cases among women age 18 to 24 years and among women who paid their own medical expenses. Since cases and controls were matched on age, the effect of this discordance cannot be estimated. It is not known in what direction the deficit of control women who paid for their own medical expenses may have influenced the ORs, since an underrepresentation in that group could result in an overrepresentation in both higher (privately insured) and lower (public aid) socioeconomic groups. Adjustment for the nonnutrient factors in the logistic models should mitigate bias resulting from nonrandom distribution of cases and controls on socioeconomic factors resulting from differential response rates.

Cases and controls were comparable on the matching variables, as well as on educational level, employment status, marital status, history of hospitalizations, oral contraceptive use, and age at first intercourse. After control-

Table 2  Quartiles of serum and red cell folate

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Quartile</th>
<th>Deficiency level*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum folate level (ng/ml)</td>
<td>1-2</td>
<td>3.4</td>
</tr>
<tr>
<td>Controls</td>
<td>26</td>
<td>22</td>
</tr>
<tr>
<td>Cases*</td>
<td>36</td>
<td>23</td>
</tr>
<tr>
<td>Red cell folate level (ng/ml)</td>
<td>12-19</td>
<td>150-190</td>
</tr>
<tr>
<td>Controls</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Cases*</td>
<td>26</td>
<td>18</td>
</tr>
</tbody>
</table>

* Deficiency levels are those defined by the laboratory.

A decreased odds of disease for those in the highest quartile of red cell folate relative to those in the lowest quartile are apparent in both the crude and adjusted models. The pattern of decreasing ORs with increasing quartile of red cell folate is also evident in both models.

Table 4 presents the ORs, ORA, and 95% CIs for dietary intake of folate and vitamin C. Decreasing ORs with increasing quartile of dietary folate and vitamin C are evident in both the crude and adjusted analyses. For dietary vitamin C, a statistically significant decrease in the odds of CIN is evident in both the crude and adjusted models for those in the highest quartile of intake relative to those in the lowest quartile.

To assess possible bias due to differential participation rates of cases and controls, information on age, ethnic origin, zip code, and type of payment for medical services was collected for all women asked to participate in the study. Response rates were lower (P < 0.05) for controls than for cases among women age 18 to 24 years and among women who paid their own medical expenses. Since cases and controls were matched on age, the effect of this discordance cannot be estimated. It is not known in what direction the deficit of control women who paid for their own medical expenses may have influenced the ORs, since an underrepresentation in that group could result in an overrepresentation in both higher (privately insured) and lower (public aid) socioeconomic groups. Adjustment for the nonnutrient factors in the logistic models should mitigate bias resulting from nonrandom distribution of cases and controls on socioeconomic factors resulting from differential response rates.

Cases and controls were comparable on the matching variables, as well as on educational level, employment status, marital status, history of hospitalizations, oral contraceptive use, and age at first intercourse. After control-
ling for smoking and income, cases and controls were also comparable on number of sexual partners and parity. The similarity of the cases and controls on these factors and the selection of all participants from clinics serving primarily low-income individuals suggests that the sample was selected from a high-risk population. The ability to generalize the study findings to other populations may be limited.

With control for assay group, the statistically significant decrease in the OR, for those in the highest quartile of serum folates was attenuated. This adjustment did not substantively modify the dose gradient results or the findings for red cell folates. Because of the unequal distribution of cases and controls in the assay groups, a dichotomous variable was used to control for interassay variability. While this procedure crudely adjusts for assay group, residual confounding may be present and the possibility of bias cannot be entirely excluded.

Bias in the findings for the red cell folate may have resulted from the exclusion of the 34 pairs from the analyses. On the dietary and serum folate measures, the controls who were excluded were similar to those included. However, the distribution of excluded cases by quartile of both dietary and serum folate differed from that of cases included in the red cell folate analyses, with more than the expected number of cases in the highest quartiles and fewer than expected in the lowest quartiles. To the extent that the serum and dietary folate measures reflect red cell folate, the findings for red cell folates may be overstated.

Folate deficiency has been reported in patients with a diversity of malignancies (17). The question of whether this is a cause or a consequence of cancer remains unanswered. However, it has been suggested that because tumors exhibit rapid cell multiplication, those with cancer may be at increased risk for folate deficiency (18). Although CIN may represent an early stage of the neoplastic process, the length of time required for progression from CIN I and II to cancer (19) argues against rapid cell multiplication at this stage of the disease. Since 79% of the cases were diagnosed with CIN I or II, it seems unlikely that the elevated ORs for those in the lower quartiles of serum and red cell folate are a result of folate sequestration by the dysplastic cells.

Similarly, while anorexia is often a symptom of those with cancer, it has not been documented as a feature of CIN. It is unlikely that cases exhibited either poorer folate status or lower intake of vitamin C as the result of dietary intake depression in sick patients with poor appetites. Additionally, because participants were asked to record their average frequency of consumption over the last 5 years, the potential for reported dietary intake reflecting changes subsequent to the onset of disease seems unlikely. Nonetheless, given the case-control design of this study, the possibility that the lower levels of serum folate, red cell folate, and dietary vitamin C for the cases resulted from the disease process cannot be ruled out.

Levels of serum retinyl palmitate indicated high levels of compliance with the fasting requirement among both cases and controls. For the 98 pairs included in the serum folate assays, one control and two cases had levels of retinyl palmitate indicative of nonfasting. Therefore, bias due to differential compliance among cases and controls regarding fasting is unlikely.

**Hematological and Dietary Factors.** Red cell folate shows a strong, statistically significant inverse association with CIN. Additionally, the estimates of effect for both serum and dietary folate offer supporting evidence for the role of inadequate folate nutritional status in the development of CIN.

Some of the difference in the findings for the folate variables may be attributable to characteristics of the measurements. Greater variability is expected in the serum compared to the red cell folate measure, because serum folate indicates short-term changes in folate balance, while red cell folate reflects changes over several months (16). For folates, there is a greater potential for misclassification from dietary intake measures compared to laboratory data because of inaccuracies in reporting and converting food to nutrients, the destruction of fo-
Hematological measures have been log-transformed. Pearson product-moment correlations. Folate assays from serum and red cell folate in women with cervical dysplasia versus hospital employee controls. Due to the lack of statistically significant results possibly related to the relatively small sample size of 34 cases and 40 controls, Butterworth’s findings remain suggestive. Brock et al. noted an inverse association between folic acid and cervical carcinoma in situ on crude analysis. This protective effect disappeared in the adjusted model, which included nonnutrient risk factors, as well as dietary carotene, retinol, vitamin C, and energy. However, the validity of the simultaneous addition of dietary folate and vitamin C into a logistic regression model must be questioned, given the generally high correlation between the two measures (Table 5 and Ref. 20). Of two studies on invasive cervical cancer and folate intake, one (5) showed an inverse association between dietary folate and disease in an analysis which did not control for other risk factors, and the second (6) revealed a protective effect for folate among heavy smokers. These two studies focused on white women whose socioeconomic status and risk of disease are not described. Therefore, these samples may have differed in a significant fashion from that of the present study, which included primarily low-income, nonwhite women from a population at high risk of disease.

Two of the many mechanisms suggested for folate deficiency causing altered DNA and subsequent carcinogenesis are based on the observed misincorporation of uracil into DNA in place of thymine, the de novo synthesis of which is folate dependent. One theory suggests that this leads to methyl-poor regions in the DNA strand, which preclude the coiling necessary for the proper configuration of the DNA molecule (21). The second hypothesis proposes that efficient DNA repair mechanisms cause chromosome breakage through repeated excision-repair cycles, which aim at removing the misincorporated uracil, but which are futile in the low-thymine environment of folate deficiency (22).

That folate may play an antitumorigenic role by preventing preneoplastic epithelial cellular changes is suggested in both the Butterworth et al. study (3) and the preliminary results of a chemopreventive trial with men at high risk of lung cancer (23). The findings from the latter study indicate regression of bronchial squamous metaplasia with folic acid and vitamin B12 supplementation. Folate supplementation has also been reported to be protective against the development of colon cancer and dysplasia in patients with chronic ulcerative colitis (24).

Evidence for an inverse association between dietary vitamin C and risk of CIN is consistent with the findings from a similar investigation (25). Of an additional three studies, one found a statistically significant inverse association between dietary vitamin C and invasive cervical cancer (5); one found no association between vitamin C intake and invasive cervical cancer (6); and one was suggestive of an increase in risk of cervical carcinoma in situ with decreased consumption of vitamin C (4). The evidence of an inverse association between CIN and vitamin C is also consistent with the finding of lower levels of serum vitamin C in women with CIN than in age- and clinic-matched controls (26). This latter study, however, failed to control for several possible confounders, including smoking. The suggestions of an association, combined with the inconsistencies of previous investigations, indicate the need for further research on the relationship of vitamin C to cervical dysplasia. Vitamin C functions as an antioxidant and enhances cellular immunity, both of which may play a role in cancer prevention (27).

Due to the relatively high correlations between the folate and vitamin C measures, it is not possible to delineate the relative importance of these nutrients as possible preventive agents in the etiology of CIN. When quartiles of dietary vitamin C and red cell folate are entered simultaneously into a logistic model with the nonnutrient variables, there continues to be a statistically significant protective effect evident for those in the highest quartiles of both measures, and a dose gradient is evident. The decreases in odds of CIN for those in the highest quartiles of red cell folate and dietary vitamin C are also evident controlling for quartile of serum α-carotene, β-carotene, lycopene, and lutein.

That dietary intake of folate is highly correlated with fruits, while the serum and red cell folate measures are more strongly correlated with vegetables, may indicate an inadequacy in the dietary folate measure. Although it was not part of the study design, serum vitamin B12 assays were conducted simultaneously with the folate assays. A preliminary review of these analyses suggests that vitamin B12 may play a role in a subset of women with cervical dysplasia. However, controlling for quartile of vitamin B12 did not substantively alter the odds ratios associated with serum and red cell folate.

The findings from this study support dietary recommendations, such as those of the American Cancer Society, the National Cancer Institute, and the U.S. Dietary Guidelines, which allow for adequate intake of folate and vitamin C, both of which are found in good quantity in fruit and vegetables. Increased consumption of legumes and whole grains is also in accord with current dietary

Table 5  Correlation* among vitamin assays* and dietary intake measures†

<table>
<thead>
<tr>
<th>Folate assays</th>
<th>Dietary intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cell folate</td>
<td>Serum folate</td>
</tr>
<tr>
<td>Nutrient intake</td>
<td></td>
</tr>
<tr>
<td>Folate</td>
<td>0.27*</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.14</td>
</tr>
<tr>
<td>Food intake</td>
<td></td>
</tr>
<tr>
<td>Total fruit</td>
<td>0.00</td>
</tr>
<tr>
<td>Citrus fruit</td>
<td>−0.01</td>
</tr>
<tr>
<td>Total vegetables</td>
<td>0.21*</td>
</tr>
<tr>
<td>Vegetables excluding rice and potatoes</td>
<td>0.25*</td>
</tr>
</tbody>
</table>

* Pearson product-moment correlations.  
* Hematological measures have been log-transformed.  
† Dietary intake measures were adjusted for total energy intake using linear regression methodology (10) prior to correlation procedure.

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recommendations, and both of these types of foods are good sources of folates.

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References

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