Effects of folic acid supplementation on overall and site-specific cancer incidence during the randomised trials: meta-analyses of data on 50 000 individuals

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Summary

Background Some countries fortify flour with folic acid to prevent neural tube defects but others do not, partly because of concerns about possible cancer risks. We aimed to assess any effects on site-specific cancer rates in the randomised trials of folic acid supplementation, at doses higher than those from fortification.

Methods In these meta-analyses, we sought all trials completed before 2011 that compared folic acid versus placebo, had scheduled treatment duration at least 1 year, included at least 500 participants, and recorded data on cancer incidence. We obtained individual participant datasets that included 49 621 participants in all 13 such trials (ten trials of folic acid supplementation, at doses higher than those from fortification).

Findings During a weighted average scheduled treatment duration of 5·2 years, allocation to folic acid quadrupled plasma concentrations of folic acid (57·3 nmol/L for the folic acid groups vs 13·5 nmol/L for the placebo groups), but had no significant effect on overall cancer incidence (1904 cancers in the folic acid groups vs 1809 cancers in the placebo groups, RR 1·06, 95% CI 0·99–1·13, p=0·10). There was no trend towards greater effect with longer treatment. There was no significant heterogeneity between the results of the 13 individual trials (p=0·23), or between the two overall results in the cardiovascular prevention trials and the adenoma trials (p=0·13). Moreover, there was no significant effect of folic acid supplementation on the incidence of cancer of the large intestine, prostate, lung, breast, or any other specific site.

Interpretation Folic acid supplementation does not substantially increase or decrease incidence of site-specific cancer during the first 5 years of treatment. Fortification of flour and other cereal products involves doses of folic acid that are, on average, an order of magnitude smaller than the doses used in these trials.

Funding British Heart Foundation, Medical Research Council, Cancer Research UK, Food Standards Agency.

Introduction

Results from epidemiological studies of pregnant women showed that the intake of folate and the plasma concentration of folate early in pregnancy were both inversely associated with the incidence of neural tube defects, suggesting protection. Non-randomised and randomised intervention trials confirmed a protective effect. Folate is now routinely recommended as a supplement before and during pregnancy. Furthermore, population-wide folate fortification of flour for prevention of neural tube defects has been mandatory since 1998 in North America, resulting in a two-fold increase in population plasma concentrations of folate. It is also mandatory in some other countries, including Chile, Argentina, Brazil, South Africa, and Australia, but not in New Zealand or in western European countries, partly because of concerns about possible adverse effects on cancer incidence or prognosis.

Results from epidemiological studies in other adult populations have also shown inverse (ie, apparently protective) associations of folate intake, and consequently of plasma concentrations of folate, with the incidence of cardiovascular disease and colorectal cancer. To test whether any real protective effect exists against cardiovascular disease, placebo-controlled trials of about 5 years of folic acid supplementation were undertaken in some 47 000 adults at high risk of vascular disease, and a collaborative meta-analysis of individual patient data on cancer incidence from all these trials was agreed prospectively in 2004, before any results emerged. These trials did not suggest any protective effect of folic acid supplementation against cardiovascular disease or against mortality from any cause during the scheduled trial treatment period. To test whether there is any real protective effect against progression of colorectal adenomas, another...
Methods

Trial eligibility

We identified trials by searching PubMed using the search terms “randomized trials”, “folic acid”, “B-vitamins” or “homocysteine-lowering treatment”, and by scanning reference lists of trial reports (appendix p 3). Trials were eligible for inclusion if (1) at least one randomised comparison was folic acid versus placebo with scheduled treatment duration of at least 1 year (irrespective of whether any other treatment was tested factorially); (2) the trial included at least 500 participants; and (3) data on cancer incidence had been recorded. We sought for unpublished trials completed before 2011 through electronic searches and discussions with other experts in the field, but did not find any. (As of Jan 1, 2013, we still know of no such trials completed since 2010.) We obtained individual participant datasets for all 49621 participants in the 13 trials that completed the end of 2010 (table 1, appendix p 6). Information about cancer incidence was not recorded in two other trials, with a total of 5992 participants. The protocol for trial identification, analysis and involvement of trialists was agreed following discussion with all collaborators before any cancer results emerged.19,20

Baseline and follow-up data

For each participant, we requested information about characteristics recorded before randomisation, allocated

<table>
<thead>
<tr>
<th>Number of patients randomised</th>
<th>Previous disease</th>
<th>Main countries</th>
<th>Mean (SD) scheduled duration of treatment (years)*</th>
<th>Daily dose of folic acid (mg)</th>
<th>Total number of incident cancers</th>
<th>Number of cancers (%) with known site of origin</th>
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</thead>
<tbody>
<tr>
<td>Colorectal adenoma trials</td>
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<td></td>
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<td>UK CAP</td>
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<td>USA</td>
<td>5 (3.1)</td>
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<td>USA</td>
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<td>1.0</td>
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<tr>
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<td></td>
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<tr>
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<td>2501</td>
<td>CVD</td>
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<tr>
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<td></td>
<td>5.2 (1.3)</td>
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<td>3545</td>
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<td></td>
<td></td>
<td>5.2 (1.3)</td>
<td>2.0†</td>
<td>3713</td>
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</table>

CVD=previous cardiovascular disease or increased risk of cardiovascular disease. *Mean (SD) in each trial, and weighted average of means (and of SDs) in subtotals and total (weighted by trial-specific variance of log rank [D-E] for cancer). †Median value.
treatment, and the type and date (or time from randomisation) of any cancer incidence or mortality during the scheduled treatment period. Information about admissions to hospital and cancer incidence was obtained in each trial at 3–6 month intervals during the scheduled treatment period. Self-reported cancer was recorded, and additional information on cancer incidence was, where possible, obtained from national cancer registries. In one trial, the site of cancer onset was not recorded in the primary database, so we searched all relevant databases of adverse events in that trial to identify incident cancers. The other trials all sought verification of incident cancers from hospital electronic records or by writing to hospital or family physicians. Sites of cancer onset were available for 93% (3446/3713) of the cases.

We checked analyses of the individual participant data for consistency with any published reports and checked them with the trialists to ensure that the data were incorporated correctly into the meta-analysis. We asked investigators to confirm summary data for every treatment group on the number of randomised participants, on plasma concentrations of folate and homocysteine before and after start of treatment, and on the number of participants who developed each of the predefined outcomes.

The main outcome was incident cancer, defined as the first occurrence after randomisation, but during the scheduled treatment period, of any cancer (ignoring non-melanoma skin cancer). Where cancer was diagnosed only at death and no other information was available, we recorded the date of diagnosis as the date of death. Where cancer site was available only for mortality, we used this for analyses of incidence as well as mortality. We subdivided cancers into the 17 most common types, on the basis of the International Classification of Disease-10 (ICD-10), with the aggregate of all other types as the 18th category, and missing cancer code as the 19th category. Having individual participant data from every trial facilitated uniform categorisation of cancer types (and permitted analyses of treatment effects in prespecified subgroups).

**Statistical analyses**

We based comparisons of cancer rates by allocated treatment on intention-to-treat analyses of first events.

![Figure 1: Effects of folic acid allocation on overall first cancer incidence](chart.png)

RR = rate ratio. The black squares denote the RRs and horizontal lines the 99% CIs. Each square has an area inversely proportional to the variance of the log of the RR. The diamonds represent the summary estimates and their corresponding 95% CIs. *p=0.07. †p=0.20. ‡p=0.10.
during the scheduled treatment period to calculate the event rate ratio (RR). The log-rank observed minus expected (o−e) statistics from each trial and their variances (v) were separately summed to produce, respectively, a grand total o−e statistic (G) and its variance (V). The one-step estimate of the log of the RR is then G/V with variance 1/V (and 95% CI G/V±1.96/√V). For n trials, a χ² statistic for heterogeneity with n−1 degrees of freedom (χ²n−1) is S−G²/V, where S is the sum over all trials of (o−e)²/v. We assessed the effects on cancer incidence in subgroups of year of follow-up (first 3 years or later), age, sex, plasma folate concentration, plasma homocysteine concentration, and whether or not there was a nationwide folic acid fortification programme. We investigated heterogeneity of the RRs in these subgroups by a global χ² test to reduce the chance of misinterpreting any false positive results arising from multiple comparisons.9 We used 99% CIs for individual trials or subgroups (again to avoid misinterpreting false positive results), but used 95% CIs for the overall findings. To correct for multiple comparisons, p values for particular types of cancer were multiplied by the number of types investigated (to a maximum corrected p value of 1.0).40,41

To help reassess the hypotheses of increased incidence of colorectal adenoma and prostate cancer raised by AFPPS,42 we assessed the effects of folic acid on colorectal and prostate cancer with and without exclusion of the AFPPS trial.43,44 The provision of (o−e) for each trial facilitates sensitivity analyses that exclude or include particular trials. Folate reduces homocysteine, and the mean reduction in all trials was the weighted mean of study-specific percent reductions in homocysteine, with weights proportional to the variances of the log-rank statistics for overall cancer incidence. We used Statistical Analysis System (SAS) version 9.2.

### Role of the funding source
The sponsors had no role in study design, data collection, data analysis, data interpretation, or writing or submission of the report. The Clinical Trial Service Unit and Epidemiological Studies Unit (CTSU) authors had full access to all the data and analyses and accept responsibility for this report. Final analyses and a draft report were circulated to all authors, revised and re-circulated. All authors are responsible for the decision to submit for publication.

### Results
Individual participant datasets were obtained from all 13 trials45–57 that met the inclusion criteria, including in total 49 621 participants (2652 from three trials46–48 in patients with a previous colorectal adenoma and 46 969 from ten trials49–55 in people with, or at high risk of, cardiovascular disease; table 1). Two-thirds of the participants were men, and the mean age at entry was 64 (SD 10) years (appendix p 6). The daily doses of folic acid ranged from 0.5 mg to 5 mg, except in one trial49 of a 40 mg daily dose (table 1). All trials compared the effects of folic acid versus placebo, except one trial50 that compared analyses of 2.5 mg versus 0.02 mg (5% of the recommended dietary intake; roughly equivalent to placebo). Mean scheduled treatment duration in different trials varied from 1-8 to 7-4 years, with weighted average 5-2 years.

Allocation to folic acid was associated with quadrupling median plasma concentrations of folate (57·3 nmol/L for folic acid vs 13·5 nmol/L for placebo; except in the one trial of high-dose folic acid,51 where treatment produced more than a hundred-fold increase in plasma folate). It was also associated with a reduction by a quarter in plasma homocysteine concentrations (9·3 μmol/L for folic acid vs 12·3 μmol/L for placebo; except in the one trial where the pre-treatment homocysteine was already low,52 where treatment produced little further effect on it; appendix p 7). As expected, effects on plasma homocysteine concentrations appeared to be somewhat greater in populations not fortified with folic acid (27% reduction) than in fortified populations (20% reduction; appendix p 7).53

Information was available on 3713 patients with an incident cancer during the scheduled treatment period. Allocation to folic acid treatment did not have any significant effect on overall cancer incidence, with 1904 (7.7%) first events in 24 799 participants allocated folic acid versus 1809 (7.3%) in 24 822 allocated control (RR 1·06; 95% CI 0·99–1·13, p=0.10: figure 1). No significant heterogeneity was noted between the results of all 13 trials or between the two subtotals for adenoma trials and vascular trials (figure 1). Importantly, we found no evidence of an increasing effect of folic acid with increasing duration of treatment (figure 2), although only four of the trials lasted more than 5 years. There were no significant differences
by sex, age, pretreatment blood concentration of folate, pretreatment blood concentration of homocysteine, folic acid fortification in the population, folate acid dose, or percent homocysteine reduction (appendix pp 4–5). Even in the trial of 40 mg/day of folic acid, which produced more than a hundred-fold increase in plasma folate, no apparent increase was noted in overall cancer incidence (65 cancers in the folic acid group vs 72 cancers in the placebo group, RR=0·94; 99% CI 0·61–1·47; figure 1).

We classified the 3713 cancers into 18 main types. There was no significant effect of folic acid allocation compared with placebo on the incidence of colorectal cancer, lung, breast cancer, prostate cancer, any of the less common types, or cancer of an unknown type, either overall or by treatment duration (figure 3, table 2).

Although there were conventionally significant protective effects against oesophageal cancer during the first 3 years but not later, and against breast cancer after 3 years but not during the first 3 years (figure 3), these unanticipated period-specific protective effects ceased to be significant when corrected for multiple comparisons (table 2). For adverse effects, however, even before any such corrections, there were no conventionally significant hazards for any type of cancer in either time period.

The appendix (pp 9–10) gives the log-rank statistics for colorectal and prostate cancer separately for each trial. We repeated the analyses after exclusion of the AFPPS trial, which had reported a significant excess risk of colorectal and prostate cancer. Overall, the remaining trials did not support a significant adverse effect on colorectal cancer (1·11; 95% CI 0·95–1·30; heterogeneity [AFPPS vs the other trials] $\chi^2=0·28$, p=0·6) or prostate cancer (1·11; 95% CI 0·95–1·30; heterogeneity [AFPPS vs the other trials] $\chi^2=4·46$, p=0·03). The provision of $\chi^2$ for each trial (appendix pp 9–11) facilitates additional sensitivity analyses that exclude particular trials.

Discussion

Both the hopes for rapid cancer prevention and the fears about rapidly increased cancer risk from folic acid supplementation were not confirmed by this meta-analysis of the trials of folic acid supplementation. Although the point estimate for overall cancer incidence was slightly increased, this finding was compatible with a chance effect, and the risk did not seem to increase with duration of treatment or daily dose of folic acid. Taking all studies together, allocation to folic acid for an average duration of 5 years had no significant effect on overall or site-specific cancer incidence during the scheduled treatment period.
Particularly, supplementation had no significant effect on the incidence of cancers of the large intestine (despite the epidemiological evidence of protection), prostate, lung, breast, or any other specific site, either over all time periods or during the period more than 3 years after randomisation, although the power to detect differences for cancer at particular sites at varying intervals of follow-up was limited. The results of the present meta-analysis, including 49621 participants, are unlikely to be biased by the unavailability of data on cancer incidence from two small trials, one of 1882 participants testing treatment with folic acid for 2 years and the other of 4110 participants testing treatment with folic acid for 4 years. Inclusion of the few cancer deaths recorded in those two trials would not materially alter the present meta-analyses of cancer incidence in all other trials.

A previous meta-analysis using summary data from a subset of the trials suggested a marginally significant excess of prostate cancer. The present meta-analysis, however, which used individual participant data in a time-to-event analysis from all large trials, showed no significant excess of prostate cancer or of any other type of cancer. Thus, the apparent excess risk of prostate cancer from folic acid supplementation in the results of the AFPPS trial was most likely produced or exaggerated by the play of chance. Appropriate interpretation of such findings requires avoidance of unduly selective emphasis on particular trials, which can be achieved by analysing all trials, and by testing the hypothesis after excluding the results of the trial that generated the hypothesis. For, a striking excess of some type(s) of cancer in some individual trial(s) can be expected by chance alone when many different types of cancer are analysed separately in many different trials.

Although many of the trials used combinations of B vitamins (vitamin B12 and vitamin B6 in addition to folic acid), it is unlikely that this would have concealed any effects of folic acid alone on cancer rates. The median daily dose of folic acid in the trials was 2·0 mg, which is greater than in most widely used vitamin supplements (0·1–0·8 mg) and an order of magnitude greater than the dose typically delivered by flour fortification programmes (0·1–0·4 mg).

Despite the low doses provided by fortification, it had been suggested that transient increases in colorectal cancer incidence in Canada and the USA in 1996–98 (during the 3-year period in which nationwide introduction of folate fortification was being established) might have been due to folic acid. Although the increases in incidence of colorectal cancer recorded in the SEER cancer registries in
...the USA during 1996–98 are unexplained, they occurred too soon to be plausibly ascribable to the introduction of folate fortification during 1996–98, and did not persist after 1998. Moreover, national trends in mortality from colorectal cancer in the USA at ages 35–69 years (which are unlikely to be influenced by any artifactual trends in cancer detection or registration rates) showed no evidence of any new hazard after the introduction of fortification (figure 4). Likewise, examination of US mortality rates at ages 35–69 years from the other main types of cancer (data not shown) provides no good evidence of any hazard following fortification.

Our meta-analyses included all large trials of folic acid, but the power to exclude beneficial or adverse effects on cancer at individual sites was limited by the number of cancers and the short duration of follow-up in these trials. Although the present meta-analyses address the effects of folic acid supplementation on cancer during the scheduled trial treatment period, they do not address the question of whether any beneficial or harmful effects on cancer incidence will eventually emerge among the participants many years after the trials all ended. Follow-up for decades after the end of the trials might be feasible, especially in populations with automated record linkage to cancer registries and causes of death, but again, it will be important not to place unduly data-dependent emphasis on the results for specific types of cancer in individual trials after particular follow-up durations.

Nevertheless, the human evidence about folic acid and cancer that has impeded folate acid fortification in the UK and some other countries involved possible increases in incidence of colorectal and prostate cancer within just the first few years of starting treatment, which, if real, should have been detectable during the trials. The present meta-analyses (which include the hypothesis-generating trial) address this issue directly, showing that in aggregate, the trials provide no significant evidence of short-term effects of folate acid supplementation on overall cancer incidence, or on the incidence of any particular type of cancer. The present meta-analysis rules out moderate increases in overall cancer incidence from folic acid supplementation during the trials. Large increases during the trials in any of the common types of cancer are similarly unlikely. Nationwide dietary fortification involves doses of folic acid that are an order of magnitude lower than the doses studied in these trials.

Contributors
The authors accept full responsibility for the content of this paper. All authors contributed to either the collection or analysis of the data, or both, and to preparation of the report. All authors had an opportunity to contribute to the interpretation of the results and to a critical review of the final draft of the manuscript.

B-Vitamin Treatment Trialists’ (BVTT) Collaboration

Conflicts of interest
Sources of funding for individual trials are described in their separate publications. The Clinical Trial Service Unit and Epidemiological Studies Unit (CTSU), where the BVTT secretariat is located, has a policy of not accepting fees, honoraria, or paid consultancies directly or indirectly from any industry. It receives its core funding from the British Heart Foundation, UK Medical Research Council, and Cancer Research UK. Support for this project was also provided by a grant from the UK Food Standards Agency (N05072).

Acknowledgments
We thank the many participants in the trials, and the trialists who cared for them and shared the data. We thank Jill Boreham for providing figure 4.

References


