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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 for prevention of cardiovascular disease [n=46 969] and three trials in patients with colorectal adenoma [n=2652]). All these trials were evenly randomised. The main outcome was incident cancer (ignoring non-melanoma skin cancer) during the scheduled treatment period (among participants who were still free of cancer). We compared those allocated folic acid with those allocated placebo, and used log-rank analyses to calculate the cancer incidence rate ratio (RR).

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 cadiovascular 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.

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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.[1–3] Non-randomised[4,5] and randomised[6,7] intervention trials confirmed a protective effect. Folate is now routinely recommended as a supplement before and during pregnancy.[8] 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.[9,10] It is also mandatory in some other countries,[9] including Chile, Argentina, Brazil, South Africa, and Australia;[11–13] but not in New Zealand or in western European countries, partly because of concerns about possible adverse effects on cancer incidence or prognosis.[13–15]

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[16,17] and colorectal cancer.[18] 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.[19,20] 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.[20]

To test whether there is any real protective effect against progression of colorectal adenomas, another

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See **Online** for appendix

three such trials[21–23] were undertaken in about 3000 patients. One of these trials, the Aspirin and Folic Acid Polyp Prevention Study (AFPPS),[21] reported in 2007 unexpected increases in the incidence of advanced colorectal adenomas and of prostate cancer during 7 years of treatment with folic acid. Also in 2007, it was suggested that transient increases in colorectal cancer incidence in Canada and the USA during 1996–98 might have been due to the 1996–98 introduction of folic acid fortification programmes in North America.[24] Taken together, these reports (and awareness that anti-folate drugs such as methotrexate are used for the treatment of some cancers) prompted concerns about possible risks of cancer associated with folic acid supplements and folic acid fortification. Studies in animals had previously suggested the possibility that high intakes of folate could suppress the development of early lesions in normal tissue, but enhance the growth of established neoplasms.[25]

To see whether, in aggregate, the randomised trials of folic acid show an increase or decrease in cancer risk over a period of just a few years, we present collaborative meta-analyses of site-specific cancer incidence during the scheduled treatment period among 50 000 individuals from all available large cardiovascular and adenoma trials. We do not address the question of whether any effects on cancer incidence will emerge some years or decades after the trials have ended.

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 49 621 participants in the 13 trials[21–23,26–35] completed by the end of 2010 (table 1, appendix p 6). Information about cancer incidence was not recorded in two other trials[36,37] 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

	Number of patients randomised	Previous disease	Main countries	Mean (SD) scheduled duration of treatment (years)*	Daily dose of folic acid (mg)	Total number of incident cancers	Number of cancers (%) with known site of origin
Colorectal adenoma trials							
UK CAP[22]	939	Adenoma	UK	2·4 (1·3)	0·5	27	27 (100%)
Harvard[23]	692	Adenoma	USA	5·3 (1·4)	1·0	49	48 (98%)
AFPPS[21]	1021	Adenoma	USA	7·4 (1·7)	1·0	92	89 (97%)
Subtotal	2652	..	..	6·0 (1·6)	..	168	164 (98%)
Vascular disease trials							
VITRO[26]	701	CVD	Netherlands	2·3 (0·7)	5·0	19	18 (95%)
HOST[27]	2056	Renal	USA	2·9 (1·2)	40·0	137	135 (99%)
WENBIT[28]	3090	Coronary heart disease	Norway	3·1 (1·0)	0·8	144	141 (98%)
NORVIT[29]	3749	Coronary heart disease	Norway	3·0 (0·8)	0·8	149	135 (91%)
SU-FOL-OM3[30]	2501	CVD	France	4·4 (1·1)	0·6	171	170 (99%)
VISP[31]	3680	Stroke	Canada and USA	1·8 (0·4)	2·5	187	88 (47%)
VITATOPS[32]	8164	CVD	Australia, India, and UK	3·7 (2·3)	2·0	345	317 (92%)
WAFACS[33]	5442	CVD	USA	6·9 (1·1)	2·5	414	384 (93%)
HOPE-2[34]	5522	CVD or diabetes mellitus	Canada and USA	4·6 (1·1)	2·5	662	650 (98%)
SEARCH[35]	12 064	Coronary heart disease	UK	6·7 (1·5)	2·0	1317	1244 (94%)
Subtotal	46 969	..	..	5·2 (1·3)	..	3545	3282 (93%)
Total	49 621	..	..	5·2 (1·3)	2·0†	3713	3446 (93%)

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 logrank [O–E] for cancer). †Median value.

Table 1: **Design and eligibility criteria of included trials**

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,[28–30,35] obtained from national cancer registries. In one trial,[31] 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

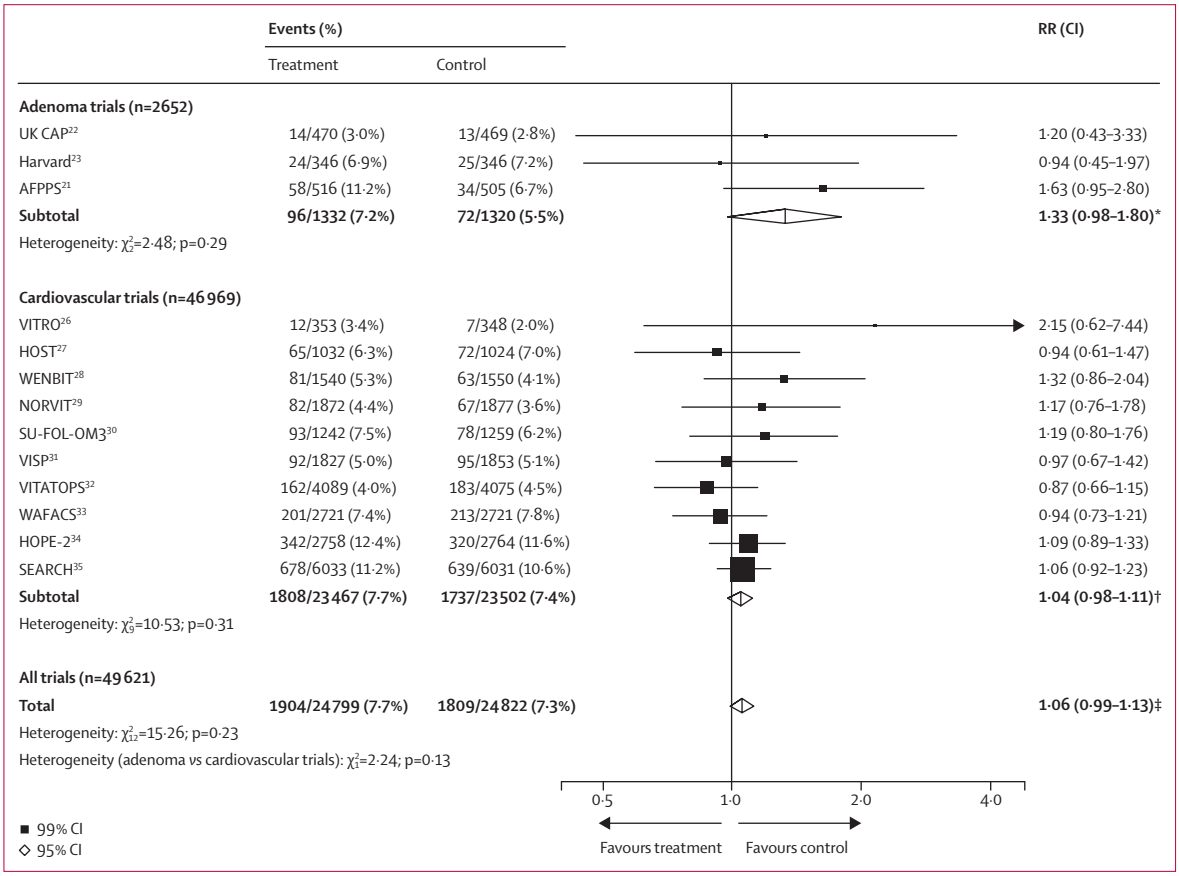

Figure 1: **Effects of folic acid allocation on overall first cancer incidence**
RR=rate ratio. The black squares denote the RRs and horizontal lines the 99% CIs. Each square has an area inversely proportional to the variance of the log of the RR. The diamonds represent the summary estimates and their 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).[38] 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 χ^2 statistic for heterogeneity with n–1 degrees of freedom (χ^2_{n-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 χ^2 test to reduce the chance of misinterpreting any false positive results arising from multiple comparisons.[39] 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,[21] we assessed the effects of folic acid on colorectal and prostate cancer with and without exclusion of the AFPPS trial.[40,41] 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.

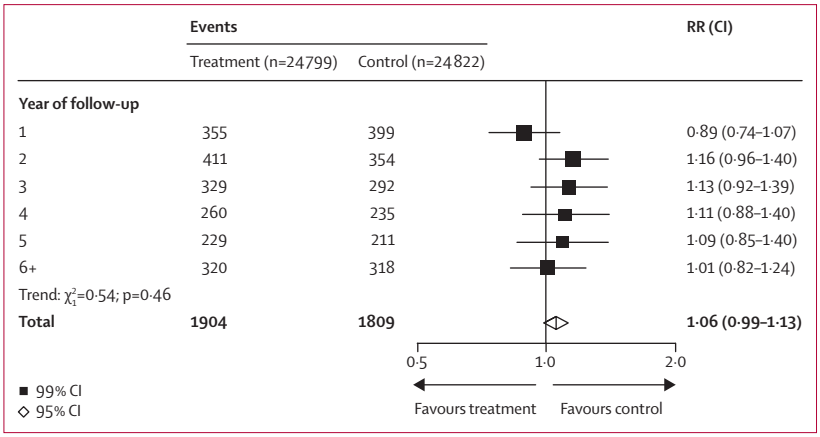

Figure 2: **Effects of folic acid on cancer incidence in all available trials, by year of follow-up**
RR=rate ratio. The black squares denote the RRs and horizontal lines the 99% CIs. Each square has an area inversely proportional to the variance of the log of the RR. The diamonds represent the summary estimates and their corresponding 95% CIs.

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 trials[21–23,26–35] that met the inclusion criteria, including in total 49 621 participants (2652 from three trials[21–23] in patients with a previous colorectal adenoma and 46 969 from ten trials[26–35] 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 trial[27] of a 40 mg daily dose (table 1). All trials compared the effects of folic acid versus placebo, except one trial[31] 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,[27] 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,[21] 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).[20]

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 24799 participants allocated folic acid versus 1809 (7·3%) in 24822 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[21,23,33,35] of the trials lasted more than 5 years. There were no significant differences

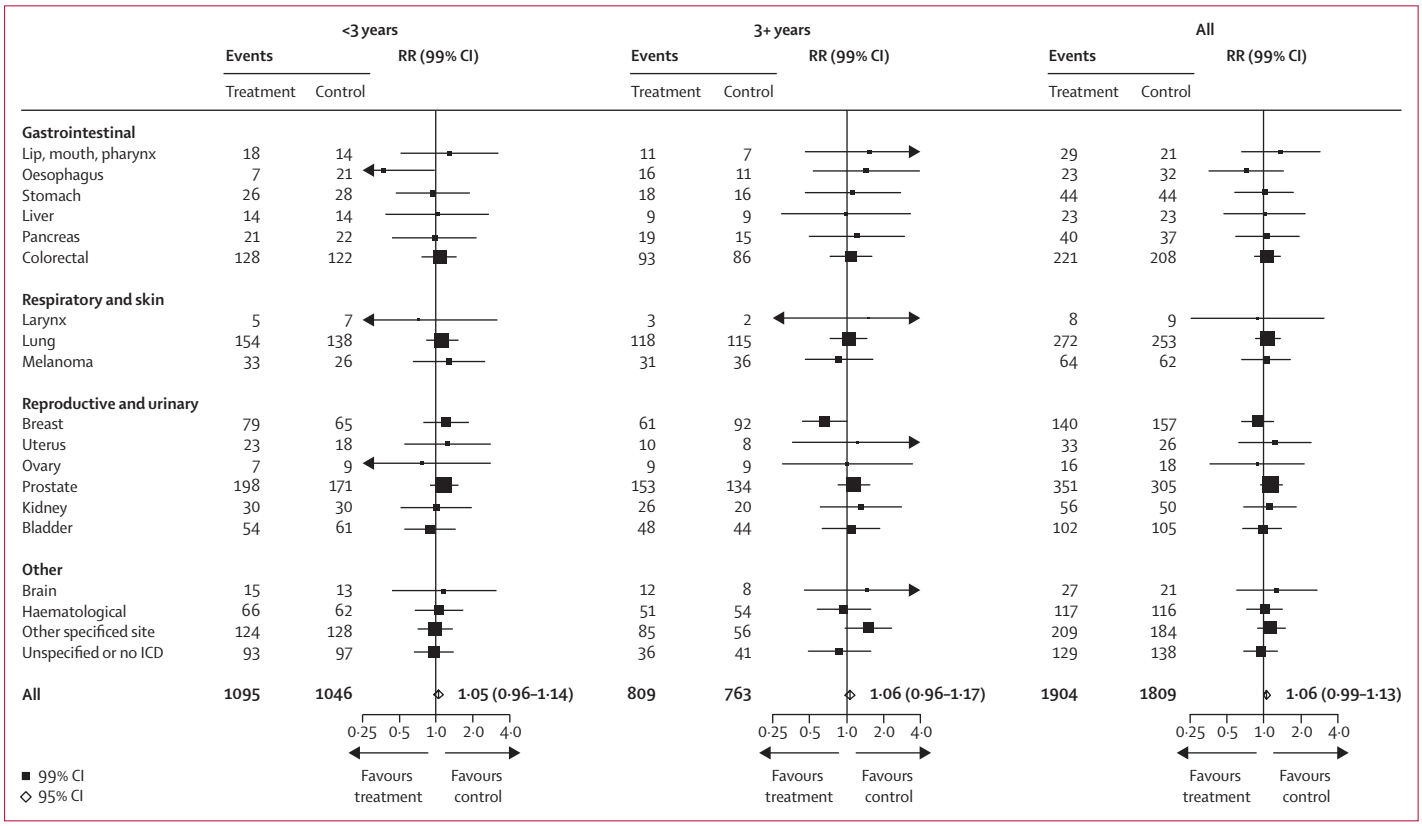

Figure 3: **Effects of folic acid on first cancer incidence, by type and duration of treatment**
RR=rate ratio. ICD=International Classification of Diseases. The black squares denote the RRs and horizontal lines the 99% CIs. Each square has area inversely proportional to the variance of the log of the RR. The diamonds represent the summary estimates and their corresponding 95% CIs.

by sex, age, pretreatment blood concentration of folate, pretreatment blood concentration of homocysteine, folic acid fortification in the population, folic acid dose, or percent homocysteine reduction (appendix pp 4–5). Even in the trial[27] 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 oesophagus 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,[21] which had reported a significant excess risk of colorectal adenomas and prostate cancer. Overall, the remaining trials did not support a significant adverse effect on colorectal cancer (1·08; 95% CI 0·89–1·30; heterogeneity [AFPPS[21] *vs* the other trials] χ^2_1=0·28, p=0·6) or prostate cancer (1·11; 95% CI 0·95–1·30; heterogeneity [AFPPS[21] *vs* the other trials] χ^2_1=4·46, p=0·03). The provision of (o–e) 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.

	<3 years after randomisation				≥3 years after randomisation				All years				
	Folic acid	Control	Uncorrected p value*	Corrected p value†	Folic acid	Control	Uncorrected p value*	Corrected p value†	Folic acid	Control	RR (CI)‡	Uncorrected p value*	Corrected p value†
Number of participants at start of time period	24799	24822	..	..	17292	17363	..	..	24799	24822	..	..	..
Lip, mouth, pharynx	18	14	0·47	1·00	11	7	0·35	1·00	29	21	1·38 (0·66–2·86)	0·26	1·00
Oesophagus	7	21	0·01	0·14	16	11	0·34	1·00	23	32	0·72 (0·36–1·44)	0·22	1·00
Stomach	26	28	0·82	1·00	18	16	0·73	1·00	44	44	1·01 (0·58–1·75)	0·97	1·00
Liver or gall bladder	14	14	0·96	1·00	9	9	0·98	1·00	23	23	1·01 (0·47–2·15)	0·98	1·00
Pancreas	21	22	0·91	1·00	19	15	0·59	1·00	40	37	1·07 (0·59–1·93)	0·78	1·00
Colorectal	128	122	0·66	1·00	93	86	0·58	1·00	221	208	1·07 (0·83–1·37)	0·49	1·00
Larynx	5	7	0·56	1·00	3	2	0·64	1·00	8	9	0·89 (0·25–3·11)	0·81	1·00
Lung	154	138	0·35	1·00	118	115	0·78	1·00	272	253	1·08 (0·86–1·35)	0·37	1·00
Melanoma	33	26	0·35	1·00	31	36	0·55	1·00	64	62	1·04 (0·66–1·64)	0·84	1·00
Breast	79	65	0·25	1·00	61	92	0·01	0·17	140	157	0·89 (0·66–1·20)	0·30	1·00
Uterus	23	18	0·49	1·00	10	8	0·68	1·00	33	26	1·23 (0·63–2·41)	0·43	1·00
Ovary	7	9	0·60	1·00	9	9	0·99	1·00	16	18	0·88 (0·37–2·15)	0·72	1·00
Prostate	198	171	0·16	1·00	153	134	0·26	1·00	351	305	1·15 (0·94–1·41)	0·07	1·00
Kidney	30	30	1·00	1·00	26	20	0·37	1·00	56	50	1·12 (0·68–1·85)	0·56	1·00
Bladder	54	61	0·52	1·00	48	44	0·69	1·00	102	105	0·97 (0·68–1·39)	0·83	1·00
Brain	15	13	0·69	1·00	12	8	0·41	1·00	27	21	1·27 (0·60–2·69)	0·40	1·00
Haematological	66	62	0·74	1·00	51	54	0·80	1·00	117	116	1·01 (0·72–1·42)	0·94	1·00
Other sites	124	128	0·90	NA	85	56	0·02	NA	209	184	1·15 (0·88–1·49)	0·18	NA
Missing ICD/unspecified	93	97	0·78	NA	36	41	0·55	NA	129	138	0·94 (0·68–1·28)	0·58	NA
Total§	1095	1046	0·26	NA	809	763	0·23	NA	1904	1809	1·06 (0·99–1·13)	0·10	NA

RR=rate ratio. ICD=International Classification of Diseases. NA=not applicable. *p values are two-sided, from log-rank analyses. †Corrected p values have been multiplied by the number of tests (17 sites) to allow for making multiple comparisons. ‡All are 99% CIs, except for "all cancers", which is a 95% CI. §If non-melanoma skin cancer had been included, the number of people developing a cancer would have been 1989 for the folic acid group versus 1890 for the control group. No deaths were attributed to this cause.

Table 2: **Number of people with incident cancers at specific sites, by duration of treatment**

Particularly, supplementation had no significant effect on the incidence of cancers of the large intestine (despite the epidemiological evidence of protection),[18] 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[36] and the other[37] 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.[42] 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[43] 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.[40,41] 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).[8]

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.[24] Although the increases in incidence of colorectal cancer recorded in the SEER cancer registries in

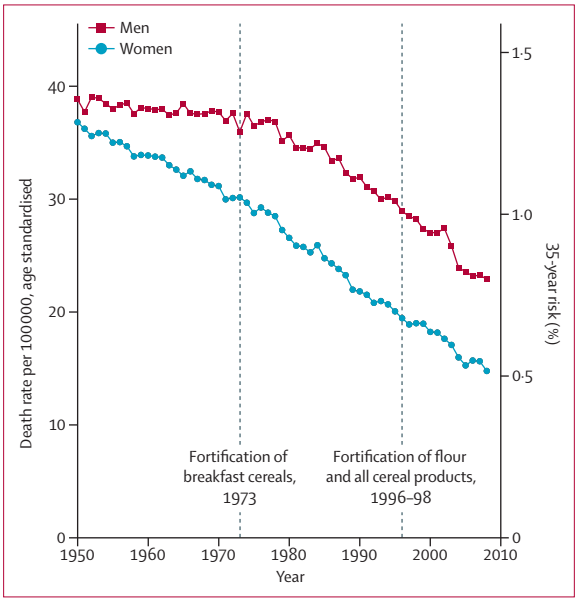

Figure 4: **Annual mortality from colorectal cancer in the USA, 1950–2008, at ages 35–69 years**

Mortality rates were calculated as the mean of the annual rates in the seven component 5-year age groups. Source: WHO mortality database and UN population tables. Mortality rates were standardised for age by averaging the seven component 5-year age groups. If the annual rate per 100 000, standardised in this way, is R, then the 35-year death rate from colorectal cancer, ignoring competing risks, is 1–exp(–35R /100 000).

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 folic 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 folic 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.

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Conflicts of interest

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References

1 Hibbard BM. The role of folic acid in pregnancy, with particular reference to anaemia, abruption and abortion. *J Obstet Gynaecol Br Common W* 1964; **71:** 529–42.
2 Molloy AM, Kirke P, Hillary I, Weir DG, Scott JM. Maternal serum folate and vitamin B12 concentrations in pregnancies associated with neural tube defects. *Arch Dis Child* 1985; **80:** 660–65.
3 Bower C, Stanley FJ. Dietary folate as a risk factor for neural tube defects: evidence from a case-control study in Western Australia. *Med J Aust* 1989; **150:** 613–19.
4 Smithells RW, Sheppard S, Schorah CJ, et al. Possible prevention of neural tube defects by periconceptional vitamin supplementation. *Lancet* 1980; **1:** 339–40.
5 Berry RJ, Li Z, Erickson JD, et al. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. *N Engl J Med* 1999; **341:** 1485–90.

6 MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet* 1991; **338:** 131–37.

7 Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* 1992; **327:** 1832–35.

8 Scientific Advisory Committee on Nutrition. Folate and disease prevention. London: The Stationery Office, 2006.

9 Centres for Disease Control and Prevention (CDC). Trends in wheat flour fortification with folic acid and iron worldwide, 2004 and 2007. *MMWR Morb Mortal Wkly Rep* 2008; **57:** 8–10.

10 Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *N Engl J Med* 1999; **340:** 1449–54.

11 Sayed AR, Bourne D, Pattinson R, Nixon J, Henderson B. Decline in the prevalence of neural tube defects following folic acid fortification and its cost-benefit in South Africa. *Birth Defects Res Clin Mol Teratol* 2008; **82:** 211–16.

12 López-Camelo JS, Castilla EE, Orioli IM; INAGEMP (Instituto Nacional deGenética Médica Populacional); ECLAMC (Estudio Colaborativo Latino Americano de Malformaciones Congénitas). Folic acid flour fortification: impact on the frequencies of 52 congenital anomaly types in three South American countries. *Am J Med Genet* 2010; **152:** 2444–58.

13 Australian Institute of Health and Welfare (AIHW). Mandatory folic acid and iodine fortification in Australia and New Zealand: baseline report for monitoring. Canberra: AIHW, 2011.

14 EFSA Meeting summary report. Folic acid: an update on scientific developments. European Food Safety Authority, 2010.

15 Scientific Advisory Committee on Nutrition. Folic acid and colorectal cancer risk: review of recommendations for folic acid mandatory fortification. London: Stationery Office, 2009.

16 Clarke R, Daly L, Robinson K, et al. Hyperhomocysteinemia: an independent risk factor for vascular disease. *N Engl J Med* 1991; **324:** 1149–55.

17 Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *JAMA* 1995; **274:** 1049–57.

18 Sanjoaquin MA, Allen NA, Couto E, Roddam AW, Key TJ. Folate intake and colorectal cancer risk: a meta-analytical approach. *Int J Cancer* 2005; **113:** 825–38.

19 B-Vitamin Treatment Trialists' Collaboration. Homocysteine-lowering trials for prevention of cardiovascular events: a review of the design and power of the large randomized trials. *Am Heart J* 2006; **151:** 282–87.

20 Clarke R, Halsey J, Lewington S, et al. Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality. Meta-analysis of 8 randomized trials involving 37485 individuals. *Arch Intern Med* 2010; **170:** 1622–31.

21 Cole BF, Baron JA, Sandler RS, et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. *JAMA* 2007; **297:** 2351–59.

22 Logan RF, Grainge MJ, Shepherd VC, Armitage NC, Muir KR. Aspirin and folic acid for the prevention of recurrent colorectal adenomas. *Gastroenterology* 2008; **134:** 29–38.

23 Wu K, Platz EA, Willett WC, et al. A randomized trial of folic acid supplementation and risk of recurrent colorectal adenoma. *Am J Clin Nutr* 2009; **90:** 1623–31.

24 Mason JB, Dickstein A, Jacques PF, et al. A temporal association between folic acid fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis. *Cancer Epidemiol Biomarkers Prev* 2007; **16:** 1325–29.

25 Kim YI. Will mandatory folic acid fortification prevent or promote cancer? *Am J Clin Nutr* 2004; **80:** 1123–28.

26 den Heijer M, Willems HP, Blom HJ, et al. Homocysteine lowering by B vitamins and the secondary prevention of deep vein thrombosis and pulmonary embolism: a randomized, placebo-controlled, double-blind trial. *Blood* 2007; **109:** 139–44.

27 Jamison RL, Hartigan P, Kaufman JS, et al. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: a randomized controlled trial. *JAMA* 2007; **298:** 1163–70.

28 Ebbing M, Bønaa KH, Nygård O, et al. Cancer incidence and mortality after treatment with folic acid and vitamin B12. *JAMA* 2009; **302:** 2119–26.

29 Bonaa KH, Njolstad I, Ueland PM, et al. Homocysteine lowering and cardiovascular events after acute myocardial infarction. *N Engl J Med* 2006; **354:** 1578–88.

30 Galan P, Kesse-Guyot E, Czernichow S, et al. Effects of B-vitamins and omega-3 fatty acids on cardiovascular diseases: a randomised placebo-controlled trial. *BMJ* 2010; **341:** C6273 doi:10.1136.

31 Toole JF, Malinow MR, Chambless LE, et al. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. *JAMA* 2004; **291:** 565–75.

32 VITATOPS Trial Study Group. B-vitamins in patients with recent transient ischaemic attack or stroke in the Vitamins To Prevent Stroke (VITATOPS) trial: a randomised, double-blind, parallel, placebo-controlled trial. *Lancet Neurology* 2010; **9:** 855–65.

33 Zhang SM, Cook NR, Albert CM, Gaziano JM, Buring JE, Manson JE. Effect of folic acid, vitamin B6, and vitamin B12 on cancer risk in women: a randomized trial. *JAMA* 2008; **300:** 2012–21.

34 Lonn E, Yusuf S, Arnold MJ, et al. Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med* 2006; **354:** 1567–77.

35 Armitage JM, Bowman L, Clarke RJ, et al. Effects of homocysteine-lowering with folic acid plus vitamin B12 vs placebo on mortality and major morbidity in myocardial infarction survivors: a randomized trial. *JAMA* 2010; **303:** 2486–94.

36 Baker F, Picton D, Blackwood S, et al. Blinded comparison of folic acid and placebo in patients with ischaemic heart disease: an outcome trial. *Circulation* 2002; **106** (suppl 2)**:** 741S (abstr).

37 Bostom AG, Carpenter MA, Kusek JW, et al. Homocysteine-lowering and cardiovascular disease outcomes in kidney transplant recipients: primary results from the Folic Acid for Vascular Outcome Reduction In Transplantation trial. *Circulation* 2011; **123:** 1763–70.

38 Yusuf S, Peto R, Lewis J, Collins R, Sleight P. Beta-blockade during and after myocardial infarction: an overview of the randomized trials. *Prog in Cardiovasc Dis* 1985; **27:** 335–71.

39 Sankoh AJ, Huque MF, Dubey SD. Some comments on frequently used multiple end-point adjustment methods in clinical trials. *Stat Med* 1997; **16:** 2529–42.

40 Peto R, Emberson J, Landray M, et al. Analyses of cancer data from three ezetimibe trials. *N Engl J Med* 2008; **359:** 1357–66.

41 Baigent C, Peto R, Gray R, Parish S, Collins R. Large-scale randomized evidence: trials and meta-analyses of trials. In: Warrell D, Cox TM, Firth JD, eds. Oxford Textbook of Medicine, 5th edn. Oxford: Oxford University Press, 2011.

42 Wien TN, Pike E, Wisloff T, Staff A, Smeland S, Kemp M. Cancer risk with folic acid supplements: a systematic review and meta-analysis. *BMJ Open* 2012; **2:** e000653.

43 Figueiredo JC, Grau MV, Haile RW, et al. Folic acid and risk of prostate cancer: results from a randomized clinical trial. *J Natl Cancer Inst* 2009: **101:** 432–35.