Folate and the risk of colorectal, breast and cervix cancer: the epidemiological evidence

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Abstract: It is only recently that folate deficiency has been implicated in the development of cancer. The mechanisms by which folate might protect against cancer are not clear but may relate to its role in DNA methylation and DNA synthesis. All case-control, cohort and intervention trials reported in English, French, or German, on folate intake or blood levels in relation to the risk of colorectal, breast, and cervix cancer were reviewed. Twenty case-control, and 12 nested case-control or cohort studies were identified. The epidemiological studies consistently show an inverse association between intake and/or levels of folate and the frequency of colorectal carcinomas, and less clearly of adenomas. Long-term use of supplements of folate seems to be of greater benefit than dietary intake. The effect of folate seems to be modulated by alcohol, methionine, and MTHFR polymorphisms. Results from animal studies suggest that folate supplementation might decrease or increase cancer risk depending on dosage and timing. Recent studies also suggest an inverse association between folate intake and breast cancer among women who regularly consume alcohol. Conversely, epidemiological evidence remains uncertain for the role of folate in cervical cancer prevention; the results of two intervention trials on rates of cervical intraepithelial neoplasia regression or progression were negative. An effect of folate later in carcinogenesis is not supported by the few (nested) case-control studies on invasive cervical cancer. Some of the conflicting results may be due to the fact that dietary intake or blood levels of folate do not accurately reflect folate concentrations in the cells of cancer origin. Furthermore, only a few studies have taken into account the modulating effect of alcohol, methionine, and MTHFR polymorphisms in their analyses. The observed inverse associations between folate and risk of cancer, on the other hand, may be confounded by various factors, especially by other potentially protective constituents in fruits and vegetables. Ongoing intervention studies can strengthen evidence for causality by excluding such confounding, but the optimal dose, duration, and stage of carcinogenesis and the appropriate (genetically predisposed) study group for folate chemoprevention are not yet defined.
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Summary

It is only recently that folate deficiency has been implicated in the development of cancer. The mechanisms by which folate might protect against cancer are not clear but may relate to its role in DNA methylation and DNA synthesis. All case-control, cohort and intervention trials reported in English, French, or German, on folate intake or blood levels in relation to the risk of colorectal, breast, and cervix cancer were reviewed. Twenty case-control, and 12 nested case-control or cohort studies were identified. The epidemiological studies consistently show an inverse association between intake and/or levels of folate and the frequency of colorectal carcinomas, and less clearly of adenomas. Long-term use of supplements of folate seems to be of greater benefit than dietary intake. The effect of folate seems to be modulated by alcohol, methionine, and MTHFR polymorphisms. Results from animal studies suggest that folate supplementation might decrease or increase cancer risk depending on dosage and timing. Recent studies also suggest an inverse association between folate intake and breast cancer among women who regularly consume alcohol. Conversely, epidemiological evidence remains uncertain for the role of folate in cervical cancer prevention; the results of two intervention trials on rates of cervical intraepithelial neoplasia regression or progression were negative. An effect of folate later in carcinogenesis is not supported by the few (nested) case-control studies on invasive cervical cancer. Some of the conflicting results may be due to the fact that dietary intake or blood levels of folate do not accurately reflect folate concentrations in the cells of cancer origin. Furthermore, only a few studies have taken into account the modulating effect of alcohol, methionine, and MTHFR polymorphisms in their analyses. The observed inverse associations between folate and risk of cancer, on the other hand, may be confounded by various factors, especially by other potentially protective constituents in fruits and vegetables. Ongoing intervention studies can strengthen evidence for causality by excluding such confounding, but the optimal dose, duration, and stage of carcinogenesis and the appropriate (genetically predisposed) study group for folate chemoprevention are not yet defined.

Key words: folate; cervical; colorectal; breast cancer prevention; gene-nutrition interaction; MTHFR polymorphism

Introduction

Folate deficiency has been associated with neural tube defects, cardiovascular disease, and anaemia [1, 2]. More recently, it has been hypothesized that folate may modulate cancer risk [3–7], notably risk of cervix and colorectal cancer, and less well studied breast cancer and a rapidly growing number of other cancer sites such as lung, pancreas, stomach, oesophagus, leukaemia, skin, and endometrium [8–13].

Mechanisms

Two possible mechanisms by which low folate may increase cancer risk are likely. First, in mediating the transfer of one-carbon moieties, folate is critical for the synthesis of S-adenosylmethionine (SAM), an important compound for DNA methylation. DNA methylation is an epigenetic determinant in gene expression, DNA stability, and mutagenesis. Second, folate is important for normal DNA synthesis and repair. Consequently, folate
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As well as in other studies (see below), compared more, in the case-control study by Levine et al. [27] colorectal carcinogenesis (see below) [16]. Further, hol intake, and the MTHFR 677 genotype in colorectal neoplasia. In agreement with this hypothesis, in three (nested) case-control studies [24–26] individuals with the homozygous mutant MTHFR genotype (677TT) had a 40–50% reduction in colorectal cancer risk compared with those with the heterozygous (677CT) or normal (677CC) genotype. But this reduced risk was only observed in those subjects with adequate folate status. In individuals with inadequate folate status, or with high alcohol consumption, the risk reduction conferred by the MTHFR 677TT genotype was abolished, suggesting possible gene-nutrition interactions between folate status, alcohol intake, and the MTHFR 677 genotype in colorectal carcinogenesis (see below) [16]. Furthermore, in the case-control study by Levine et al. [27] as well as in other studies (see below), compared with those with at least one wild-type allele, TT homozygotes in the lowest quartiles of RBC or plasma folate showed an approximate doubling of adenoma risk, whereas adenoma risk in TT homozygotes in the highest folate quartile was decreased by 20% (RBC folate) or 50% (plasma folate). Thus, when folate intake is adequate, those with MTHFR 677TT genotype may have a reduced risk because of adequate provision of methyl donors. This would enhance DNA synthesis affected by inhibition of the 5-methyltetrahydrofolate pathway due to diminished MTHFR enzyme activity, and result in a decreased DNA damage. However, when folate intake is low, both impaired DNA methylation and DNA synthesis/repair may become the primary mechanism of carcinogenesis in those who have the variant MTHFR genotype [28]. Accordingly, for paediatric leukaemia it is hypothesized that in individuals with the MTHFR 677TT genotype decreased risk should be more pronounced in subgroups of leukaemia characterized by chromosomal translocations [29]. Thus, polymorphism is another means to show the folate metabolism’s involvement in carcinogenesis. A second common polymorphism of MTHFR involves an A→C substitution at nucleotide 1298, which causes glu→ala substitution in the MTHFR protein [16]. According to Hanson et al. [30], this mutation results in a small decrease in MTHFR activity but no increased thermodurability and no interaction with plasma folate is observed. Further, a recent study showed normal fasting total homocysteine levels in subjects homozygous for this polymorphism [30]. In a recent study a decrease in risk of acute lymphatic leukaemia was observed in individuals with the MTHFR 677TT genotype as well as with the MTHFR 1298 CC genotype. In addition double heterozygotes (677CT/1298AC) showed a non-significant decreased risk of developing acute lymphocytic leukaemia compared with 677CC/1298AA individuals [16, 31]. Conversely, in the case-control study by Song et al. [28] the 1298CC genotype was associated with elevated risk of oesophageal squamous cell carcinoma compared with the 1298AA genotype. The same was true for the 677TT compared with the 677CC genotype. Moreover, in the study by Chen et al. [32] a newly identified polymorphism (asp919gly) of the methionine synthase gene revealed a non-significant decrease in risk for colorectal adenomas (OR 0.66; 95% CI 0.26–1.70).

Epidemiological evidence

A number of epidemiological study designs are available, and this has to be born in mind when assessing results of such studies. Of these, case-control studies are of shorter duration and less expensive to perform than cohort studies, but resulting risk estimates may be distorted by selection and recall bias. Cohort studies are less susceptible to such bias, since information is collected before a disease develops. However, both types of studies are susceptible to confounding. When the results of case-control and cohort studies are repeatedly consistent, the case for causal links is strengthened. On
the other hand, known and unknown confounding is avoided in randomised, placebo-controlled, intervention trials whereby subjects are allocated at random to either active treatment or placebo. Thus intervention trials may produce strong conclusions, but they are limited by the fact that they can often only be interpreted for a particular study population, and findings are valid only for the particular dose of a substance provided during the trial, the duration of the trial, and the combinations of agents used in a particular study [2]. The epidemiologic evidence of a relationship between folate status and cancer presented below will be limited to cancer of the cervix, colon/rectum and of the breast, the best studied cancer sites. Evidence in relation to precursor lesions, such as adenomatous polyps and cervical intraepithelial neoplasia is presented for better understanding and is not tabulated.

Methods

All case-control, cohort, and intervention trials in humans reported in English, French, or German on folate (intake or blood levels) in relation to the risk of colorectal, breast, and cervix cancer were considered. We excluded studies of other cancer sites and overall cancer. Four reviews [3, 5, 6, 33] were used as the basis of the bibliographic search. Further studies were found in the MEDLINE® database, or they were referenced in the identified studies. The process of cross-referencing was continued until no new studies were found.

Among 32 epidemiological studies, we identified 10 case-control and 8 nested case-control or cohort studies that reported on the relationship between intake or blood levels of folate and colorectal cancer, 5 case-control and 3 nested case-control or cohort studies that reported on the association with pre- and/or postmenopausal breast cancer, and 5 case-control and 1 nested case-control studies that reported on the relationship between folate and invasive cervical cancer. The methods and results of these surveys are presented in Tables 1 to 3.

Studies were summarized by type of design, year published, number of cancer cases, exposure measurement, relative risk (RR) with 95% confidence intervals or p-values, potential confounders controlled for by matching or in analyses, and population/country. The presented RR were those for the cancer rate in the highest intake or blood level of folate divided by the rate of the lowest. In case-control studies, the odds ratio was used to estimate the relative risk. Studies comparing only mean values of cases and controls but not estimating relative risks or odds ratios were excluded from the analysis. Only the newest results of a cohort or a case-control study were considered. No intervention studies with colorectal, breast or invasive cervix cancer as endpoints were found. Due to the limited number of studies available, small studies were also included in the analysis, and no attempt was made to exclude studies for the methods that were used. Due to the heterogeneity of study design, exposure measurement and analysis, no overall summary estimate of effect was attempted.

For better understanding, evidence in relation to the precursor lesions, adenomatous polyps, and cervical intraepithelial neoplasia was added, but it was not tabulated and no attempt of completeness was made. Other biomarkers of cancer risk were not considered.

Results

Folate and colorectal neoplasia

Colorectal carcinogenesis is a multistage process comprising alterations in DNA methylation, hyperproliferation, adenoma formation, and malignant transformation. Adenomatous polyps (about two-thirds of all polyps) are considered precursors of colorectal cancer. International differences, migrant data, and recent rapid changes in incidence rates in several countries indicate that colon cancer is dependent on environmental changes. Colorectal cancer is also known to occur more frequently in certain families, in patients with ulcerative colitis, or rare genetic syndromes.

It has been suggested that dietary constituents such as vegetables, red and processed meat, alcohol, etc., physical activity, aspirin, and smoking may act as risk or protective factors [34–38].

Folate and adenomatous polyps

Four of eight (nested) case-control studies [19, 32, 37–42] found a significant inverse association between dietary [19, 38, 42] or blood folate [39] and risk of adenomatous polyps. In one study a non-significant decreased risk was observed especially in women [40]. Bird et al. [39] revealed inverse associations between red blood cells (RBC), serum, and dietary folate (including supplements) among men but not among women, the former two associations being statistically significant. High dietary folate (including supplements), but not folate from foods only, was inversely associated with risk of colorectal adenoma in women (RR = 0.66; 95% CI, 0.46–0.95) of the Nurses’ Health Study, and in men (RR = 0.63; 95% CI, 0.41–0.98) of the Health Professionals Follow-up Study [19]. The relative risk of those with a high alcohol and low methionine and folate intake compared with those with low alcohol and high folate and methionine consumption was 3.17 (95% CI, 1.69–5.95) (men and women combined). The Nurses’ Health Study and the Health Professionals Follow-up Study are both large, well-designed, ongoing cohort studies started in 1976 with 121,700 US registered nurses and in 1986 with 51,529 US men working in the
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In the Nurses’ Health Study [55], women who used supplements had a reduced risk for colon cancer (multivariate RR = 0.25; 95% CI, 0.13–0.51). Dietary folate plus supplements for less than 15 years did not result in a significant risk reduction. Strengths of this study include its prospective design, repeated dietary assessments, validation of folate intake, comprehensive data on potential confounders, and high follow-up response rate. But because it was an observational rather than a randomised study, the results cannot definitely be attributed to folate.

Alcohol and methionine intake and genotype may modulate the observed association between folate and colorectal cancer. In two prospective studies, the association of a statistically significant increase in risk of colorectal cancer and lower folate intake was only observed when this was combined with higher alcohol and lower methionine/protein intake [20, 51]. In the Women’s Health Study [53] the risk of colorectal cancer was almost twice as high in subjects with serum folate below the median and total alcohol above the median compared with higher serum folate and lower alcohol consumption (OR = 1.99; 95% CI, 0.92–4.29). The non-significant results may be due to the fact that alcohol intake in this cohort was relatively low, so that it may not be the most appropriate for a study of the interaction with alcohol.

Gene-nutrition interactions: A decreased risk of colon cancer among men homozygous for the $677C\rightarrow T$ polymorphism of MTHFR was reported in the Physicians’ Health [24] and the Health Professionals Follow-up Study [26]. The former study is a prospective case-control study nested in the Physicians’ Health Study, a randomised trial of aspirin and beta-carotene among 22071 healthy US male physicians. These two studies also revealed that this inverse association was absent in individuals with a low dietary intake of folate (or a low plasma folate level), and that low methionine intake or high alcohol consumption appeared to weaken the inverse association. Similarly, in a recent case-control study by Slattery et al. [25] the lowest risk was observed in the group with the MTHFR TT genotype and high intake of folate (OR = 0.6; 95% CI, 0.26–1.70).

Folate and colorectal cancer

Ten case-control and eight nested case-control or cohort studies have been reported [20, 24–26, 42–55]. Six case-control and six prospective studies found a statistically significant inverse association between intake (dietary, supplements) or blood levels of folate and colorectal cancer at least in subgroups [20, 24, 25, 43, 44, 46, 48, 49, 51–53, 55]. The methods and the results of these studies are described in table 1. The case-control study by Levi et al. [50] was the only one showing an increased risk with high folate intake, but the results were statistically not significant (OR = 1.54; 95% CI, 0.8–3.1). Of the six studies showing no significant association, three were based on rather small numbers of cancer cases [26, 42, 54]. In the Nurses’ Health Study [55], women who used supplements for more than 15 years had a reduced risk for colon cancer (multivariate RR = 0.25; 95% CI, 0.13–0.51). Dietary folate plus supplements for less than 15 years did not result in a significant risk reduction. Strengths of this study include its prospective design, repeated dietary assessments, validation of folate intake, comprehensive data on potential confounders, and high follow-up response rate. But because it was an observational rather than a randomised study, the results cannot definitely be attributed to folate.

Gene-nutrition interactions: The effect of the $677C\rightarrow T$ polymorphism of MTHFR (see “mechanisms”) on the risk of colorectal adenomas was investigated within the Minnesota CPRU case-control study [37]. Even though overall individuals homozygous for the thermolabile mutation (TT genotype) were at decreased risk for adenoma compared with those with the wild type CC genotype (OR = 0.8; 95% CI, 0.6–1.3), individuals with the TT genotype and with folate intake in the lowest tertile were at increased risk for adenomas compared with those with the CC genotype with high intake (OR = 1.5; 95% CI, 0.6–3.5). Conversely, among individuals with a MTHFR TT genotype and high folate intake, a slightly decreased risk was observed (OR = 0.7; 95% CI, 0.3–1.3). In the already mentioned case-control study by Levine et al. [27] the odds ratio for the $677T$ genotype was slightly increased, compared with the presence of at least one wild-type allele; apart from that the results are comparable with the findings of the study by Ulrich et al. [37]. In the Nurses’ Health Study [32] a non-significant direct association between the risk of adenomas and the $677TT$ genotype (RR = 1.35; 95% CI, 0.84–2.17) was observed. Further, there was no significant interaction between this polymorphism and intake of either folate, methionine, or alcohol. In the same study [32] polymorphism (2756A→G; asp→gly) in the gene for methionine synthase (MTR) was also not significantly associated with risk of colorectal adenomas (RR = 0.66; 95% CI, 0.26–1.70).

Folate and colorectal cancer

Ten case-control and eight nested case-control or cohort studies have been reported [20, 24–26, 42–55]. Six case-control and six prospective studies found a statistically significant inverse association between intake (dietary, supplements) or blood levels of folate and colorectal cancer at least in subgroups [20, 24, 25, 43, 44, 46, 48, 49, 51–53, 55]. The methods and the results of these studies are described in table 1. The case-control study by Levi et al. [50] was the only one showing an increased risk with high folate intake, but the results were statistically not significant (OR = 1.54; 95% CI, 0.8–3.1). Of the six studies showing no significant association, three were based on rather small numbers of cancer cases [26, 42, 54]. In the Nurses’ Health Study [55], women who used supplements...
Table 1  Folate and risk of colorectal cancer.

<table>
<thead>
<tr>
<th>Study design and reference</th>
<th>folate measure</th>
<th>no. of colorectal cancer cases</th>
<th>assoc.</th>
<th>relative risk/odds ratio (95% CI)</th>
<th>adjusted/matched for</th>
<th>country/population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case-control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freudenheim et al. 1991 diet [43]</td>
<td></td>
<td>428 colon 372 rectal cancer NS NS</td>
<td>δ 1.03 (0.56–1.89) (\hat{\circ}) 0.69 (0.36–1.30) (\hat{\circ}) 0.31 (0.16–0.59) (\hat{\circ}) 0.50 (0.24–1.03)</td>
<td>A, D, L</td>
<td>Western New York</td>
<td></td>
</tr>
<tr>
<td>Benito et al. 1991 diet [44]</td>
<td></td>
<td>286 males and females (\downarrow ptrend)</td>
<td>0.61 (ptrend &lt;0.05)</td>
<td>A-D</td>
<td>Mallorca, Spain</td>
<td></td>
</tr>
<tr>
<td>Meyer et al. 1993 diet [45]</td>
<td></td>
<td>colon 424 males and females NS NS</td>
<td>δ trend OR 1.19 (0.91–1.46) (\hat{\circ}) trend OR 0.89 (0.66–1.22)</td>
<td>A, D, G, I, K</td>
<td>Western Washington State</td>
<td></td>
</tr>
<tr>
<td>Ferraroni et al. 1994 diet [46]</td>
<td></td>
<td>828 males and females (\downarrow \downarrow)</td>
<td>δ 0.63 (0.44–0.91) (\hat{\circ}) 0.37 (0.24–0.57)</td>
<td>A-E, J</td>
<td>Northern, Italy</td>
<td></td>
</tr>
<tr>
<td>Slattery et al. 1997 diet (folate, alcohol, methionine) [47]</td>
<td></td>
<td>colon 1993 males and females NS</td>
<td>0.99 (0.68–1.43) (\hat{\circ})</td>
<td>A, C, D, E, H, O-Q, Aspirin use excluded</td>
<td>Kaiser Permanent Center</td>
<td></td>
</tr>
<tr>
<td>White et al. 1997 supplements colon, 251 males and females (\downarrow ptrend)</td>
<td></td>
<td>0.59 (0.34–1.01) 0.44 (0.24–0.80)</td>
<td>A</td>
<td>Seattle area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>La Vecchia et al. 1997 diet [49]</td>
<td></td>
<td>1953 males and females (\downarrow ptrend 0.06)</td>
<td>0.83 (0.6–1.1)</td>
<td>A, B, D, E, H, I, M</td>
<td>Italy</td>
<td></td>
</tr>
<tr>
<td>Boutron et al. 1996 diet [42]</td>
<td></td>
<td>171 colorectal cancer colon NS</td>
<td>1.0 (0.5–2.0)</td>
<td>A, D</td>
<td>Burgundy, France</td>
<td></td>
</tr>
<tr>
<td>Slattery et al. 1999 diet and MTHFR genotype colon 1467 males and females (i)</td>
<td></td>
<td>0.6 (0.4–1.0)</td>
<td>A-D, F, H, I</td>
<td>Kaiser Permanent Medical Care program</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Levi 2000 [50] diet</td>
<td></td>
<td>223 males and females NS</td>
<td>1.54 (0.8–3.1)</td>
<td>A-I</td>
<td>Vaud, Switzerland</td>
<td></td>
</tr>
<tr>
<td>Nested case-control</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Chen et al. 1996 diet and MTHFR genotype 144 men</td>
<td></td>
<td>0.44 (0.13–1.55)</td>
<td>A</td>
<td>Health Professionals Follow-up Study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glynn et al. 1996 diet + supplements colon 91 rectum 33 NS NS NS col. 0.51 (0.20–1.31) col. 2.12 (0.43–10.54)</td>
<td></td>
<td>D, H, V, W</td>
<td>baseline ATBC trial heavy smokers 50–69 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ma et al. 1997 plasma and MTHFR genotype colon 202 males NS</td>
<td></td>
<td>1.78 (0.93–3.42)</td>
<td>A</td>
<td>Physicians’ Health Study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ma et al. 1999 plasma and MTHFR genotype and MTR genotype colon 356 males NS</td>
<td></td>
<td>0.29 (0.12–0.73) 0.57 (0.24–1.88)</td>
<td>A</td>
<td>US male Health Professionals plus Physicians’ Health Study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kato et al. 1999 serum total intake 105 females NS</td>
<td></td>
<td>0.52 (0.27–0.97) 0.88 (0.46–1.69)</td>
<td>A, G, H, J, R, S</td>
<td>New York Women’s Health Study</td>
<td></td>
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<tr>
<td>Cohort</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Lashner et al. 1997 supplements folate acid 1 mg or multivitamin 0.4 mg</td>
<td></td>
<td>4 males with colon cancer and ulcerative colitis NS</td>
<td>0.45 (0.05–3.80) vs. no dysplasia</td>
<td>unadjusted</td>
<td>Chicago</td>
<td></td>
</tr>
<tr>
<td>Giovannucci et al. 1995 diet (folate, alcohol, methionine) colon 205 males</td>
<td></td>
<td>3.30 (1.58–6.88)</td>
<td>A, C, D, F, H, N-Q, Aspirin use excluded</td>
<td>US male Health Professionals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Giovannucci et al. 1998 diet +&lt;15 y suppl. multivitamin suppl. colon 442 women NS</td>
<td></td>
<td>0.82 (0.56–1.20) a15 years: 0.25 (0.13–0.51)</td>
<td>A, C, F-H, J, O, T, U</td>
<td>US Nurses’ Health Study</td>
<td></td>
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</tr>
</tbody>
</table>
MTHFR polymorphisms and adequate folate intake a decreased risk of colorectal cancer has been observed. However, no inverse association was observed in those with a diet inadequate in folate, high in alcohol, or low in methionine. Finally, the corresponding findings for colorectal adenomas are conflicting. The results of a recent cross-sectional study [57] showing that in smokers, high folate status may confer increased or decreased risk for high risk adenoma, depending on the MTHFR genotype, may explain some of the inconsistent data. Animal trials have provided considerable support for the epidemiological findings [58]. Animal studies have also shown a dose-dependent protective effect of modest levels of dietary folate supplementation up to few times the dietary requirements did not convey further benefits; in fact, there was a nonsignificant trend towards increased colorectal tumorigenesis in rats fed a supraphysiological dose of folate [59, 60]. In addition, the timing of folic acid supplementation may be important. In a mouse model for colon cancer, folic acid supplementation given before microscopic neoplastic foci were established suppressed the development of intestinal adenoma, while supplementation given after the establishment of neoplastic foci appeared to have an opposite effect on ileal polyps [61, 62]. In addition, Leu et al. found that folate deficiency reduced the development of tumorigenesis right through to colorectal cancer in azoxymethane-treated rats. The authors considered it as likely that the lower tissue folate concentrations of folate deficient animals may have inhibited the promotion and/or progression of tumorigenesis [63].

Folate and breast cancer

Several risk factors for breast cancer have been established, most of which relate to reproductive events. An increase in a reproductive lifetime that includes later and fewer births results in an increase in risk, i.e. endogenous hormones, particularly oestrogens have been implicated as underlying determinants. A family history of breast cancer and inheritance of mutations in specific genes increase the risk. The same is true for ionising radiation. A number of dietary factors have been hypothesized as risk or protective factors (alcohol, body weight, vegetables, fruits, etc.) but convincing evidence is lacking for all of them [35].

The methods and the results of epidemiological studies on the relationship between folate and breast cancer are summarised in table 2. Numbers of breast cancer cases varied considerably between studies, i.e. from several hundreds [64–67, 69] to several thousands [68, 70, 71]. Of five case-control studies [64–68], all but one [66] found a significantly decreased risk of pre- and postmenopausal breast cancer with a higher intake of folate. In two of these studies, the OR were no longer significant when adjusted for vegetable intake [65, 67]. On the other hand in the study by Ronco et al. [67] the inverse association remained statistically significant when vegetable intake was adjusted for dietary folate. Furthermore, in the survey by Freudenheim et al. [65] the use of folic acid supplements was not associated with reduced risk in premenopausal women with breast cancer. Similarly, in the study by Potischman et al. [66] neither dietary folate (OR = 0.89; 95% CI, 0.7–1.2) nor folate from food plus supplements were associated with early stage breast cancer. Considering prospective studies, a nested case-control study [69] showed no association between serum folate levels and the incidence of breast cancer. Also in the very large, well-designed cohort study with 3483 breast cancer cases by Zhang et al. [70] total folate intake was not associated with overall risk of breast cancer. However, higher total folate intake or multivitamin use was associated with a lower risk of breast cancer among women of the Nurses’ Health Study who regularly consumed alcohol. These data suggest that alcohol consumption modified the association of folate intake with breast cancer risk in a similar manner to the interaction between folate and alcohol observed for colon cancer. Similarly, the results of a large case-cohort analysis by Rohan et al. [71] suggest that dietary folate consumption might be associated with reduced risk of breast cancer at relatively high levels of alcohol intake, particularly in postmenopausal women. Also the case-control study by Negri et al. [68] based on 2569 breast cancer cases confirms that high folate intake may have a favourable effect in women consuming two or more alcoholic beverages per day. Other studies of folate and breast cancer have not evaluated risk in relation to levels of alcohol [64–67]. With respect
to the $^{67}\text{C}\rightarrow\text{T}$ polymorphism of MTHFR it appears to enhance the risk of breast cancer [72].

In summary, recent studies suggest an inverse association between folate intake and breast cancer among women who regularly consume alcohol.

**Folate and cervical intraepithelial neoplasia and cervical cancer**

Invasive squamous cell carcinoma (95% of cervical cancers) arises from precursor lesions of the cervix known as cervical intraepithelial neoplasia (CIN). CIN I represents very mild dysplasia with a high rate of spontaneous regression, CIN II and III, moderate to severe dysplasia, with a considerably higher rate of progression to invasive cancer. The main causal factor for cervix cancer is thought to be sexually transmitted infectious agents, almost certainly the human papillomaviruses (HPVs). During infection HPV must be integrated into the host DNA, which occurs preferentially at fragile sites. Low tissue folate levels increase the frequency of such fragile sites on DNA. Less well established risk factors for cervix cancer are smoking and use of oral contraceptives (OC) [33–35].

**Table 2**

<table>
<thead>
<tr>
<th>Study design and reference</th>
<th>menopausal status</th>
<th>folate measure</th>
<th>no. of cases</th>
<th>association relative risk/odds ratio (95% CI)</th>
<th>adjusted/ matched for*</th>
<th>country/population</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Case-control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graham et al. 1991 [64]</td>
<td>post</td>
<td>dietary</td>
<td>419</td>
<td>↓ p trend 0.03</td>
<td>0.70 (0.48–1.02)</td>
<td>A-H Erie, Niagara Counties</td>
</tr>
<tr>
<td>Freudenheim et al. 1996 [65]</td>
<td>pre</td>
<td>dietary</td>
<td>297</td>
<td></td>
<td>0.50 (0.31–0.82)</td>
<td>A-F, F, I</td>
</tr>
<tr>
<td></td>
<td></td>
<td>intake</td>
<td></td>
<td></td>
<td>0.76 (0.43–1.37)</td>
<td>A-F, F, I, ZZ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>supplements</td>
<td></td>
<td></td>
<td>0.97 (0.67–1.42)</td>
<td>A-F, F, J</td>
</tr>
<tr>
<td>Potschman et al. 1999 [66]</td>
<td>pre</td>
<td>diet plus</td>
<td>568</td>
<td>1.11 (0.8–1.5)</td>
<td></td>
<td>B, C, K-P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>supplements</td>
<td></td>
<td></td>
<td></td>
<td>3 US centers</td>
</tr>
<tr>
<td>Ronco et al. 1999 [67]</td>
<td>pre and post</td>
<td>dietary</td>
<td>400</td>
<td>↑ p trend 0.01</td>
<td>0.70 (0.46–1.07)</td>
<td>A, D, E, G, I, Q, Uruguay R, S plus ZZ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>intake</td>
<td></td>
<td></td>
<td>0.98 (0.60–1.59)</td>
<td></td>
</tr>
<tr>
<td>Negri et al. 2000 [68]</td>
<td>pre and post</td>
<td>diet</td>
<td>2569</td>
<td>all: 0.73 (0.60–0.88)</td>
<td>0.57 (0.41–0.78)</td>
<td>A, B, G, I, L, S six Italian areas</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pre: 0.79 (0.62–0.99)</td>
<td>(0.32–0.74)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥ 25 g alcohol/d: 0.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nested case-control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wu et al. 1999 [69]</td>
<td>pre and post</td>
<td>serum</td>
<td>195</td>
<td>1974: 1.08 (0.50–2.37)</td>
<td></td>
<td>A, M, S, T Washington County</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1989: 0.79 (0.33–1.90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cohort</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zhang et al. 1999 [70]</td>
<td>pre and post</td>
<td>diet plus</td>
<td>3483</td>
<td>all: 0.93 (0.83–1.03)</td>
<td></td>
<td>A, C-II (N), S, U-Y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>suppl.</td>
<td></td>
<td>≥ 15 g alcohol: 0.56</td>
<td>(0.41–0.79)</td>
<td>Nurses’ Health Study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multi-</td>
<td></td>
<td>current vs. never +</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>vitamins</td>
<td></td>
<td>≥ 15 g alcohol: 0.74</td>
<td>(0.59–0.93)</td>
<td></td>
</tr>
<tr>
<td>Rohan et al. 2000 [71]</td>
<td>pre and post</td>
<td>diet</td>
<td>1469</td>
<td>all: 0.99 (0.79–1.25)</td>
<td>0.34 (0.18–0.61)</td>
<td>A, D, E, G, I, L (N), S, Z Canada</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt; 14 g alcohol: 0.34</td>
<td>(0.18–0.61)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>post + ≤ 14 g alcohol: 0.28 (0.14–0.55)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* high vs. low
↓ statistically significant inverse association. NS, statistically non-significant association.

As long ago as 1982 a small trial, in which 47 OC users randomly received 10 mg of either folic acid or ascorbic acid/day for 3 months, revealed that treatment with folic acid was significantly associated with CIN regression [73]. Conversely, two more recent folic acid intervention trials reported negative results. In one of these, 235 women with CIN I or CIN II lesions randomly received either 10 mg folic acid or vitamin C daily for 6 months. Although RBC folate significantly increased in the intervention group, no significant differences in rates of either CIN regression or progression were observed [74]. In the other trial [75], 331 patients with koilocytic atypia, CIN I, or CIN II received 5 mg folic acid or placebo randomly. Regression was of borderline statistical significance after 3 months, and no difference was seen between the groups after 6 months of treatment.

**Folate and invasive cervical cancer**

None of three case-control studies on diet and invasive cervical cancer showed a dose-response association with folate intake [76–78]. Similarly
Potischman et al. [80] observed no association between folate serum levels and invasive cervical cancer in the case-control study in four Latin American countries. In the small nested case-control study by Alberg et al. [79] adjusted odds ratios (OR), based on only 39 cases, were 1.0; 0.62; and 0.6 (95% CI, 0.19–1.88) for the low to high tertiles of serum folate concentrations, respectively, an inverse trend which was not statistically significant. The methods and the results of these studies are described in table 3. Recently, in a multicenter, community-based case-control study in the US [81] low serum and red cell folate were each moderately, but non-significantly, associated with increased invasive cervical cancer risk. In addition, a strong significant positive association between serum homocysteine and cervical cancer was observed, providing evidence that the moderate folate association was real [82].

In summary, the effect of folate on carcinogenesis in the cervix remains uncertain. Two trials showed no significant effect of folic acid on the rates of cervical intraepithelial neoplasia regression or progression. An inverse association between folate and later stages of carcinogenesis is not suggested by the few (nested) case-control studies on invasive cervical cancer.

### Discussion and conclusions

With the important exception of cervix cancer, only recently it has been hypothesized that folate may modulate cancer risk. In summary, epidemiological studies support an inverse association between folate status and the rate of colorectal adenomas and carcinomas. Folate taken long term as supplements seems to be of greater benefit than higher intake in food. The corresponding findings for colorectal adenomas are conflicting. Moreover, recent studies suggest an inverse association between folate intake and breast cancer among women who regularly consume larger amounts of alcohol. On the contrary, the effect of folate on carcinogenesis in the cervix remains uncertain. Two prevention trials showed no significant effect of folic acid on rates of cervical intraepithelial neoplasia regression or progression. An inverse association between folate and later stages of carcinogenesis is not suggested by the few (nested) case-control studies on invasive cervical cancer.

Some of the conflicting results may be due to the fact that folate deficiency has not been assessed accurately. The epidemiological studies have relied on either dietary folate or blood folate concentrations as estimates of folate intake and that there is substantial variation within and across methods of the analysis of serum and whole-blood folate [4]. When measurement errors are in-

### Table 3

<table>
<thead>
<tr>
<th>Study design and reference</th>
<th>folate measure</th>
<th>number of cases</th>
<th>association</th>
<th>relative risk/odds ratio (95% CI)</th>
<th>adjusted/matched for</th>
<th>country/population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case-control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verreault et al. 1989 [76]</td>
<td>diet</td>
<td>189</td>
<td>NS</td>
<td>0.8 (0.3–1.7)</td>
<td>A-G, K</td>
<td>Seattle, USA</td>
</tr>
<tr>
<td></td>
<td>supplements</td>
<td></td>
<td>NS</td>
<td>not reported</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ziegler et al. 1990 [77]</td>
<td>diet</td>
<td>218</td>
<td>NS</td>
<td>0.85 (0.3–1.5)</td>
<td>A-D, E, G, M</td>
<td>white women, USA</td>
</tr>
<tr>
<td></td>
<td>supplements</td>
<td></td>
<td>not reported</td>
<td>RR = 1.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herrero et al. 1991 [78]</td>
<td>diet</td>
<td>748</td>
<td>NS</td>
<td>0.95 (0.7–1.3)</td>
<td>A, B, E, G-I, M</td>
<td>4 Latin American countries</td>
</tr>
<tr>
<td></td>
<td>serum</td>
<td>330</td>
<td>NS</td>
<td>1.05 (0.7–1.6)</td>
<td>A, B, E, G-I</td>
<td>4 Latin American countries</td>
</tr>
<tr>
<td>Weinstein et al. 2001 [81]</td>
<td>serum</td>
<td>183</td>
<td>NS</td>
<td>1.3 (0.8–2.9)</td>
<td>A-E, G-J, M, O</td>
<td>5 US areas</td>
</tr>
<tr>
<td></td>
<td>(microbiol.)</td>
<td></td>
<td>NS</td>
<td>1.6 (0.9–2.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>radiobinding</td>
<td></td>
<td>NS</td>
<td>1.2 (0.6–2.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>red cell</td>
<td></td>
<td>NS</td>
<td>1.5 (0.8–2.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(microbiol.)</td>
<td></td>
<td>NS</td>
<td>1.5 (0.8–2.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>radiobinding</td>
<td></td>
<td>NS</td>
<td>1.5 (0.8–2.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nested case-control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alberg et al. 2000 [79]</td>
<td>serum</td>
<td>39</td>
<td>NS</td>
<td>0.60 (0.19–1.88)</td>
<td>A, C-E, I, J, L, N</td>
<td>Washington County, Maryland, USA</td>
</tr>
</tbody>
</table>

NS statistically non-significant association

a high vs. low

b low vs. high

c A, age; B, sexual activity; C, smoking; D, oral or barrier contraceptive use; E, socioeconomic status, education, income; F, history of genital infection; G, time since last Pap smear or frequency of Pap smears; H, parity; I, HPV infection (HPV-16 and/or HPV-18); J, race, ethnicity; K, total energy; L, data of blood donation; M, study center, telephone exchange; N, time since last menstrual period; O, age at first intercourse
dependent of outcome their tendency is to bias results toward the null [55].

Also, it is not clear whether such measurements accurately assess the concentration of folate in the cells of cancer origin, which is likely to be more critical [83]. Tissue-specific susceptibility to folate deficiency has for example been shown in smokers; buccal mucosal cells were low in folate when systemic folate concentrations were normal [84]. Furthermore, Meenan et al. [85] described the lack of association between erythrocyte folate levels and colonic biopsy specimens in healthy individuals, indicating the potential difficulty in predicting localized folate deficiency. In a subsequent report [86], epithelial cell folate depletion occurred in neoplastic but not in adjacent normal colonic mucosa. Conversely, in patients with polyps the folate content of colon biopsy samples was significantly correlated with serum and red cell folate concentrations [87].

Some of the negative results may also be due to the fact that the association between folate and cancer seems to be modulated by other dietary factors (e.g. alcohol, methionine), as well as genetic polymorphisms, aspects which have not been taken into account in many of the previous studies. Caucasian and Asian populations show frequency rates of MTHFR of about 12% for those who are homozygous, and up to 50% for those who are heterozygous [16]. In addition, polymorphism of a potentially wide range of other enzymes involved in folate metabolism may modulate cancer risk (see above).

Taking all the present evidence into account it remains to be established whether folate itself is directly linked to the risk of cancer of various sites. Importantly, the observed inverse associations between folate and cancer may be confounded by numerous factors, especially by other potentially protective constituents in fruit and vegetables, an important dietary source of folate. Intervention studies can exclude such confounding. For colorectal cancer at least four large, randomised, placebo-controlled chemoprevention trials are ongoing in the United States [3], but the optimal dose of folate, the duration and stage of carcinogenesis, and the appropriate (genetically predisposed) study group for folate chemoprevention are not yet defined. Results from animal trials suggest that folate supplementation might decrease or increase cancer risk depending on dosage and timing. The emerging picture is one of complex interaction of multiple nutritional and genetic factors whose fine balance can be disturbed leading to important predisposition for a range of common diseases such as neural tube defects, vascular disease and now cancer.

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Folate and the risk of colorectal, breast and cervix cancer: the epidemiological evidence


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