Association between Low Dietary Folate Intake and Suboptimal Cellular DNA Repair Capacity

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Abstract

Both reduced DNA repair capacity (DRC) and folate deficiency are associated with increased cancer risk. Furthermore, folate is involved in DNA repair through de novo DNA synthesis and methylation. To determine whether low dietary folate intake is associated with low cellular DRC in humans, we assessed total dietary folate intake using a food frequency questionnaire in 559 non-Hispanic white cancer-free subjects enrolled from 1995 through 2001 as controls for ongoing molecular epidemiological studies from among enrollees in a community-based multispecialty physician practice in the Houston metropolitan area. We assessed cellular DRC using the host-cell reactivation assay that measures nucleotide-excision repair capacity in peripheral blood lymphocytes. The distribution of DRC was approximately normal in this study population. In univariate analysis, subjects in the lowest tertile of total dietary folate intake (<170 µg/1000 kcal/day) exhibited a significant reduction (−18%) in DRC compared with those in the upper tertile (>225 µg/1000 kcal/day; P < 0.001). In multivariate linear regression analysis, calorie-adjusted total folate intake remained an independent predictor of DRC (P < 0.001). Additional stratification analysis indicated that this association was more pronounced in those who did not use folate supplementation (n = 230; P < 0.001) compared with those who did (n = 329; P = 0.177). Our findings suggest that low dietary folate intake is associated with suboptimal cellular DRC. Once replicated by other investigators, this finding has public health implications by reinforcing the need for folate supplementation or dietary modification for the at-risk population.

Introduction

Numerous epidemiological studies have shown that select dietary nutrients can provide protection against cancer (1). One of the most intensively investigated dietary nutrients is folate, derived from folic acid-rich food such as vegetables, legumes, and whole grains (2). Reduced folate intake in pregnancy is associated with increased risk of neural tube defects (3) and has been implicated in malignant brain tumors in children (4). In adults, reduced folate intake is associated with increased risk of developing several cancers including breast (5) and colon cancer (6). In an accompanying article from our ongoing lung cancer study, we also found that low dietary folate intake is a significant risk factor for lung cancer in former smokers (7).

Folate participates in both DNA methylation and nucleotide biosynthesis reactions. Folate is involved in the de novo formation of methyl groups during interconversion of one carbon group in the intermediary metabolism of S-adenosylmethionine, which serves as a methyl donor in the methylation of DNA (2). Folate also plays an important role in de novo synthesis of purines and pyrimidines, which are needed during replication and repair of DNA (8). Evidence for folate deficiency-related DNA damage and repair is mostly derived from animal experiments. Folate deficiency induces DNA damage because of impaired nucleotide-excision repair in rats (9) and increases genetic damage in Chinese hamster ovary cells (10), perhaps also because of impaired DNA repair activities.

Although deficiencies in micronutrients including folate have been suggested as a major cause of DNA damage (11), there is limited information on the relationship between folate deficiency and DNA repair in humans. Normal human lymphocytes grown in low folate medium exhibit excessive DNA strand breaks and increased uracil misincorporation even at folate concentrations within the normal range observed in human plasma (12). In humans, folate deficiency causes both massive incorporation of uracil into DNA and chromosome breaks, which can be reversed by folate administration (13). DNA damage and chromosomal aberrations may be the underlying mechanism of folate deficiency-associated cancer risk in humans (11). Therefore, understanding such mechanisms and the impact of folate deficiency on DNA repair in humans has important implications in cancer prevention.

The HCR assay (14) measures the ability of the cell to remove bulky DNA damage (mainly by the nucleotide-excision repair pathway) in a bacterial drug-resistance gene, cat, that does not exist in human cells (15). This phenotypic assay has been successfully used to measure the repair of DNA damage induced by UV light and benzo(a)pyrene diol epoxide in studies...
of susceptibility to skin (16) and lung cancer (17), respectively. In this assay, unrepaired DNA damage (such as a bulky adduct on a plasmid) induced before transfection can effectively block gene expression of the plasmid in the host cells (18, 19). The CAT expression level of the reporter gene harbored in the damaged plasmids relative to that of the undamaged ones reflects the activities of repair enzymes in the host cells, referred to as DRC (14). Therefore, this assay provides a quantitative measurement of the nucleotide-excision repair (DNA repair phenotype) of host cells. Using this assay in short-term cultures of peripheral lymphocytes, we investigated the association between dietary intake of folate and cellular DRC in a cancer-free population to provide additional evidence for possible underlying molecular mechanisms of folate deficiency-related lung carcinogenesis.

Materials and Methods

Study Subjects. This study included 559 non-Hispanic white subjects with no previous history of cancer, who were recruited between July 1, 1995 and July 17, 2001 as controls for ongoing molecular epidemiological case-control studies. All of the subjects were enrollees in a community-based multispecialty physician practice in the Houston metropolitan area (20) and denominator a one-time 20-ml blood sample that was drawn into two 10-ml heparinized vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) for the DNA repair assay. Each subject was blood transfusion in the past 6 months. “Smokers” were defined and alcohol consumption. The exclusion criterion was any interviewers to collect information on demographic data, diet-related, and a structured questionnaire was administered by interviewed a community-based multispecialty physician practice in the Houston metropolitan area (20) and donates a one-time 20-ml blood sample that was drawn into two 10-ml heparinized vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) for the DNA repair assay. Each subject was scheduled for an interview after informed consent was obtained, and a structured questionnaire was administered by interviewers to collect information on demographic data, dietary information, and other risk factors such as smoking status and alcohol consumption. The exclusion criterion was any blood transfusion in the past 6 months. “Smokers” were defined as those who had smoked >100 cigarettes in their lifetimes, and they were additionally divided into “former smokers” (those who had quit smoking >1 year previously) and “current smokers” (including those who had quit smoking within the previous year). Pack-years smoked were calculated from duration and amount of smoking. BMI was defined as: weight in kg/height in m². The study protocol was approved by M. D. Anderson and Kelsey Seybold Institutional Review Boards.

The HCR Assay. Blood sample processing, preparation of plasmids with BPDE-induced adducts, transfection, and the HCR assay have been described in detail previously (17). Briefly, lymphocytes were isolated from whole peripheral blood by Ficoll gradient centrifugation, cryopreserved within 24 h by using freezing medium, and then stored in a −80°C freezer in 1.5-ml aliquots until thawed for the assays. The cryopreserved cells were thawed and cultured in T-25 flasks at 37°C in a 5% CO2 atmosphere in regular RPMI 1640 (Life Technologies, Inc., Grand Island, NY) supplemented with 20% fetal bovine serum (Life Technologies, Inc.). The assays were performed in batches of 10 samples each. The frozen cells in each vial (1.5 ml) were quickly thawed and mixed before the last trace of ice disappeared with 8.5 ml of thawing medium (50% fetal bovine serum, 40% RPMI 1640, and 10% dextrose; Sigma Chemical Co.), which ensured a cellular viability of >80% as tested by 0.4% trypan blue (Sigma Co., St. Louis, MO) exclusion test (21). After being washed with the thawing medium, the cells were incubated in RPMI 1640 supplemented with 20% fetal bovine serum and 56.25 μg/ml phytohemagglutinin (Murex Diagnostics, Norcross, GA) for stimulation at 37°C for 72 h. Only stimulated lymphocytes will uptake the plasmids (22) and exhibit active nucleotide excision repair activity that removes the BPDE-induced DNA adducts.

After harvesting, the cells from each subject were divided into four aliquots, each having ~1 × 10^6 cells, for duplicate transfections with untreated plasmids (as the baseline for comparison) and duplicate transfections with BPDE-treated plasmids. The transfections were performed by the DEAE-dextran (Pharmacia Biotech Inc., Piscataway, NJ) method (23) with ~0.25 μg of either untreated plasmids or plasmids treated with 60 μM BPDE. The CAT expression was measured at the 40th h of continued culture in the same medium after transfection. The DRC value (%) is a ratio of the level of CAT expression of the reactivated BPDE-treated plasmids relative to that of untreated plasmids (100%; Ref. 14). The assay covariables such as blastogenetic rate (percentage of lymphocytes that respond to phytohemagglutinin stimulation), the actual amount of radioactivity of 3H-labeled acetyl base that has been transferred by CAT (the baseline expression level, in cpm) from untreated plasmids, and cell storage time (months) were also recorded.

Dietary Folate Assessment. Trained interviewers collected dietary data using a modified version of the National Cancer Institute HHHQ (24). The HHHQ is a food frequency questionnaire developed for etiologic cancer research that includes a food frequency list, an open-ended food section, and other food-behavior questions pertaining to consumption of vitamin supplements, eating at restaurants, and food preparation methods, including folate-ricit fortified food. The validity and reliability of this questionnaire are well documented in the DietSys User’s Guide (25, 26). The modified questionnaire used in this study lists 135 food and beverage items, including ethnic foods commonly consumed in the Houston area. Interviewers asked study participants about their diet during the previous year and recorded the data in a noncategorical format.

DietSys (version 4.01), the nutrient analysis program designed to accompany the HHHQ (27), was used for double key entry of all of the completed questionnaires. Folate was fortified in the United States food supply as of January 1998 (4). Therefore, DietSys (27) was used for dietary analysis of questionnaires completed before January 1998 because the nutrient file for this program version contained total folate values before the United States food supply folate fortification. For respondents who completed questionnaires after January 1998, dietary analysis was conducted using the program DIETSYS + Plus dietary analysis program (Version 5.9; Block Dietary Data Systems, Berkeley CA; 1999), an updated version of DietSys that contained postfortification total folate values for food items. Postfortification values for natural folate and folic acid were obtained and added to the nutrient data file used by DIETSYS + Plus because the software did not contain separate values for naturally occurring folate and folic acid supplied by food fortification. The primary source for postfortification folate values was the SR14 (28). For multi-ingredient food items on the food frequency that were not available in SR14, appropriate recipes from the Continuing Survey of Food Intakes by Individuals, 1994–96, 1998 were used to derive natural folate, folic acid, and total folate using values obtained from SR14 for each recipe ingredient (29). As needed, recipe adjustments for moisture changes and nutrient loss because of cooking were also made. Details of procedures used to update folate values in a nutrient data file were presented previously (30). For analysis, we used total dietary folate intake that included both natural folate from food and folic acid from fortification. Folate intake
RBCs were not available for measuring the folic acid level for a few subjects of other ethnic groups. Therefore, these data were not used for additional comparisons. Homocysteine was dramatically decreased over the storage time in the stored plasma samples, but unfortunately the amount of homocysteine was adjusted by daily calorie intake and expressed as dietary intake.

Statistical Analysis. Both dietary folate intake and DRC were analyzed as continuous variables before and after natural logarithmic transformation. Student’s t tests and χ² tests were used to compare the differences between groups. Whenever the variance of the groups varied significantly, Student’s t tests with unequal variances were used for comparisons. Correlation analyses were performed on select covariates. Because the DRC had an approximately normal distribution, a generalized linear regression model was fit by using DRC as the dependent variable. For comparison, log-transformed DRC was also used as the dependent variable to fit the generalized linear regression model. All of the statistical analyses were performed with Statistical Analysis System software (Version 8.0; SAS Institute Inc., Cary, NC).

Results

In the analysis, we focused on non-Hispanic whites, because few subjects of other ethnic groups were available for meaningful ethnic-specific analysis. The characteristics of the study population are summarized in Table 1. A total of 559 subjects were included in this analysis, of whom there were 299 men (53.5%) and 260 women (46.5%) with an overall mean age of 61.2 years (SD ±53.5), ranging from 32 to 89 years. The proportions of never, former, and current smokers were 9.8%, 53.5%, and 36.7%, respectively. The inclusion of more former smokers was based on control enrollment criteria for the ongoing lung cancer case-control study (20). History of taking supplemental multivitamins containing folate was reported by 58.9% of the subjects (329 versus 230).

We attempted to measure plasma levels of homocysteine in the stored plasma samples, but unfortunately the amount of homocysteine was dramatically decreased over the storage time of the blood samples. Therefore, these data were not used for comparison with dietary intake of folate. In addition, stored RBCs were not available for measuring the folic acid level for additional comparisons.

Table 1  Characteristics of the study population between subjects with and without supplemental folate

|                | All Subjects n = 559 | Subjects with supplemental folate n = 329 | Subjects without supplemental folate n = 230 | P
|----------------|---------------------|------------------------------------------|---------------------------------------------|---
| Males (%)      | 53.5                | 50.2                                     | 58.3                                        | 0.060 |
| Age > 60 years (%) | 58.9                | 61.7                                     | 54.8                                        | 0.102 |
| Current smokers (%) | 36.7                | 35.6                                     | 38.3                                        | 0.533 |
| Former smokers (%) | 53.5                | 53.5                                     | 53.5                                        | 0.994 |
| Ever alcohol users (%) | 65.1                | 67.8                                     | 61.3                                        | 0.114 |
| Family history of lung cancer (%) | 12.3                | 10.6                                     | 14.8                                        | 0.143 |
| Mean age in years (±SD) | 61.2 ± 10.3          | 61.9 ± 10.0                              | 60.3 ± 10.6                                 | 0.062 |
| Body mass index (BMI) | 27.3 ± 4.7          | 27.1 ± 4.5                               | 27.7 ± 5.0                                  | 0.090 |
| Pack-years smoked | 42.4 ± 31.4         | 40.4 ± 29.9                              | 45.1 ± 33.3                                 | 0.080 |
| Alcohol intake (g/1000 kcal/day) | 5.3 ± 10.0          | 5.1 ± 9.6                                | 5.5 ± 10.6                                  | 0.676 |
| Natural dietary folate (µg/1000 kcal/day) | 149.4 ± 55.5      | 150.0 ± 53.3                             | 148.6 ± 58.2                                | 0.758 |
| Folate from fortified food (µg/1000 kcal/day) | 63.0 ± 43.7        | 63.7 ± 45.7                              | 62.0 ± 40.9                                 | 0.652 |
| Total dietary folate (natural + fortified) (µg/1000 kcal/day) | 212.4 ± 81.1      | 213.7 ± 79.5                             | 210.6 ± 83.6                                | 0.650 |
| Supplemental folate* (µg/1000 kcal/day) | 112.4 ± 123.6      | 191.0 ± 104.5                            | —                                            | — |
| DRC (%)         | 9.0 ± 4.1           | 8.7 ± 3.7                                | 9.4 ± 4.5                                   | 0.032 |
| Log-DRC (%)     | 2.1 ± 0.4           | 2.1 ± 0.4                                | 2.2 ± 0.4                                   | 0.043 |

* Two-sided t tests or χ² tests between subjects with and without folate supplement.

On the basis of the questionnaire used, the most frequently consumed food items that contributed to folate intake were orange juice, high fiber, highly fortified cold cereals, all types of breads, rice and rice dishes, beans, green salads, and other cold or cooked cereals (data not shown). As shown in Table 1, the mean calorie-adjusted natural dietary folate intake was 149.4 µg/1000 Kcal/day (SD ±55.5), ranging between 47 and 437 µg/1000 Kcal/day. The mean calorie-adjusted folate acid from fortified food was 63.0 µg/1000 Kcal/day (SD ±43.7), ranging between 0 and 459 µg/1000 Kcal/day. Therefore, the calorie-adjusted total dietary folate (including natural folate and folate acid from fortification) was 212.4 µg/1000 Kcal/day (SD ±81.1). The mean DRC was 9.0% (SD ±4.1), ranging between 2.6 and 38.7% (mostly within a variation of 5-fold; i.e., 3–15%), and the distribution was approximately normal (Fig. 1).

Because more than half of the subjects (58.9%) reported taking folate supplementation, we compared the differences in variables of interest by folate supplement status. As shown in...
Table 2 Differences between selected variables by tertile of total calorie-adjusted dietary folate intake in a cancer-free population (n = 559)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lower tertile (72–170 µg/1000 kcal/day)</th>
<th>Middle tertile (171–224 µg/1000 kcal/day)</th>
<th>Upper tertile (225–668 µg/1000 kcal/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Age in years</td>
<td>58.7 ± 10.3</td>
<td>62.3 ± 10.0</td>
<td>62.6 ± 10.0</td>
</tr>
<tr>
<td>BMI*</td>
<td>27.1 ± 5.2</td>
<td>27.7 ± 5.4</td>
<td>27.2 ± 4.3</td>
</tr>
<tr>
<td>Pack-years smoked</td>
<td>44.2 ± 31.7</td>
<td>38.7 ± 28.8</td>
<td>44.1 ± 33.3</td>
</tr>
<tr>
<td>Alcohol use (g/day)</td>
<td>6.5 ± 12.6</td>
<td>5.5 ± 8.7</td>
<td>3.9 ± 8.0</td>
</tr>
<tr>
<td>DRC (%)*</td>
<td>8.2 ± 3.4</td>
<td>8.7 ± 3.2</td>
<td>10.0 ± 5.1</td>
</tr>
<tr>
<td>Log DRC (%)</td>
<td>2.04 ± 0.35</td>
<td>2.10 ± 0.36</td>
<td>2.19 ± 0.45</td>
</tr>
</tbody>
</table>

* Total dietary folate intake included natural food folate and folic acid from fortified food.

** Two-sided t tests for the differences between subgroups of lower and upper tertiles of total dietary folate intake.

Table 1, compared with nonusers, folate supplement users tended to be female (P = 0.060), older (P = 0.102), and ever alcohol users (P = 0.114) but tended to consume less alcohol (P = 0.676), to have lower pack-years smoked (P = 0.080), and lower BMI (P = 0.990), and to report a lower family history of lung cancer (P = 0.143); however, none of these differences was statistically significant. There was no significant difference in natural dietary folate intake (P = 0.758), folic acid from fortified food (P = 0.652), as well as total dietary folate (P = 0.650) between folate supplement users and non-users. Overall, those who used supplemental folate had slightly lower DRC than those who did not take supplemental folate (8.7% versus 9.4%; P = 0.032), but this difference could be because more women than men used supplemental folate, and women in general had lower DRC than men (16, 17) in this study population as shown in Table 3. It appears that males who did not use supplementation may have benefited from high dietary folate intake. It is noted that the DRC values among the nonusers had a larger variation (SD = 4.5) than that of users (SD = 3.7; Table 1), suggesting that dietary folate intake is more likely to contribute to the variation in DRC among non-users of supplemental folate.

To evaluate the association between calorie-adjusted total dietary folate intake and DRC, we first examined the distribution of tertiles of calorie-adjusted total dietary folate intake by select variables, including DRC that could possibly be modulated by folate intake. As shown in Table 2, there were significant differences in the calorie-adjusted total dietary folate intake by age (P < 0.001) and alcohol use (P = 0.018) between the low and upper tertiles of folate intake, whereas no differences were observed for BMI (P = 0.776) and pack-years smoked (P = 0.982). The average DRC was 8.2% and 10.0% among subjects having the lowest and highest tertile of dietary folate intake, respectively, and the difference was statistically significant (P < 0.001), representing an 18% reduction because of low dietary folate intake. There was a dose-response relationship between decreased dietary folate intake and decreased DRC, with DRCs of 10.0%, 8.7%, and 8.2% in upper, middle, and lower tertiles of total dietary folate intake, respectively (trend test: t = 4.24; P < 0.001) in a multivariate general linear regression model with adjustment for age, sex, smoking status, pack-years, and alcohol use. The same trend was also observed for log-transformed DRC (Table 2). A reverse trend was evident for alcohol use (t = 3.07; P < 0.001; Table 2).

Because both dietary folate intake and DRC may be modified by sex, age, smoking, alcohol use, and folate supplement, we evaluated the association between dietary folate intake and DRC stratified by these covariates. As shown in Table 3, the DRC values were decreased in every subgroup with lower total dietary folate intake. Compared with the subjects in the upper tertile of dietary folate intake, those in the low tertile exhibited significantly lower DRC in all of the subgroups except for never-smokers (small sample size: n = 55; P = 0.205) and those reporting use of supplemental folate (P = 0.081). A statistically significant trend (tested in a multivariate general linear regression model) of a decrease in DRC associated with decrease of calorie-adjusted total dietary folate intake was particularly evident in nonusers of supplemental folate (trend test: t = 4.71; P < 0.001), but this trend was not significant for those who used folate supplementation (trend test: P = 0.177).

Finally, in the multivariate linear regression analysis, the continuous untransformed and log-transformed DRC values were used as the dependent variable. As shown in Table 4, two different models were fit to evaluate the association between the calorie-adjusted total dietary folate intake and DRC. When age, sex, BMI, smoking status, pack-years smoked, and alcohol use were included in the model, calorie-adjusted total dietary folate intake remained a statistically significant predictor for DRC (both untransformed and log-transformed values) in a dose-response relationship for all of the subjects (P < 0.001) and for nonfolate-supplement users (P < 0.001). However, this trend was not significant for folate supplement users (P = 0.177 for untransformed DRC and P = 0.115 for log-transformed DRC). We also performed correlation analysis and consistently found that DRC was significantly correlated with total dietary folate intake among subjects without folate supplementation (correlation coefficient r = 0.30; P < 0.001) but not among those taking folate supplementation (correlation coefficient r = 0.09; P = 0.083; data not shown).

Discussion

Although reduced DNA repair has been implicated in susceptibility to cancer (16, 17), and impaired DNA repair has been associated with folate deficiency in animals (10), no population studies have linked dietary folate deficiency with reduced DRC in humans. In this study in a cancer-free population, we observed a statistically significant association between decreased total dietary folate intake and reduced DRC after adjustment for smoking and alcohol use, as well as other relevant variables. This finding is biologically plausible and has important impli-
Defective or impaired DRC in peripheral lymphocytes has been demonstrated consistently in fibroblastoid and lymphoblastoid cell lines established from XP patients, as well as in primary lymphocytes from patients with XP and nonhematological cancers. XP is a recessive genetic disease involving defective nucleotide excision repair. Similar observations in population studies are consistent with the notion that DRC is genetically determined (16, 17, 35, 36). However, our findings in this study suggest that DRC can also be modulated epigenetically in humans. This view is supported by a series of reports on the effects of folate deficiency on lymphocytes in animals. In rats, folate-deficient lymphocytes exhibit altered nucleotide metabolism, reduced deoxynucleotide precursor pools, and increased DNA damage (37, 38). These changes result in altered DNA synthesis and delayed cell cycles (39), leading to inefficient DNA repair. A decrease in the pool of methyl donors can lead to imbalance in DNA methylation (40, 41), and insufficient purines and pyrimidines for DNA repair synthesis (42), and, thus, can increase the mutation rate (43, 44). Our findings additionally suggest that insufficient dietary folate intake may reduce DRC in humans, thereby reducing effective response to exposure to environmental carcinogens, and leading to DNA instability and subsequent carcinogenesis.

The impact of low dietary folate intake in this study population appeared to have a long-term effect. The cells were cultured in standard RPMI 1640, which contains physiological levels of folate, methionine, a precursor of S-adenosylmethionine, and nucleotide precursors such as thymidine and hypoxanthine. Therefore, under this cell culture condition, the folate-containing medium should have provided sufficient folic acid for short-term correction. It is reported that folate-induced abnormalities of chromosomes in vitro (45) and alteration of DNA damage or repair in vivo (9) can be reversed by supplementation with folate and/or deoxynucleotide pool precursors (46). Because the damage to the plasmids induced by BPDE before cell culture is not because of folate deficiency, and the plasmids are not replicating after transfection, the difference in removal of the BPDE-adducts in these cultured cells may be attributable to active repair activities in the cells but not to folate supplement in the culture medium. On the other hand, folate can be a methyl donor for DNA methylation, and thus, can alter DNA methylation levels. This view is supported by studies showing that folate deficiency can lead to increased DNA damage (47) and reduced DNA repair (48). However, our findings suggest that folate deficiency may not be the sole factor contributing to DNA damage and repair in humans, as other factors such as smoking, alcohol use, and diet may also play a role in this process.
hand, the functions of the genes coding for repair enzymes may be modulated by methylation status and the rate of DNA synthesis, which can be altered by folate availability (47). Therefore, it is likely that epigenetic changes such as methylation of the repair genes that may have been induced by the long-term low folate intake may not be easily corrected by the short-term supplement of folate in the culture medium. Nevertheless, the exact mechanisms of the effect of low dietary folate intake on DRC remains to be unraveled.

This study has inherent limitations. Dietary folate intake and folic acid from fortified food were assessed only from food frequency questionnaires, and the exact dosage of folate supplementation among the subjects could only be estimated from the generic forms of multivitamins and folate consumed as a single vitamin. The folate concentration in serum or plasma reflecting recent dietary intake or RBCs may be more reflective of tissue status (48) but was not available to validate the reported dietary folate intake. Although dietary folate assessed by a food frequency questionnaire may be imprecisely correlated with systemic measures of folate status (49), RBC folate and plasma homocysteine concentrations have been demonstrated to correlate with total dietary folate intake including supplements (50, 51). It has also been shown that high dietary folate intake, as assessed by food frequency questionnaires, correlates with high plasma folate and low homocysteine concentrations among 885 elderly subjects (52). Because of the bioavailability of dietary folate and tissue specificity, tissue concentrations may not always correlate with dietary folate intake, although the food frequency questionnaire can adequately measure dietary folate intake. However, in the context of a retrospective study, it is likely that the pattern of dietary folate intake assessed by the food frequency questionnaire may provide a better estimate of long-term dietary folate intake than a one-time measurement in RBCs.

Smokers and alcohol users were over-represented in this study population; therefore, it may not be representative of the general population. Because DRC was measured by the HCR assay from frozen cells and assayed in batches, the effects of freezing and storage of the cells on DRC may be reflected in blastogenic rate (%) after mitogen stimulation. In addition, the cell storage time may have an effect on DRC. However, the association between DRC and dietary folate intake was independent of these covariates in our analysis, consistent with our previous report that none of these variables were correlated with DRC in a case-control study (17). The observed 18% reduction of DRC in those with low dietary folate intake compared with those with high dietary folate intake may be biologically significant, because this is close to what was observed between case patients and cancer-free subjects in previous case-control studies (16, 17). Furthermore, this reduction in DRC could be underestimated because the cell culture medium contains folate that may enhance the DRC in cells from subjects with low dietary folate intake. An 18% reduction in nucleotide excision repair capacity may increase the risk of cancer in the general population (16, 17), because even a small reduction in nucleotide excision repair may lead to a crisis at the molecular level, particularly in response to exposure to environmental carcinogens (53).

In summary, our finding of an adverse effect of low dietary folate intake on DRC has important implications in cancer prevention, because reduced DRC and the resultant increase in the level of genetic damage associated with low dietary folate intake can be easily corrected. However, our results should be additionally substantiated by measuring serum or plasma levels of homocysteine or folate levels in the RBCs in future prospective studies.

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References
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