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Folate and the risk of colorectal, breast and cervix cancer: the epidemiological evidence[1]

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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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deficiency may lead to an imbalance in DNA precursors, misincorporation of uridylate for thymidylate in DNA synthesis, and chromosome breakage [6, 7, 14–18].

Interactions between nutrients

Epidemiological findings suggest that subjects who consume high amounts of alcohol, with a low methionine and low folate diets may be at higher risk for colon cancer than those consuming small amounts of alcohol, with a diet high in folate and methionine (see below). SAM is synthesised from methionine which as well as being present in the diet is synthesised by the transmethylation of homocysteine by methyltetrahydrofolate. Thus deficiency of folate and/or methionine can lead to a decrease of SAM and subsequently to DNA hypomethylation. Alcohol may decrease DNA methylation by interfering with folate absorption, metabolism and excretion and/or through the antagonistic action of its related metabolite acetaldehyde on methionine synthetase [19–22].

Gene-nutrition interactions

The possible involvement of a number of enzymic reactions involved in folate metabolism and folate-mediated carcinogenesis has recently been summarized by Kim [16]. Methylenetetrahydrofolate reductase (MTHFR) catalyses the biologically irreversible reduction of 5,10-methylenetetrahydrofolate (5,10-methyleneTHF) to 5 methyltetrahydrofolate (5-methylTHF). 5-methylTHF provides the methyl group for de novo methionine synthesis and indirectly for DNA methylation, whereas 5,10-methyleneTHF is required for the conversion of deoxyuridylate to thymidylate needed for DNA synthesis. A common mutation (^{677}C→T, alanine→valine) has been detected in the MTHFR gene. This mutation leads to thermolability and slightly reduced activity of MTHFR, resulting in lower levels of 5-methylTHF [23] and higher levels of 5,10-methyleneTHF for thymidilate and DNA synthesis, etc. Some evidence exists for an inverse association between MTHFR polymorphism and 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 thermolability 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

health sector, respectively. In the study by Baron et al. [41] both dietary and supplemental folate intake were not significantly associated with the risk of recurrence of large-bowel adenoma. Individuals with folate intake below the median level and alcohol intake above the median level exhibited an increased risk for adenoma compared with an intermediate group (one of the two variables at risk) (OR = 1.85; 95% CI, 1.15–2.97). However, subjects with folate intake above the median level and alcohol intake below the median level and potentially of low risk did not show lower risk compared to the subjects within the intermediate group.

Gene-nutrition interactions: The effect of the ^{677}C→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 677TT 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 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 then 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 mean 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 ^{677}C→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, 0.4–1.0). In the two large cohorts Health Professionals and Physicians' Health [51] polymorphism (2756A→G; asp→gly) in the methionine synthase gene (MTR) was associated with a statistically non-significant 50% decreased risk of colorectal cancer, but was not correlated with plasma levels of folate. Further evidence of the link between folate and gastrointestinal cancer stems from the encouraging preliminary intervention trials in humans on folate and colorectal neoplasia [5, 56].

In summary, epidemiological studies support an inverse association between folate status and the rate of colorectal adenomas and carcinomas. Long-term supplement use seems to be of greater benefit than dietary intake. The association between folate intake and colorectal neoplasia seems to be modulated by dietary factors such as alcohol, and methionine. Furthermore, in individuals with

Table 1
Folate and risk of colorectal cancer.

Study design and reference	folate measure	no. of colorectal cancer cases	assoc.	relative risk/odds ratio[a] (95% CI)	adjusted/ matched for[c]	country/ population
Case-control						
Freudenheim et al. 1991 [43]	diet	428 colon 372 rectal cancer	NS NS ↓ NS	♂ 1.03 (0.56–1.89) ♀ 0.69 (0.36–1.30) ♂ 0.31 (0.16–0.59) ♀ 0.50 (0.24–1.03)	A, D, L	Western New York
Benito et al. 1991 [44]	diet	286 males and females	↓ ptrend	0.61 ptrend <0.05	A-D	Mallorca, Spain
Meyer et al. 1993 [45]	diet	colon 424 males and females	NS NS	♂trend OR 1.19 (0.91–1.56) ♀trend OR 0.89 (0.66–1.22)	A, D, G, I, K	Western Washington State
Ferraroni et al. 1994 [46]	diet	828 males and females	↓ ↓	♂ 0.63 (0.44–0.91) ♀ 0.37 (0.24–0.57)	A-E, J	Northern Italy
Slattery et al. 1997 [47]	diet (folate, alcohol, methionine)	colon 1993 males and females	NS	0.99 (0.68–1.43)[e]	A, C, D, F, H, O-Q, Aspirin use excluded	Kaiser Permanent Center
White et al. 1997 [48]	supplements	colon, 251 males 193 females	↓ ptrend ↓	0.59 (0.34–1.01) 0.44 (0.24–0.80)	A	Seattle area
La Vecchia et al. 1997 [49]	diet	1953 males and females	↓ ptrend 0.06	0.83 (0.6–1.1)	A, B, D, E, H, I, M	Italy
Boutron et al. 1996 [42]	diet	171 colorect. cancer	NS	1.0 (0.5–2.0)	A, D	Burgundy, France
Slattery et al. 1999 [25]	diet and MTHFR genotype	colon 1467 males and females	(↓)	0.6 (0.4–1.0)[f]	A-D, F, H, I	Kaiser Permanent Medical Care program
Levi 2000 [50]	diet	223 males and females	NS	1.54 (0.8–3.1)	A-I	Vaud, Switzerland
Nested case-control						
Chen et al. 1996 [26]	diet and MTHFR genotype	144 men	NS	0.44 (0.13–1.55)[d]	A	Health Professionals Follow-up Study
Glynn et al. 1996 [51]	diet + supplements serum alcohol, protein + dietary folate serum folate	colon 91 rectum 53	NS NS NS NS ↑ NS	colon: 0.51 (0.20–1.31) rectum: 2.12 (0.43–10.54) colon: 0.96 (0.40–2.30) rectum: 2.94 (0.84–10.33) colon: 4.79 (1.36–16.93)[i] colon: 1.28 (0.34–4.88)	D, H, V, W	baseline ATBC trial heavy smokers 50–69 years
Ma et al. 1997 [24]	plasma and MTHFR genotype	202 males	NS ↓	1.78 (0.93–3.42)[b] 0.32 (0.15–0.68)[g]	A	Physicians' Health Study
Ma et al. 1999 [52]	plasma and MTHFR genotype and MTR genotype	356 males	↓ NS NS	0.29 (0.12–0.73)[g] 0.57 (0.24–1.38)[h] 0.51 (0.14–1.90)[j]	A	US male Health Professionals plus Physicians' Health Study
Kato et al. 1999 [53]	serum total intake	105 females	↓ NS	0.52 (0.27–0.97) 0.88 (0.46–1.69)	A, G, H, J, R, S	New York Women's Health Study
Cohort						
Lashner et al. 1997 [54]	supplements folic acid 1 mg or multivitamin (0.4 mg)	4 males with colon cancer and ulcerative colitis	NS	0.45 (0.05–3.80) vs. no dysplasia	unadjusted	Chicago
Giovannucci et al. 1995 [20]	diet (folate, alcohol, methionine)	colon 205 males	↑	3.30 (1.58–6.88)[e]	A, C, D, F, H, N-Q, Aspirin use excluded	US male Health Professionals
Giovannucci et al. 1998 [55]	diet + <15 y. suppl. multivitamin suppl.	colon 442 women	NS ↓	0.82 (0.56–1.20) ≥15 years: 0.25 (0.13–0.51)	A, C, F-H, J, O, T, U	US Nurses' Health Study

[a] high vs. low
↓ statistically significant inverse association. (↓), higher 95% CI = 1.↑, statistically significant direct association.
NS, statistically non-significant association
[b] low vs. high
[c] A, age; B, sex; C, weight; D, total energy intake; E, education; F, smoking; G, alcohol; H, physical activity; I, fiber; J, family history of colorectal cancer; K, interviewer; L, neighborhood; M, center; N, history of polyps; O, red meat consumption; P, multivitamin use; Q, history of endoscopy; R, menopausal status; S, prior occult blood testing; T, aspirin; U, methionine; V, vitamin A; W, starch
[d] high folate intake-MTHFR TT vs. low folate intake-MTHFR CC or CT
[e] high alcohol-low folate-low methionine vs. low alcohol-high folate-high methionine
[f] high folate intake-MTHFR TT vs. low folate intake-MTHFR CC
[g] high plasma folate-MTHFR TT vs. high plasma folate-MTHFR CC or CT
[h] low plasma folate-MTHFR TT vs. high plasma folate-MTHFR CC or CT
[i] high alcohol, low folate, low protein vs. low alcohol, high folate, high protein
[j] high plasma folate-MTR gly/gly vs. high plasma folate-MTR gly/asp or asp/asp

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 ^{677}C→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].

Folate and cervical intraepithelial neoplasia

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 koliocytic 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

Table 2
Folate and risk of breast cancer.

Study design and reference	menopausal status	folate measure	no. of cases	association	relative risk/odds ratio[a] (95% CI)	adjusted/ matched for[b]	country/ population
Case-control							
Graham et al. 1991 [64]	post	dietary intake	439	↓ ptrend 0.03	0.70 (0.48–1.02)	A-H	Erie, Niagara Counties
Freudenheim et al. 1996 [65]	pre	dietary intake supplements	297	↓ NS NS	0.50 (0.31–0.82) 0.76 (0.43–1.37) 0.97 (0.67–1.42)	A-F, H, I A-F, H, I, ZZ A-F, H, J	Erie, Niagara Counties
Potischman et al. 1999 [66]	pre	diet plus supplements	568	NS	1.11 (0.8–1.5)	B, C, K-P	3 US centers
Ronco et al. 1999 [67]	pre and post	dietary intake	400	↓ ptrend 0.01 NS	0.70 (0.46–1.07) 0.98 (0.60–1.59)	A, D,E, G-I, Q, R, S plus ZZ	Uruguay
Negri et al. 2000 [68]	pre and post	diet	2569	↓ ↓ ↓ ↓	all: 0.73 (0.60–0.88) pre: 0.57 (0.41–0.78) post: 0.79 (0.62–0.99) ≥25 g alcohol/d: 0.49 (0.32–0.74)	A, B, G, I, L, S	six Italian areas
Nested case-control							
Wu et al. 1999 [69]	pre and post	serum	195	NS NS	1974: 1.08 (0.50–2.37) 1989: 0.79 (0.33–1.90)	A, M, S, T	Washington County
Cohort							
Zhang et al. 1999 [70]	pre and post	diet plus suppl. Multi-vitamins	3483	NS ↓ ↓	all: 0.93 (0.83–1.03) ≥ 15 g alcohol: 0.56 (0.41–0.79) current vs. never + ≥ 15 g alcohol: 0.74 (0.59–0.93)	A, C-II (N), S, U-Y	Nurses' Health Study
Rohan et al. 2000 [71]	pre and post	diet	1469	NS ↓ ↓	all: 0.99 (0.79–1.25) > 14 g alcohol: 0.34 (0.18–0.61) post + >14 g alcohol: 0.28 (0.14–0.55)	A, D, E, G, I, L (N), S, Z	Canada

[a] high vs. low
↓ statistically significant inverse association. NS, statistically non-significant association.
[b] A, age; B, education; C, age at first pregnancy; D, age at menarche; E, relative with breast cancer; F, benign breast disease; G, number of pregnancies; H, body weight; I, total energy intake; J, dietary intake of folate; K, age at diagnosis; L, study site; M, ethnicity, race; N, alcohol; O, oral contraceptive use; P, smoking; Q, residence; R, urban/rural; S, menopausal state; T, date of blood donation; U, length of follow-up; V, weight change; W, age at menopause; X, hormone replacement therapy; Y, beta carotene; Z, practice of breast examination; ZZ, total vegetable intake

Table 3
Folate and risk of invasive cervical cancer.

Study design and reference	folate measure	number of cases	association	relative risk/odds ratio[a] (95% CI)	adjusted/ matched for[c]	country/ population
Case-control						
Verreault et al. 1989 [76]	diet supplements	189	NS NS	0.8 (0.3–1.7) not reported	A-G, K	Seattle, USA
Ziegler et al. 1990 [77]	diet supplements	218	NS not reported	0.85 (0.5–1.5)[b] RR = 1.03	A-D, F, G, M	white women, USA
Herrero et al. 1991 [78]	diet	748	NS	0.95 (0.7–1.3)	A, B, E, G-I, M	4 Latin American countries
Potischman et al. 1991 [80]	serum	330	NS	1.05 (0.7–1.6)	A, B, E, G-I	4 Latin American countries
Weinstein et al. 2001 [81]	serum (microbiol.) radiobinding red cell (microbiol.) radiobinding	183	 NS NS NS NS	 1.3 (0.8–2.9)[b] 1.6 (0.9–2.9)[b] 1.2 (0.6–2.2)[b] 1.5 (0.8–2.7)[b]	A-E, G-J, M, O	5 US areas
Nested case-control						
Alberg et al. 2000 [79]	serum	39	NS	0.60 (0.19–1.88)	A, C-E, I, J, L, N	Washington County, Maryland, USA

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

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 status. It is for example recognized that food folate composition data provide inaccurate estimations 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-

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 $^{677}C \rightarrow T$ polymorphism 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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References

1 Tönz O, Lüthy J, Raunhardt O. Folsäure zur Verhütung von Neuralrohrdefekten. Schweiz Med Wochenschr 1996;126: 177–87.
2 Eichholzer M, Lüthy J, Gutzwiller F, Stähelin HB. The role of folate, antioxidant vitamins and other constituents in fruit and vegetables in the prevention of cardiovascular disease: the epidemiological evidence. Int J Vitam Nutr Res 2001;71:5–17.
3 Kim YI. Folate and cancer prevention: a new medical application of folate beyond hyperhomocysteinemia and neural tube defects. Nutr Rev 1999;57:314–21.
4 Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B_6, folate, vitamin B_{12}, pantothenic acid, biotin, and choline: a report of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B vitamins, and Choline and Subcommittee on Upper Reference Levels of Nutrients. Washington, D.C.: National Academy Press; 1998.
5 Mason JB, Levesque T. Folate: effects on carcinogenesis and the potential for cancer chemoprevention. Oncology (Huntingt) 1996;10:1727–43.
6 Glynn SA, Albanes D. Folate and cancer: a review of the literature. Nutr Cancer 1994;22:101–19.
7 Duthie SJ. Folic acid deficiency and cancer: mechanisms of DNA instability. Br Med Bull 1999;55:578–92.
8 van Dam RM, Huang Z, Giovannucci E, Rimm EB, Hunter DJ, Colditz GA, et al. Diet and basal cell carcinoma of the skin in a prospective cohort of men. Am J Clin Nutr 2000;71:135–41.
9 Voorrips LE, Goldbohm RA, Brants HAM, van Poppel G, Sturmans F, Hermus R, et al. A prospective cohort study on antioxidant and folate intake and male lung cancer risk. Cancer Epidemiol Biomarkers Prevention 2000;9:357–65.
10 Stolzenberg-Solomon R, Pietinen P, Barrett M, Taylor P, Virtamo J, Albanes D. Dietary and other methyl-group availability factors and pancreatic cancer risk in a cohort of male smokers. Am J Epidemiol 2001;153:680–7.
11 Esteller M, Garcia A, Martinez-Palones J, Xercavins J, Reventos J. Germ line polymorphisms in cytrochrome–P450 1A1 (C4887 CYP1A1) and methylenetetra-hydrofolate reductase (MTHFR) genes and endometrial cancer susceptibility. Carcinogenesis 1997;18:2307–11.
12 Botterweck AA, van den Brandt PA, Goldbohm RA. Vitamins, carotenoids, dietary fiber, and the risk of gastric carcinoma: results from a prospective study after 6.3 years of follow-up. Cancer 2000;88:737–48.
13 Zhang ZF, Kurtz RC, Yu GP, Sun M, Gargon N, Karpeh M, et al. Adenocarcinomas of the esophagus and gastric cardia: the role of diet. Nutr Cancer 1997;27:298–309.
14 Fowler BM, Giuliano AR, Piyathilake C, Nour M, Hatch K. Hypomethylation in cervical tissue: is there a correlation with folate status? Cancer Epidemiol Biomark Prev 1998;7:901–6.
15 Kim Y, Giuliano A, Hatch KD, Schneider A, Nour MA, Dallal GE, et al. Global DNA hypomethylation increases progressively in cervical dysplasia and carcinoma. Cancer 1994;74: 893–9.
16 Kim Y. Methylenetetrahydrofolate reductase polymorphisms, folate, and cancer risk: a paradigm of gene-nutrition interactions in carcinogenesis. Nutr Rev 2000;58:205–17.
17 Kim Y, Pogribny IP, Basnakian AG, Miller JW, Selhub J, James SJ, et al. Folate deficiency in rats induces DNA strand breaks and hypomethylation with the p53 tumor suppressor gene. Am J Clin Nutr 1997;65:46–52.

18 Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci USA 1997; 94:3290–5.

19 Giovannucci E, Stampfer MJ, Colditz GA, Rimm EB, Trichopoulos D, Rosner BA, et al. Folate, methionine, and alcohol intake and risk of colorectal adenoma. J Natl Cancer Inst 1993; 85:875–84.

20 Giovannucci E, Rimm EB, Ascherio A, Stampfer MJ, Colditz GA, Willett WC. Alcohol, low-methionine-low folate diets, and risk of colon cancer in men. J Natl Cancer Inst 1995;87:265–73.

21 Anonymous. Folate, alcohol, methionine, and colon cancer risk: is there a unifying theme? Nutr Rev 1994;52:18–28.

22 Kenyon S, Nicolaou A, Gibbons W. The effect of ethanol and its metabolites upon methionine synthase activity in vitro. Alcohol 1998;15:305–9.

23 Todesco L, Angst C, Litynski P, Loehrer F, Fowler B, Haefeli WE. Methylenetetra-hydrofolate reductase polymorphism, plasma homocysteine and age. Eur J Clin Invest 1999;29: 1993–9.

24 Ma J, Stampfer MJ, Giovannucci E, Artigas C, Hunter DJ, Fuchs C, et al. Methylenetetrahyrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Res 1997;57:1098–1102.

25 Slattery ML, Potter JD, Samowitz W, Schaffer D, Leppert M. Methylenetetra-hydrofolate reductase, diet, and risk of colon cancer. Cancer Epidemiol Biomarkers Prev 1999;8:513–8.

26 Chen J, Giovannucci E, Kelsey K, Rimm EB, Stampfer MJ, Colditz GA, et al. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. Cancer Res 1996;56:4862–4.

27 Levine A, Siegmund K, Ervin C, Diep A, Lee E, Frankl H, Haile R. The methylenetetrahydrofolate reductase 677C→T polymorphism and distal colorectal adenoma risk. Cancer Epidemiol Biomarkers Prev 2000;9:657–63.

28 Song C, Xing D, Tan W, Wei Q, Lin D. Methylenetetrahydrofolate reductase polymorphisms increase risk of esophageal squamous cell carcinoma in a Chinese population. Cancer Res 2001;61:3272–5.

29 Wiemels J, Smith R, Taylor G, Eden O, Alexander F, Greaves M. Methylenetetrahydrofolate reductase (MTHFR) polymorphisms and risk of molecularly defined subtypes of childhood acute leukaemia. Proc Natl Acad Sci USA 2001;98:4004–9.

30 Hanson N, Aras Ö, Fang Y, Tsai M. C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase gene: incidence and effect of combined genotypes on fasting and post-methionine load homocysteine in vascular disease. Clin Chem 2001;47:661–6.

31 Skibola CF, Smith MT, Kane E, Roman E, Rollinson S, Cartwright RA, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. Proc Natl Acad Sci USA 1999;96: 12810–5.

32 Chen J, Giovannucci E, Hankinson SE, Ma J, Willett WC, Spiegelman D, et al. A prospective study of methylenetetrahydrofolate reductase and methionine synthase gene polymorphisms, and risk of colorectal adenoma. Carcinogenesis 1998; 19:2129–32.

33 Giuliano AR, Gapstur S. Can cervical dysplasia and cancer be prevented with nutrients? Nutr Rev 1998;56:9–16.

34 Higginson J, Muir CS, Munoz N. Human cancer: epidemiology and environmental causes. Cambridge Monographs on Cancer Research. Cambridge: University Press; 1992.

35 World Cancer Research Fund and American Institute for Cancer Research. Food, nutrition and the prevention of cancer: a global perspective. AICR, NW Washington, 1997.

36 Potter JD. Colorectal cancer: molecules and populations. J Natl Cancer Inst 1999; 91:916–32.

37 Ulrich CM, Kampman E, Bigler J, Schwartz SM, Chen C, Bostick R, et al. Colorectal adenomas and the C677T MTHFR polymorphism: evidence for gene-environment interaction? Cancer Epidemiol Biomarkers Prev 1999;8:659–68.

38 Benito E, Cabeza E, Moreno V, Obrador A, Bosch FX. Diet and colorectal adenomas: a case-control study in Majorca. Int J Cancer 1993;55:213–9.

39 Bird CL, Swendseid ME, Witte JS, Shikany JM, Hunt IF, Frankl HD, et al. Red cell and plasma folate, folate consumption, and the risk of colorectal adenomatous polyps. Cancer Epidemiol Biomarkers Prev 1995;4:709–14.

40 Tseng M, Murray SC, Kupper LL, Sandler RS. Micronutrients and the risk of colorectal adenomas. Am J Epidemiol 1996;144: 1005–14.

41 Baron JA, Sandler RS, Haile RW, Mandel JS, Mott LA, Greenberg ER. Folate intake, alcohol consumption, cigarette smoking, and risk of colorectal adenomas. J Natl Cancer Inst 1998;90: 57–62.

42 Boutron-Ruault MC, Senesse P, Faivre J, Couillault C, Belghiti C. Folate and alcohol intakes: related or independent roles in the adenoma-carcinoma sequence? Nutr Cancer 1996;26: 337–46.

43 Freudenheim JL, Graham S, Marshall JR, Haughey BP, Cholewinski S, Wilkinson G. Folate intake and carcinogenesis of the colon and rectum. Int J Epidemiol 1991;20: 368–74.

44 Benito E, Stiggelbout A, Bosch FX, Obrador A, Kaldor J, Mulet M, et al. Nutritional factors in colorectal cancer risk: a case-control study in Majorca. Int J Cancer 1991;49:161–7.

45 Meyer F, White E. Alcohol and nutrients in relation to colon cancer in middle-aged adults. Am J Epidemiol 1993;138: 225–36.

46 Ferraroni M, La Vecchia C, D'Avanzo B, Negri E, Franceschi S, Decarli A. Selected micronutrients and the risk of colorectal cancer. Br J Cancer 1994;70:1150–5.

47 Slattery ML, Schaffer D, Edwards SL, Ma KN, Potter JD. Are dietary factors involved in DNA methylation associated with colon cancer? Nutr Cancer 1997;28:52–62.

48 White E, Shannon JS, Patterson RE. Relationship between vitamin and calcium supplement use and colon cancer. Cancer Epidemiol Biomarkers Prev 1997;6:769–74.

49 La Vecchia C, Braga C, Negri E, Franceschi S, Russo A, Ettore C, et al. Intake of selected micronutrients and risk of colorectal cancer. Int J Cancer 1997;73:525–30.

50 Levi F, Pasche C, Lucchini F, La Vecchia C. Selected micronutrients and colorectal cancer: a case-control study from the canton of Vaud, Switzerland. Eur J Cancer 2000;36:2115–9.

51 Glynn SA. Albanes D, Pietinen P, Brown CC, Rautalahti M, Tangrea JA, et al. Colorectal cancer and folate status: a nested case-control study among male smokers. Cancer Epidemiol Biomarkers Prev 1996;5:487–94.

52 Ma J, Stampfer MJ, Christensen B, Giovannucci E, Hunter DJ, Chen J, et al. A polymorphism of the methionine synthase gene: association with plasma folate, vitamin B_{12}, homocysteine, and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev 1999;8:825–9.

53 Kato I, Dnistrian AM, Schwartz M, Toniolo P, Koenig K, Shore RE, et al. Serum folate, homocysteine and colorectal cancer risk in women: a nested case-control study. Br J Cancer 1999;79: 1917–21.

54 Lashner BA, Provencher KS, Seidner DL, Knesebeck A, Brzezinski A. The effect of folic acid supplementation on the risk for cancer or dysplasia in ulcerative colitis. Gastroenterology 1997;112:29–32.

55 Giovannucci E, Stampfer MJ, Colditz GA, Hunter DJ, Fuchs C, Rosner BA, et al. Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. Ann Intern Med 1998;129:517–24.

56 Kim Y, Mason JB. Nutrition chemoprevention of gastrointestinal cancer: a critical review. Nutr Rev 1996;54:259–79.

57 Ulvik A, Evenson E, Lien E, Hoff G, Vollset S, Majak B et al. Smoking, folate and methylenetetrahydrofolate reductase status as interactive determinants of edenomatous and hyperplastic polyps of colorectum. Am J Med Genet 2001;101:246–54.

58 Kim Y, Baik H, Fawaz K, Knox T, Lee Y, Norton R, et al. Effects of folate supplementation on two provisional molecular markers of colon cancer: a prospective, randomized trial. Am J Gastroenterol 2001;96:184–95.

59 Cravo M, Mason J, Dayal Y, Hutchinson M, Smith D, Selhub J, et al. Folate deficiency enhances the development of colonic neoplasia in dimethylhydrazine-treated rats. Cancer Res 1992; 52:5002–6.

60 Kim Y, Salomon R, Graeme-Cook F, Choi S, Smith D, Dallal G, et al. Dietary folate protects against the development of macroscopic colonic neoplasia in a dose-response manner in rats. Gut 1996;39:732–40.

61 Song J, Sohn K, Medline A, Ash C, Gallinger S, Kim Y. Chemopreventive effects of dietary folate on intestinal polyps in *Apc+/-Msh2-/-* Mice. Cancer Res 2000;60:3191–9.

62 Song J, Medline A, Mason J, Gallinger S, Kim Y. Effects of dietary folate on intestinal tumorigenesis in the Apc^{Min} Mouse. Cancer Res 2000; 60: 5434–40.

63 Leu, R, Young G, McIntosh G. Folate deficiency reduces the development of colorectal cancer in rats. Carcinogenesis 2000; 21:2261–2265.
64 Graham S, Hellmann R, Marshall J, Freudenheim J, Vena J, Swanson M, et al. Nutritional epidemiology of postmenopausal breast cancer in western New York. Am J Epidemiol 1991;134: 552–66.
65 Freudenheim JL, Marshall JR, Vena JE, Laughlin R, Brasure JR, Swanson MK, et al. Premenopausal breast cancer risk and intake of vegetables, fruits, and related nutrients. J Natl Cancer Inst 1996;88:340–8.
66 Potischman N, Swanson CA, Coates RJ, Gammon MD, Brogan DR, Curtin J, et al. Intake of food groups and associated micronutrients in relation to risk of early-stage breast cancer. Int J Cancer 1999;82:315–21.
67 Ronco A, De Stefani E, Boffetta P, Deneo-Pellegrini H, Mendilaharsu M, Leborgne F. Vegetables, fruits, and related nutrients and risk of breast cancer: a case-control study in Uruguay. Nutr Cancer 1999;35:111–9.
68 Negri E, La Vecchia C, Franceschi S. Re: Dietary folate consumption and breast cancer risk. J Natl Cancer Inst 2000;92: 1270–1.
69 Wu K, Helzlsouer KJ, Comstock GW, Hoffman SC, Nadeau MR, Selhub J. A prospective study on folate, B_{12}, and pyridoxal 5'-phosphate (B_6) and breast cancer. Cancer Epidemiol Biomarkers Prev 1999;8:209–17.
70 Zhang S, Hunter DJ, Hankinson SE, Giovannucci EL, Rosner BA, Colditz GA, et al. A prospective study of folate intake and the risk of breast cancer. JAMA 1999;281:1632–7.
71 Rohan TE, Jain MG, Howe GR, Miller AB. Dietary folate consumption and breast cancer risk. J Natl Cancer Inst 2000;92: 266–9.
72 McGlynn K et al. Methylenetetrahydrofolate reductase, methionine synthase, folate, alcohol and breast cancer (abstract). Proceedings of the American association for Cancer Research 2000;41:588.
73 Butterworth CE, Hatch KD, Gore H, Mueller H, Krumdieck CL. Improvement in cervical dysplasia associated with folic acid therapy in users of oral contraceptives. Am J Clin Nutr 1982;35: 73–82.
74 Butterworth CE, Hatch KD, Soong SJ, Cole P, Tamura T, Sauberlich HE, et al. Oral folic acid supplementation for cervical dysplasia: A clinical intervention trial. Am J Obstet Gynecol 1992;166:803–9.
75 Childers J, Chu J, Voigt LF, Feigl P, Tamimi HK, Franklin EW, et al. Chemoprevention of cervical cancer with folic acid: a phase III Southwest Oncology Group Intergroup Study. Cancer Epidemiol Biomarkers Prev 1995;4:155–9.
76 Verreault R, Chu J, Mandelson M, Shy K. A case-control study of diet and invasive cervical cancer. Int J Cancer 1989;43: 1050–54.
77 Ziegler RG, Brinton LA, Hamman RF, Lehman HF, Levine RS, Mallin K, et al. Diet and the risk of invasive cervical cancer among white women in the United States. Am J Epidemiol 1990;132:432–45.
78 Herrero R, Potischman N, Brinton LA, Reeves WC, Brenes MM, Tenorio F, et al. A case-control study of nutrient status and invasive cervical cancer. I. Dietary indicators. Am J Epidemiol 1991;134:1335–46.
79 Alberg AJ, Selhub J, Shah KV, Viscidi RP, Comstock GW, Helzlsouer KJ. The risk of cervical cancer in relation to serum concentrations of folate, vitamin B_{12}, and homocysteine. Cancer Epidemiol Biomarkers Prev 2000;9:761–4.
80 Potischman N, Brinton LA, Laiming VA, Reeves WC, Brenes MM, Herrero R, et al. A case-control study of serum folate levels and invasive cervical cancer. Cancer Res 1991;51:4785–9.
81 Weinstein S, Ziegler R, Frongillo E, Colman N, Sauberlich H, Brinton L, et al. Low serum and red blood cell folate are moderately, but nonsignificantly associated with increased risk of invasive cervical cancer in U.S. women. J Nutr 2001;131:2040–8.
82 Weinstein S, Ziegler R, Selhub J, Fears T, Strickler H, Brinton L, et al. Elevated serum homocysteine levels and increased risk of invasive cervical cancer in US women. Cancer Causes Control 2001;12:317–324.
83 Weir DG, Scott JM. Colonic mucosal folate concentrations and their association with colorectal cancer. Am J Clin Nutr 1998;68:763–4.
84 Piyathilake C, Hine RJ, Dasanayake AP, Richards EW, Freeberg LE, Vaughn WH, et al. Effect of smoking on folate levels in buccal mucosal cells. Int J Cancer 1992;52:566–9.
85 Meenan J, O'Hallinan E, Lynch S, Molloy A, McPartlan J, Scott J, et al. Folate status of gastrointestinal epithelial cells is not predicted by serum and red cell folate values in replete subjects. Gut 1996;38:410–3.
86 Meenan J, O'Hallinan E, Scott J, Weir DG. Epithelial cell folate depletion occurs in neoplastic but not adjacent normal colon mucosa. Gastroenterology 1997;112:1163–8.
87 Kim Y, Fawaz K, Knox T, Lee YM, Norton R, Arora S, et al. Colonic mucosal concentrations of folate correlate well with blood measurements of folate status in persons with colorectal polyps. Am J Clin Nutr 1998;68:866–72.

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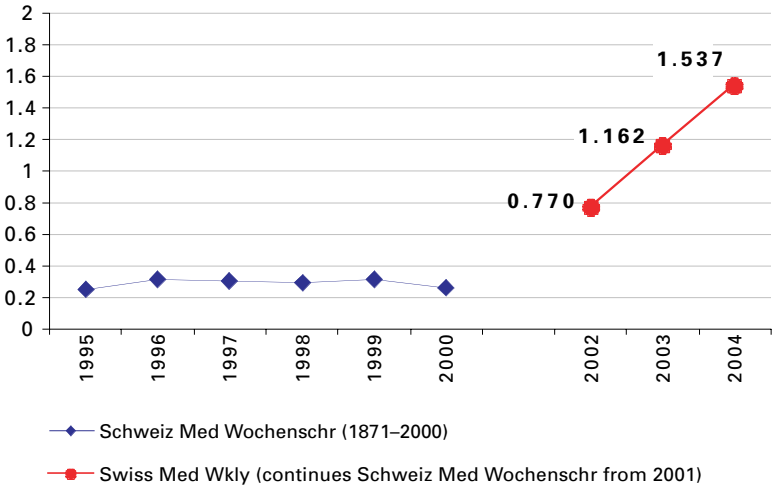

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