International Food Risk Analysis Journal

OPEN ACCESS ARTICLE

Folic Acid, Selected Cancers and All-cause Mortality: A Meta-analysis

Regular Paper

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Received 6 Jun 2013; Accepted 3 Oct 2013

DOI: 10.5772/58396

Abstract Fortification of foodstuffs with folic acid is mandatory in many countries. Although the effect of folic acid in reducing neural tube defects in utero is well accepted, questions have been raised about possible adverse effects of higher intakes of folic acid on adult health. An increase in colorectal cancer and some other cancers has been postulated. As part of a larger fortification monitoring program in Australia, we conducted a systematic review of trials of folic acid for these outcomes. We found 26 trials using between 0.4-20mg folic acid/day which reported all-cause mortality or the incidence of specified cancers or recurrence of colorectal adenoma. Using the in-trial follow-up data, the relative risks were 1.04 for total incident cancer (13 studies), 1.0 for colorectal and lung cancers, 0.82 for breast cancer, 1.16 for prostate cancer, 0.97 for the recurrence of colorectal adenoma and 1.11 for the recurrence of advanced colorectal adenoma. There was no association with all-cause mortality (relative risk=0.99, 23 studies). None of the relative risks were statistically significant at the customary alpha level. Our findings are similar to those of previous meta-analyses that have used different inclusion criteria to select studies.

Keywords Folic Acid, Cancer, Mortality, Colorectal Adenoma, Meta-analysis

1. Introduction

In mid-2007, the (then) Australia New Zealand Food Regulation Ministerial Council (the Council) approved mandatory fortification of wheat flour for bread making with folic acid in the range of no less than 2 mg/kg and no more than 3 mg/kg [1] to help reduce the prevalence of neural tube defects. This regulation commenced on 13 September 2009 [1] in Australia but was postponed in New Zealand.

During the development of the regulation, the question of a relationship between folic acid and cancer had been raised. Folate has a role in cell division. Animal studies have suggested both low levels and high levels increase the risk of cancer compared to intermediate levels. Observational studies in humans found that higher intakes of dietary folate might decrease the risk of cancer [2, 3]. When FSANZ commissioned an update of an earlier report from the UK [3], the available literature were cohort and case-control studies that examined natural folate intakes alone or with supplement use [4]. Folic acid was also hypothesised to have beneficial effects on cardiovascular disease via altered homocysteine concentrations and to reduce the recurrence of colorectal adenoma and improve cognitive outcomes. A number of

trials testing these outcomes had commenced. The majority reported their results from 2006 onwards and often included mortality and cancer data. One trial received particular media attention owing to a greatly increased incidence of prostate cancer in the group receiving folic acid [5]. A trend analysis linking a brief increase in colorectal cancer incidence to the introduction of mandatory fortification in the United States and Canada [6] also received substantial publicity.

The Council committed to monitoring the implementation and effects of mandatory fortification with folic acid in Australia. The Australian Institute of Health and Welfare was commissioned to develop a framework and report on the availability and quality of baseline data [7]. Different groups are overseeing various aspects of the implementation, such as industry compliance and the impact on the prevalence of neural tube defects. The current report fulfils FSANZ's undertaking to continue monitoring the scientific literature for further studies regarding the link between folic acid and cancer incidence.

1.1 Terminology used in this review

This review uses the language of food regulation [8] rather than epidemiology. Although there are many overlaps in concepts, some terms have different meanings and so the terms used in this report are outlined briefly.

The steps of a Risk Assessment are shown in Figure 1. 'Hazard identification' is the formal statement of the food component (i.e. hazard) being studied. In epidemiological studies, the hazard is often called the study factor or exposure. The hazard identification step also includes identification of possible consequences beneficial health consequences) of the hazard. The 'hazard characterisation' step seeks to quantify the effect of the hazard on the possible health effects, for example as a dose-response relationship, thresholds or even a determination that there is no relationship. Sometimes these two steps are merged together and called 'hazard assessment'.

The term 'exposure' is used to describe the intake (when food is being discussed) of the hazard in the population. This is a different use of the term from the common epidemiological use. Because intakes typically vary across the population, population exposure is often described using a mean and standard deviation, or median and centiles, instead of or in addition to, the percent who are exposed (prevalence). 'Risk characterisation' combines the degree of exposure (intake) in the population with information from hazard characterisation (such as dose-response) to describe the risk to the population of interest from the hazard.

Some food components, such as nutrients, have benefits as well as possible adverse effects. The term 'hazard' can encompass both although it is not usually applied to nutrients. Separate hazard characterisations could be performed to assess a range of possible beneficial and adverse effects, such as neural tube defects, cardiovascular disease, cognition, cancer and total mortality. A higher level analysis would then combine the separate hazard characterisations and the exposure (i.e. intake) into an overall risk characterisation for the population with respect to the hazard. This report focuses on hazard identification and characterisation with respect only to the outcomes listed.

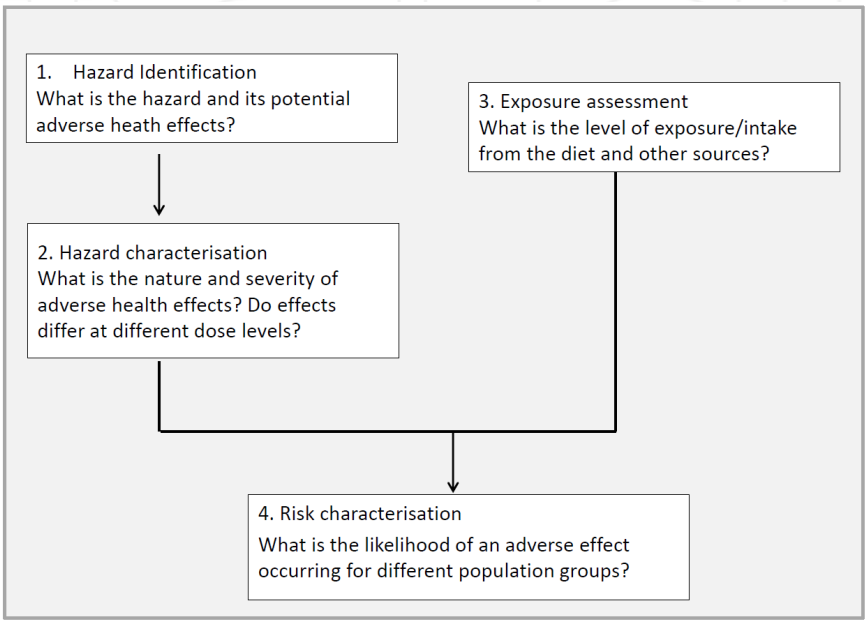

Figure 1. Steps in Risk Assessment [8]

2. Methods

2.1 Goal of the review

The purpose of this review, and the earlier reviews that it updates, is to examine the hypothesised link between higher intakes of folic acid and cancer incidence in humans (hazard characterisation) for the specific context of mandatory fortification. The relationship between folic acid intake and the incidence of all cancers combined (total incident cancer) and cancer at the four most common sites – colorectal, lung, breast and prostate – are examined. In addition, the relationship between folic acid and recurrence of colorectal adenoma is examined. The effect of folic acid on all-cause mortality, a summary of overall health, is also included.

This review is limited to randomised controlled trials that tested folic acid in humans and reported any of the outcomes of interest. Trials provide direct evidence for assessing the effects of an intervention. Others have summarised the observational literature (which provides indirect evidence when assessing the effects of an intervention). For example, Kim et al [9] released a pooled analysis that included 13 large cohort studies with follow-up of 7-20 years in more than 500,000 men and women. There was a 15% decrease in risk of colon cancer in the highest versus lowest quintile of intake for total (dietary plus supplemental) folate intakes (RR=0.85; 95% CI: 0.77–0.95).

2.2 Hazard identification: folic acid not folate

Although folic acid is converted to tetrahydrofolate in the body, and this is the same as the folate derived from food, high doses of folic acid also lead to unmetabolised folic acid in the blood [10]. It is not clear how to define 'high'. Kelly et al [11] found a threshold of approximately 0.2mg folic acid for a single oral dose. Questions remain about whether lower, more frequent doses would also lead to unmetabolised folic acid in the blood and whether there is a genetic influence that causes only a subset of the population to exhibit the effect [10]. Therefore, folic acid and natural folate from food might have different relationships with some disease outcomes.

Folic acid is the only form of folate permitted for mandatory fortification of wheat flour for bread making in Australia. Both folic acid and L-5-methylfolate (5MTHF) calcium [12] are permitted forms of folate for voluntary fortification of foods other than wheat flour for bread making. As this review relates to mandatory fortification with folic acid, only studies that tested the permitted form, folic acid, were included. Studies of other forms of folate, such as folate found naturally in food or folinic acid are out of scope. Consequently, the SU.FOL.OM3 Study which tested 0.56mg/day 5MTHF [13] was excluded.

2.3 Search strategy

This has been a living meta-analysis and updated as new papers were published. This report describes the result of a formal search conducted in May 2013. Few of the trials conducted to date have had cancer incidence as a primary outcome. Cancer incidence has generally been reported as an adverse effect and is rarely mentioned in the title or abstract of papers. Therefore the search strategy did not place any restrictions on the health or disease outcomes. Reading a number of reviews and the reference lists of papers, indicated that only two trials had been reported prior to 2001, one of which did not report relevant outcomes [14]. A third study was reported in several papers straddling 2001 [15, 16]. Therefore MEDLINE (using the PubMed portal) was searched from January 1, 2001 to 16 May 2013. Cochrane CENTRAL was also searched on 16 May 2013. The search strategies used are given in Appendix 1. Medline was searched only for English language papers. The search in CENTRAL, which contains trials identified by systematic searching accesses trials from Medline, EMBASE, other databases and by hand-searching the abstracts from many conference handbooks was not limited for language. (No relevant trials published in a language other than English were identified in CENTRAL).

The references list of each trial read at the full text screening stage was checked. The reference lists in a range of systematic reviews examining the effect of folic acid on cancer, heart disease, stroke, vascular function, cognition, Alzheimer's disease and osteoporosis [17-37] and reports by other food regulatory agencies [38, 39] were also checked.

2.3.1 Inclusion and exclusion criteria

Criteria for inclusion:

- randomised controlled trial conducted in humans
- statement that assignment to group was randomised even if no further detail of the method was included (such as description of masked allocation or central randomisation in multi-centre trials)
- at least one trial arm which used folic acid; trials which specifically described using non-folic acid forms of folate (such as 5MTHF or folinic acid) were excluded. Studies which stated that they had used folate, but did not describe the supplement further, were included because folic acid is the most common form in supplements
- co-administration of vitamin B6 and/or B12 with folic acid was permitted
- co-administration of other substances including nutrients, was permitted if they were part of a

separate randomisation stream (e.g. in a factorial design) or were given to subjects in both the intervention and control groups (e.g. statins). Arms that did not include folic acid or its placebo were excluded from the analysis (e.g. in studies that had several different treatment arms and a common placebo) when the papers reported data separately.
- any placebos for folic acid had to be either a blank or minimal dose of folic acid; factorial designs testing other substances were permitted
- trials had to administer folic acid and placebos for one year or longer and have a follow-up period of one year or longer (owing to the long latency period of cancer)
- trials had to report at least one of: all-cause mortality, total cancer incidence (exclusion of non-melanoma skin cancer was permitted), incidence of colorectal/colon/rectal cancer, breast cancer, lung cancer, prostate cancer or recurrence of colorectal adenoma

Trials were excluded if
- the subjects had cancer or were cancer survivors; had severe conditions (e.g. end stage renal disease, nephropathy, had received transplants); were on methotrexate (an anti-folate) or had rheumatoid arthritis (commonly treated with methotrexate) or HIV/AIDS
- subjects were pregnant women or children <18 years or were a mixed age population with both children and adults
- folic acid was administered in a broad multivitamin/mineral supplement

The references obtained from searches were imported into EPPI Reviewer 4 (http://eppi.ioe.ac.uk/cms/Default.aspx?tabid=2914, accessed 23 July, 2013) for screening. After removing duplicate citations, the remaining citations were screened using their title and abstract. They were coded for exclusion if it was clear from the title or abstract that they did not conform to one or more or the criteria listed above. One reviewer examined all citations and a second reviewer examined 15% as a cross-check. Citations remaining after the first screening stage were examined in detail by reading the full text or by linking conference abstracts and subsidiary papers to the primary paper describing the trial.

2.4 Data extraction

Two reviewers independently abstracted the number randomised to each arm (excluding those subsequently determined to be ineligible at baseline by the study authors), and the number of events in each arm from one or more papers describing each study. Subjects who were randomised but did not start trial vitamins or placebo were included in the denominators even if the author had excluded them in the reported analyses. Because cancer was reported as a side effect in most studies, trial entry criteria might not have excluded people with a prior history of the cancers of interest. However, randomisation, especially in the larger trials, should lead to even distribution of prior cancers and so inability to exclude these people should not bias the results.

Generally, cancer outcomes were reported in tables whereas the number of deaths was reported in the CONSORT diagram, tables or the text. When necessary, authors were contacted to clarify information or to obtain additional information, although not all responded. Any additional information that was used is described further in the relevant section of the paper.

2.5 Analysis

Some trials analysed their cancer and mortality results using hazard ratios while others presented only counts. To ensure consistency across all studies, the relative risks were calculated using counts from all studies. An overall weighted relative risk for each outcome was calculated de novo from the numbers randomised and event numbers using the DerSimonian-Laird inverse variance random effects method. Its 95% CI was calculated using the Greenland-Robins formula. Stats Direct (http://www.statsdirect.co.uk) was used for the calculations. The 'total cancer' result reported in the papers trials was used. Not all authors specified whether they had included or excluded non-melanoma skin cancer. When analysing prostate cancer, only male subjects randomised were used in the denominator and similarly only women were used in the denominator for the primary analysis of breast cancer. I^2 is reported for each analysis. I^2 describes the "percentage of total variation across studies that is due to heterogeneity rather than chance" and 0%, 25%, 50% and 75% could be interpreted as indicating no, low, medium and high heterogeneity respectively [40]. Variation in dose or study duration, for example, might cause heterogeneity (variation in results) among studies if there is a dose-response relationship or a latent period for disease development. For the meta-analyses with a large number of studies, the funnel plot was visually examined for symmetry to assess whether the result might be affected by publication bias.

Sensitivity analyses were done to explore the influence of factors that might lead to heterogeneity between trials, such as dose or co-administration of vitamin B6 or B12. Several trials reported results during the period of vitamin allocation and for follow-up several years beyond trial cessation [5, 41, 42]. The primary analysis reported here includes follow-up during the trial. A sensitivity

analysis reports whether the results change if follow-up beyond the end of the trial is substituted for the in-trial results [43, 44].

Finally, the results of the current work were compared to results of earlier systematic reviews examining the same relationships.

2.6 Data presentation in the figures

All Forest plots are ordered by increasing dose of folic acid and the daily dose is shown next to the author's name, together with a code indicating whether vitamin B6 and/or B12 were co-administered. For studies using the same dose, the ordering is from widest to narrowest confidence interval. The size of the squares within one graph indicate the relative weighting of the studies within that analysis but the size of the squares cannot be compared between graphs. Relative risks greater than 1.0 indicate that the folic acid have a higher incidence than the placebo group. The diamond indicates the combined weighted average relative risk. Sometime the confidence interval is narrower than the width of the diamond. The right hand column presents the relative risk and 95% confidence interval for each study and the combined result numerically. The relative risk shown for each study is an unadjusted relative risk (i.e. risk ratio) calculated from data in the paper. Consequently they may be different from the ratio presented in the original paper if an adjusted ratio or a hazard ratio was calculated by the authors.

3. Results

3.1 Overview of included studies

Figure 2 shows that 5357 records were identified from the two databases and the two known trials conducted prior to 2001. The majority of exclusions at the first screening were therapeutic trials in cancer patients or survivors and trials that lasted less than one year. Among the 181 remaining records, there were 26 studies described in 114 papers and conference abstracts were included after reading the full text. The major reasons for exclusion were records that were commentaries or editorials about trials, trials with inadequate duration and trials without relevant outcomes (Figure 2, Table 1).

Primary reference	Duration of follow-up (years)	Dose folic acid (mg/day)	Reason for exclusion
Coppen 1986 [14]	1	0.2	No mortality or cancer outcomes reported
Till, 2005 [45]	1	2.5	
McMahon, 2006 [46]	2	1	
Herrmann, 2007 [47]	1	2.5	
den Heijer, 2007 [48]	2.5	5	
Ntaios, 2009 [49]	1.5	5	
Vermeulen, 2000, van Dijk, 2001 [15, 16]	2	5	No deaths, 3 colon cancers reported but the papers do not state which group they occurred in
Liu, 2010 [50]	2	Not stated	Conference abstract describing a relative risk of 1.125 for stroke as "decreased risk" therefore relative risk of 12.98 for all-cause mortality could also indicate a reduction in mortality in the folic acid group. No full report found.
Richard, 2009 [51]	2	0.5	Combined intervention of aspirin and other stepped vascular care given to folic acid group but not placebo group

Table 1. Reason for excluding 20 records (9 trials) using folic acid for one year or longer at full-text reading stage

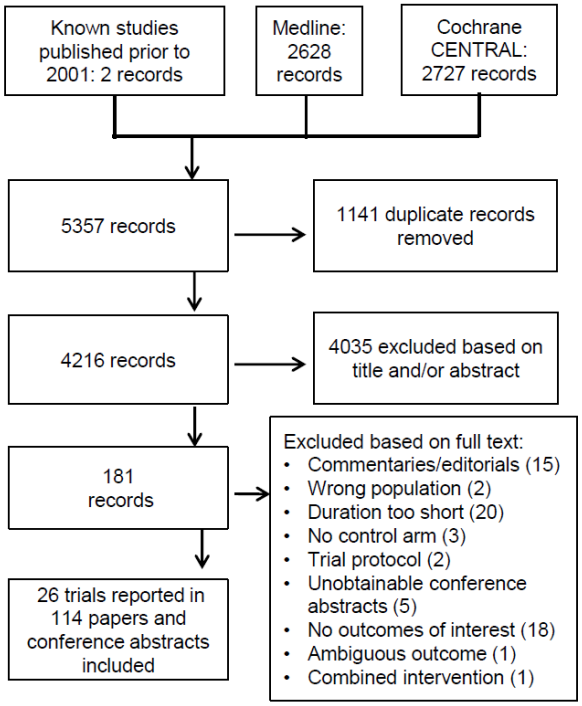

Figure 2. Flow diagram for filtering search results

3.2 Overview of trials included

The 26 included studies randomised between 20 and 12,064 subjects and lasted up to 7.3 years. In addition to trials investigating cardiovascular outcomes, there was one trial examining an intermediate vascular endpoint, seven trials investigating cognitive outcomes, six trials examining recurrence of colorectal adenoma, one trial conducted in patients with hip fractures and one trial investigating gastrointestinal lesions (Table 2).

The most frequently reported of the outcomes of interest was mortality (23 studies). The dose of folic acid ranged from 0.4mg to 20 mg per day. The trials used a range of interventions. Some used folic acid alone and others folic acid plus other B nutrients, usually vitamin B12 and often vitamin B6. Several trials had factorial designs: two adenoma trials [5, 52] also tested aspirin, Zhang et al [53] tested antioxidants and the SEARCH study [54] tested a statin.

Author, Year, (Reference) (Study Name)	**Description**	**Comparison (daily dosage unless otherwise stated)**	**Data to allow calculation of Relative risks for**			
			Total cancer	**Site specific cancer**	**Colo-rectal Ade-noma**	**Morta-lity**
Paspatis, 1994 [55]	1 year trial of 60 people with prior colorectal cancer, mean age 60 years	1 mg folic acid vs placebo	×	×	✓	×
Kim, 2001 [56]	1 year trial of 20 people with prior colorectal cancer, mean age 62 years	5 mg folic acid vs placebo	×	×	✓	×
Zhu, 2003 [57]	6 year trial, 98 Chinese adults with atrophic gastritis, mean age 55.6 years	20mg folic acid per day plus 1mg B12 intramuscularly (IM) monthly for one year; 20mg folic acid 2/week and 1mg B12/quarter IM for 2nd year vs placebo for part of follow-up	×	C	×	×
Toole, 2004 [58] Clarke, 2010 [59] (VISP)	2 year trial, 3680 stroke patients in US, Canada & Scotland, mean age 66 years	2.5mg folic acid plus 25mg B6 plus 0.4mg B12 vs low doses (0.02mg folic acid plus 0.2mg B6 plus 0.006mg B12)	✓	×	×	✓
Liem, 2004 [60] (FOLARDA)	1 year trial, 283 Dutch patients post myocardial infarct, mean age 59 years	5 mg folic acid vs control (open label study); both groups received a statin	×	×	×	✓
Liem, 2005 [61] (GOES)	3.5 year trial, 593 Dutch patients with stable coronary artery disease, mean age 65 years	0.5mg folic acid vs control (open label study); both groups received a statin	×	×	×	✓
Sato, 2005 [62]	2 year trial of hip fracture prevention in 628 Japanese stroke patients, 65 years and older	5mg folic acid plus 1.5mg B12 vs placebo	×	×	×	✓
Lonn, 2006 [63] (HOPE 2)	5 year trial conducted in 13 countries; 5,522 men and women with vascular disease or diabetes, aged 55 years and older	2.5 mg folic acid plus 50 mg B6 plus 1 mg B12 vs placebo	✓	C,B,P,L	×	✓

Author, Year, (Reference) (Study Name)	Description	Comparison (daily dosage unless otherwise stated)	Data to allow calculation of Relative risks for			
			Total cancer	Site specific cancer	Colo-rectal Ade-noma	Morta-lity
Bonaa, 2006 [41] Ebbing, 2010 [43] (NORVIT)	3.3 year factorial trial in Norway; 3,749 men and women who had survived a heart attack, mean age 63 years	0.8 mg folic acid plus 0.4mg B12 with or without 40mg B6 vs placebo (without B6)	✓	C	×	✓
Flicker, 2006 [64] and Ford, 2008 [65]	2 year trial in 299 West Australian men aged 75 and older who were clinically free of depression	2mg folic acid plus 0.4mg B12 plus 25mg B6 vs placebo	×	×	×	✓
Cole, 2007 [5] Figuerido, 2009 [44] (AFPPS)	US trial with two follow-up stages, men and women who had had a colorectal adenoma removed, aged 21-80 years, 1021 followed for 3 years and 607 followed for 6-8 years total	1.0 mg folic acid with or without aspirin vs placebo; factorial design with aspirin	✓	C,P	✓	✓
Fernandez-Miranda, 2007 [66]	3 year trial in 137 Spanish patients with elevated homocysteine & heart disease, aged less than 80 years	2.5 mg folic acid (open label study); both groups received a statin	✓	×	×	✓
Durga, 2007 [67] (FACIT)	3 year trial, 818 Dutch men and women aged 50-70 years, with elevated homocysteine	0.8mg folic acid vs placebo	×	×	×	✓
Logan, 2008 [52] (ukCAP)	3 year trial in 939 UK men and women aged <75 years who had had a colorectal adenoma removed	0.5 mg folic acid with or without aspirin vs placebo; factorial design with aspirin	✓	C	✓	✓
Zhang, 2008 [53] and Albert, 2008 [68] (WAFCS)	Up to 7.3 year factorial trial in 5442 US women (mean age 63 years) with either a history of cardiovascular disease or 3 risk factors	2.5 mg folic acid plus 50 mg B6 plus 1 mg B12 with or without antioxidants vs placebo; factorial design with antioxidants	✓	C,B,L	×	✓
Ebbing, 2008 [42] Ebbing, 2010 [43] (WENBIT)	3.2 year factorial trial, 3096 Norwegian men and women undergoing angiography, mean age 62 years	0.8 mg folic acid plus 0.4mg B12 with or without 40mg B6 vs placebo (without B6)	✓	C	×	✓
Van Uffelen, 2008 [69]	1 year trial, 179 UK elderly with mild cognitive impairment, mean age 75 years	5 mg folic acid plus 0.4mg B12 plus 50mg B6 vs placebo; factorial design with a walking program	×	×	×	✓
Jaszewski, 2008 [70]	3 year trial, 137 US veterans who had had a colorectal adenoma removed, 92% male, mean age 61 years	5mg folic acid vs placebo	×	×	✓	✓
Aisen, 2008 [71]	1.5 year trial in 409 US elderly (mean age 75 years) with probable Alzheimer's disease, mean age 75 years	5 mg folic acid plus 1mg B12 plus 25mg B6 vs placebo	×	×	×	✓
Hodis, 2009 [72] (BVAIT)	3.1 year trial of 506 US men and postmenopausal women, aged 40 years and older, without clinical signs or symptoms of CVD but with elevated homocysteine	5 mg folic acid plus 0.4 mg B12 plus 50 mg B6 vs placebo	✓	×	×	✓
Wu, 2009 [73] (NHS/HPPS Folic Acid Prevention Trial)	5.3 year trial in 672 US nurses and male health professionals with a history of previous colorectal adenoma and who were not B12 deficient, mean age 65 years	1 mg folic acid vs placebo	✓	C,B,P,L	✓	✓
SEARCH Study Group, 2010 [54] (SEARCH)	6.7 year trial, 12,064 UK post-myocardial infarction patients, aged 18-80 years	2 mg folic acid plus 1 mg B12 vs placebo; factorial design with simvastatin	✓	C, P,L	×	✓

Author, Year, (Reference) (Study Name)	Description	Comparison (daily dosage unless otherwise stated)	Data to allow calculation of Relative risks for			
			Total cancer	Site specific cancer	Colo-rectal Ade-noma	Morta-lity
Smith et al, 2010 [74] (VITACOG)	2 year trial, 271 UK patients with mild cognitive impairment, aged 70 years or older	0.8 mg folic acid plus 0.5 mg B12 plus 20mg B6 vs placebo	✓	×	×	✓
VITATOPS Trial Study Group, 2010 [75] and Hankey, 2012 [76] (VITATOPS)	3.4 year trial of 8,164 patients (across 20 countries), post-stroke or TIA, mean age 62 years	2 mg folic acid plus 0.5 mg B12 plus 25mg B6 vs placebo	✓	C,B,P,L	×	✓
Walker, 2010 [77]	2 year trial of 909 Australian adults aged 60-74 years with elevated psychological distress and low physical activity	0.4 mg folic acid plus 0.1 mg B12 vs placebo; factorial design with activity, nutrition and information interventions	×	×	×	✓
Kwok, 2011 [78]	2 year trial in 140 dementia patients in Hong Kong, aged 60 years and older	1 mg folic acid plus 1 mg B12 vs placebo	×	×	×	✓

✓ counts given in paper: × no data: C, B, P, L: counts given for colorectal, breast, prostate and lung cancer respectively

Table 2. Description of trials using folic acid and reporting relevant outcomes

	Bonaa et al, 2006 [41] NORVIT		Ebbing et al, 2008 [42] WENBIT		Ebbing et al, 2009 [43] Extended follow-up of NORVIT and WENBIT combined	
Comparison	**Total incident cancer***	**All-cause mortality**	**Total incident cancer***	**All-cause mortality**	**Total incident cancer***	**All-cause mortality**
Any folic acid plus B12 vs no folic acid, with or without B6	1.23 (0.9-1.7)	1.02 (0.8-1.3)	1.25 (0.9-1.7)	1.27 (0.9-1.8)	1.21 (1.03-1.41)	1.18 (1.04-1.33)
3 B vitamins vs placebo (excludes arms with folic acid plus B12, and B6 alone from analysis)	1.01 (0.6-1.6)	1.21 (0.9-1.6)	1.52 (0.96-2.4)#	1.19 (0.7-2.0)#	1.27 (1.02-1.57)#	1.22 (1.04-1.43)#
Folic acid plus B12 with or without B6 vs placebo (excludes arm with B6 only from analysis)	0.99 (0.7-1.5)#	1.05 (0.8-1.4)#	1.38 (0.9-2.1)#	1.11 (0.98-1.3)#	1.26 (1.04-1.52)#	1.19 (1.03-1.37)#
Folic acid plus B12 vs placebo (excludes arms with 3B vitamins and B6 alone from analysis)	0.98 (0.6-1.5)#	0.90 (0.7-1.2)#	1.29 (0.8-2.1)#	1.30 (0.8-2.1)#	1.25 (1.01-1.55)#	1.16 (0.99-1.37)#

calculated from data provided in the paper

Table 3. Possible comparisons in the two Norwegian studies as reported in the original papers [41, 42] and the combined analysis from the extended follow-up [43]

The two Norwegian trials [41, 42] used a factorial design with some subjects receiving vitamin B6 in addition to placebo or folic acid plus vitamin B12. Therefore the main analysis compares the two arms that received folic acid plus B12 (with or without B6) to the placebo (no B6) arm because this is most similar to the contrast reported by the other trials.

Table 3 shows other possible contrasts that could be considered for the Norwegian studies. There is no consistent pattern between the two trials in which of the possible contrasts had the highest relative risks and there is little difference in the relative risk for any of the possible contrasts in the combined extended follow-up in the trials [43].

Although Zhang et al [53] state that they ascertained the incidence of invasive cancer, rather than all cancer, this trial has been included because the other trials do not provide much detail about their cancer definition. Sensitivity analyses have been done by excluding the results of Zhang et al [53] to determine if they have an important influence on the direction of any effects.

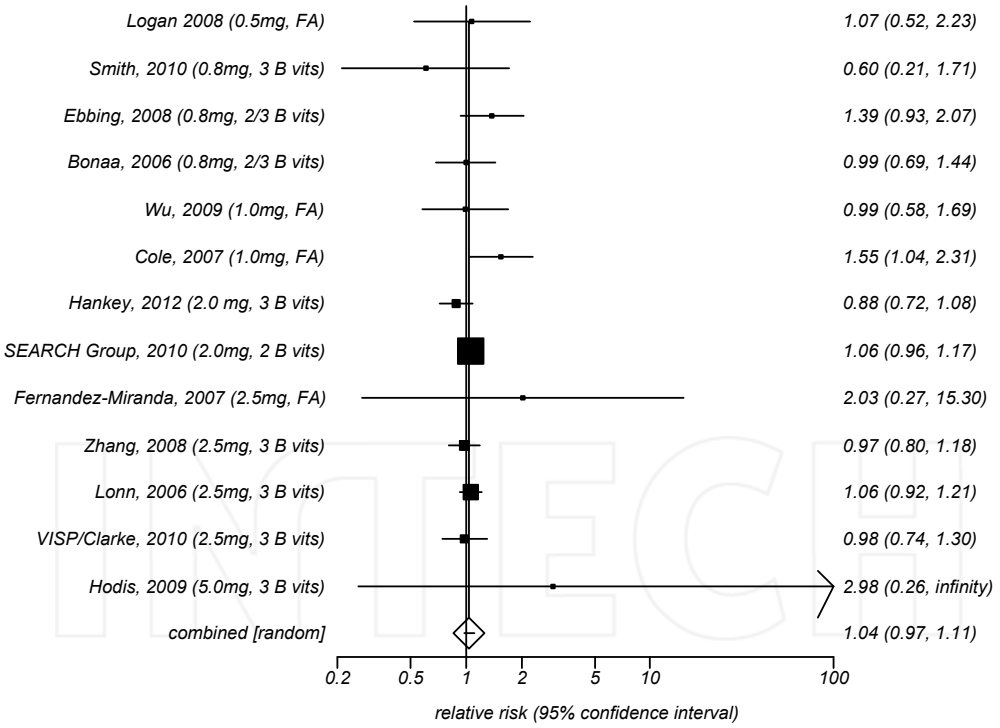

Figure 3. Meta-analysis of trials of the effect of folic acid on total cancer incidence during the trial period, relative risk <1 favours folic acid (Studies ordered by increasing dose of folic acid, amount of folic acid given shown brackets, FA: folic acid administered without other B vitamins, 2 or 3 B: folic acid given with 1 or 2 other B vitamins)

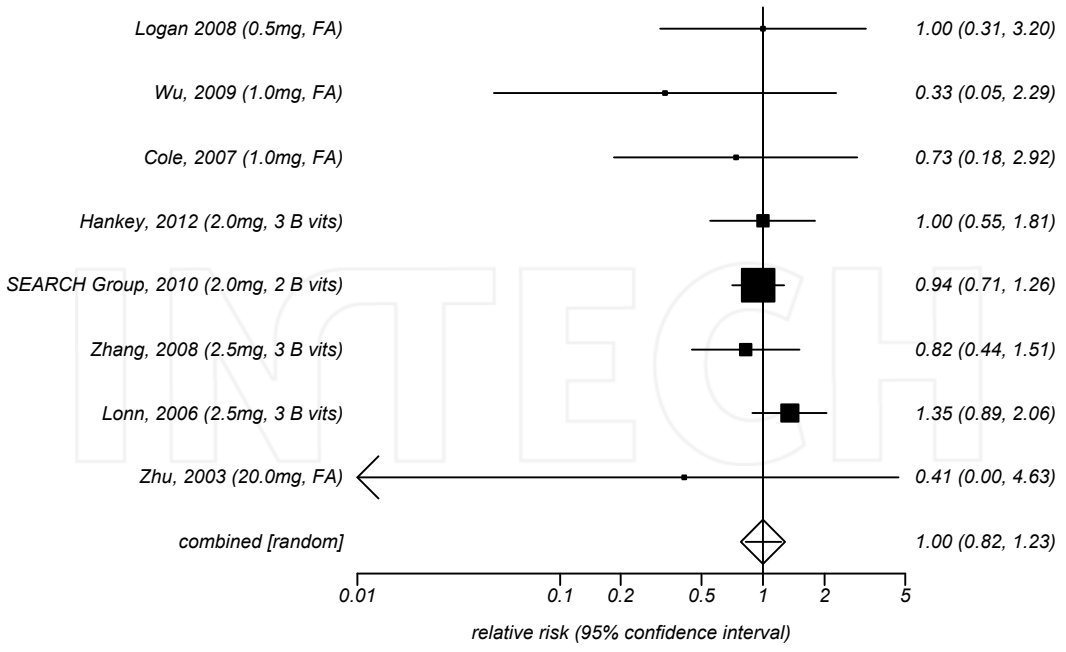

Figure 4. Meta-analysis of trials of the effect of folic acid on colorectal cancer incidence during the trial period, relative risk <1 favours folic acid (Studies ordered by increasing dose of folic acid, amount of folic acid given shown brackets, FA: folic acid administered without other B vitamins, 2 or 3 B: folic acid given with 1 or 2 other B vitamins)

Appendix 1 summarises some of the quality aspects of the included trials relating to main study outcome, rather than the mortality or cancer outcomes which were subsidiary data. The large trials and some of the smaller trials are high quality studies. They describe central randomisation by persons not involved in subject recruitment and so it can be concluded that these trials had masked allocation.

Model	Description	Weighted relative risk (95% CI)	I^2 (95% CI)
0	Figure 3	1.04 (0.97-1.11)	0% (0% to 48.6%)
1	Figure 3 with the longer post-randomisation follow-up of the Norwegian trials [43] substituted for the data reported at trial end [41, 42]	1.05 (0.98-1.14)	12.1% (0%-55.7%)
2	Figure 3 excluding Zhang et al [53]	1.05 (0.98-1.12)	0% (0%-49.8%)
3a	Studies using 0.5-1mg folic acid with or without other B vitamins [5, 41, 42, 52, 74, 73]	1.19 (0.97-1.45)	2.6% (0%-62.0%)
3b	Studies using 2-2.5mg folic acid ([58] reported in [59]), [53, 54, 63, 66, 76]	1.02 (0.96-1.09)	0% (0%-61.0%)
4	Studies using 0.5-1.0mg folic acid alone [5, 52, 73]	1.28 (0.95-1.72)	0% (0%-72.9%)

Table 4. Sensitivity analysis for total incident cancer

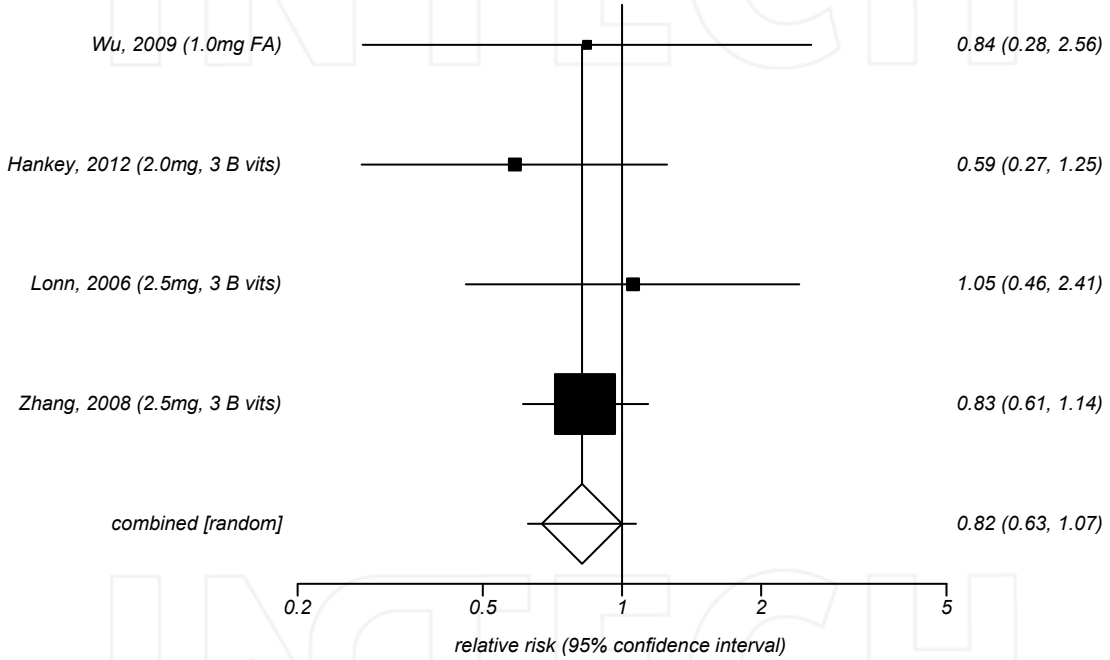

Figure 5. Meta-analysis of trials of the effect of folic acid on breast cancer incidence during the trial period, relative risk <1 favours folic acid (Studies ordered by increasing dose of folic acid, amount of folic acid given shown brackets, FA: folic acid administered without other B vitamins, 3 B: folic acid given with 2 other B vitamins)

These studies generally also had independent and blind outcome assessment committees. Only one study used a minimal dose of folic acid (20ug/day) in the control group [58]. Two trials stated that they were open label studies [60,61]. One study stated that it was double-blind although this statement appears to apply to non-folic acid arms in the trial because the folic acid group received monthly intra-muscular injections of vitamin B12 for the first year and the control group did not [57]. The lower quality studies are small in relation to the high quality studies and so have a low influence in the overall analyses.

3.2.1 Incidence of all cancer combined

When combined together, the 13 trials (43,557 subjects) yield a non-significant overall relative risk of 1.04 (95% CI: 0.97-1.11) (Figure 3, Table 4). For all studies together, I^2 was 0% (95% CI: 0-48.6%) indicating that variation among the results of the studies can be attributed to chance.

The two Norwegian studies reported a longer follow-up for their subjects [43] after the trials had finished. Substituting these results for the results at the end of the trials [41, 42] made little difference to the result (RR=1.05).

Model	Description	Weighted relative risk (95% CI)	I^2 (95% CI)
Colorectal cancer			
0	Figure 4	1.00 (0.82-1.23)	0% (0%-56.3%)
1	Figure 4 plus the post-randomisation follow-up from the two Norwegian trials [43]	1.01 (0.84-1.22)	0% (0%-54.4%)
2a	Studies using 0.5-1.0mg folic acid without other B vitamins [5, 52, 73]	0.76 (0.32-1.82)	0% (0%-72.9%)
2b	Studies using 0.5-1.0mg folic acid with or without other B vitamins [5, 52, 73] plus the post-randomisation follow-up from the two Norwegian trials [43]	0.99 (0.64-1.53)	0% (0%-67.9%)
2c	Studies using 2.0-2.5mg folic acid with or without other B vitamins [53, 54, 63, 76]	1.02 (0.83-1.26)	0% (0% to 67.9%)
3	Figure 4 excluding Zhu et al [57]	1.02 (0.84-1.23)	0% (0%-56.3%)
4	Figure 4 excluding Zhang et al [53]	1.04 (0.85-1.26)	0% (0%-56.3%)
Breast cancer			
0	Figure 5	0.82 (0.63-1.07	0% (0%-67.9%)
1	Figure 5 excluding Zhang et al [53]	0.78 (0.47-1.31)	0% (0%-72.9%)
2	Figure 5 assuming that men in Lonn et al [63] and Hankey et al [76] should be included in the denominators	0.82 (0.63-1.07)	0% (0-67.9%)
Prostate cancer			
0	Figure 6 – results during the trial	1.16 (0.85-1.60)	52.7% (0%-80.7%)
1	Figure 6 substituting the longer follow-up of Figueiredo et al [44] for Cole et al [5] plus the post-randomisation follow-up from the two Norwegian trials [43]	1.17 (0.91-1.49)	43.9% (0%-76.2%)
2	Studies using 1.0 mg folic acid without other B vitamins [5, 73]	1.56 (0.45-4.93)	Too few strata to calculate
3	Studies using 2.0-2.5mg folic acid with other B vitamins [54, 76, 63] plus the post-randomisation follow-up from the two Norwegian trials [43]	1.12 (0.93-1.35)	16.4% (0%-72.9%)
4	Studies using 1.0 mg folic acid or less with or without other B vitamins using the longest follow-up reported [43, 44, 73]	1.40 (0.74-2.63)	58.2% (0%-86.3%)
Lung cancer			
0	Figure 7 – results during the trial	1.00 (0.84-1.21)	0% (0%-64.1%)
1	Figure 7 plus the post-randomisation follow-up from the two Norwegian trials [43]	1.04 (0.88-1.24)	0% (0%-61.0%)
2	Figure 7 excluding Zhang et al [53]	1.00 (0.82-1.21)	0% (0%-67.9%)

Table 5. Sensitivity analysis for site-specific cancer analyses

Excluding Zhang et al [53] which ascertained only invasive cancers makes little difference. When divided according to the dose of folic acid given, the overall relative risk was 1.19 in studies giving 0.5-1mg and 1.02 in studies giving 2-2.5mg but the 95% confidence intervals of the combined effect of the higher dose studies lies within the 95% confidence interval of the lower dose. The single study using a higher dose [72] is small in relation to the other studies and has a 95% confidence interval that includes the confidence intervals of all the other studies (Table 4).

3.2.2 Colorectal cancer incidence

Fewer trials have reported site-specific cancer incidence than have reported total cancer incidence. Among the eight trials (33,922 subjects) that have reported colorectal cancer incidence during trial follow-up, there is an overall relative risk of 1.00 (95% CI: (0.82-1.23) (Figure 4). I^2 was 0% (95% CI = 0-56.3%) indicating that variation in the results among the studies can be attributed to chance. Including the colorectal cancers reported in the post-trial follow-up of the two Norwegian studies does not alter these results importantly (RR=1.01) (Table 5).

Excluding the Chinese study [57] because the nutritional status of that population may be different and which was not blinded or Zhang et al [53] which ascertained only invasive cancers makes little difference to the results (Table 5). The sensitivity analyses (Table 5) show that there is extensive overlap in the confidence intervals if the studies are examined by dose, or if studies using folic acid alone are considered.

3.2.3 Breast cancer incidence

Of the four trials reporting breast cancer data (Table 2), one had only female participants [53] and another specified that the breast cancers occurred in women [73]. The other two trials did not specify whether the breast cancer data are for women only [63, 76]. Because breast cancer rarely occurs in men, the primary analysis (Figure 5) assumes that all breast cancer cases in all trials occurred in women and includes only women in the denominators. The four trials (10,361 female subjects) yield a combined relative risk of 0.82 (95% CI: 0.63-1.07) (Figure 5, Table 5). I^2 is 0% which indicates that the variation in results among the studies can be attributed to chance (Table 5).

As noted above, Zhang et al [53] specifically state that they ascertained invasive cancer whereas the other reports do not specify this; excluding their results reduces the overall relative risk to 0.78 (Table 5). Including men from Lonn et al [63] and Hankey et al [76] in the relevant denominators does not alter the overall conclusion (Table 5).

3.2.4 Prostate cancer incidence

Five trials have reported the incidence of prostate cancer during the trial (Table 2). The combined relative risk is 1.16 (95% CI: 0.85-1.60) for these five studies (20,094 male subjects) (Figure 6). There is moderate heterogeneity in the results across the trials which might indicate important differences in participant or other characteristics among the trials.

Additional results beyond the period of the trial are available for Cole et al [5] reported in a subsequent paper [44]. The two Norwegian cardiovascular trials reported combined data for subjects in a follow-up that extended after the trial ceased [43]. Because the longer term follow-up result of Figuerido et al [44] is similar to the in-trial result reported by Cole et al [5] and the combined result of the two Norwegian trials [43] is similar to the primary analysis (Figure 6) using the post-trial follow-up data makes little difference to the analysis (RR= 1.17; 95% CI: 0.91-1.49). The confidence intervals for both summary relative risks exclude the relative risk of 2.59 reported by the Cole trial; therefore the results of the sensitivity analyses depend on whether they include the trial of Cole et al [5] (Table 5).

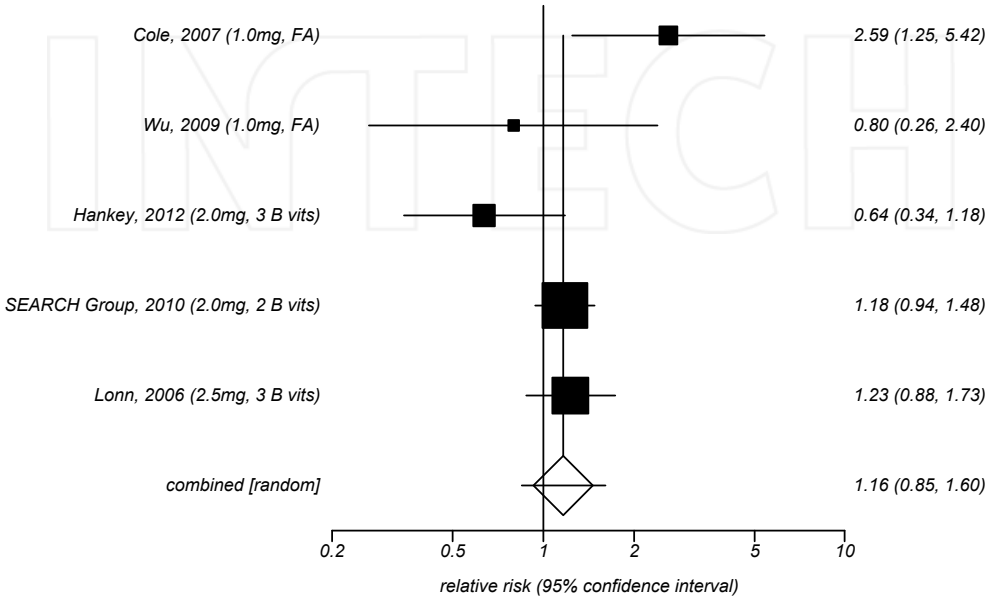

Figure 6. Meta-analysis of trials of the effect of folic acid on prostate cancer incidence during the trial period, relative risk <1 favours folic acid (Studies ordered by increasing dose of folic acid, amount of folic acid given shown brackets, FA: folic acid administered without other B vitamins, 2 or 3 B: folic acid given with 1 or 2 other B vitamins)

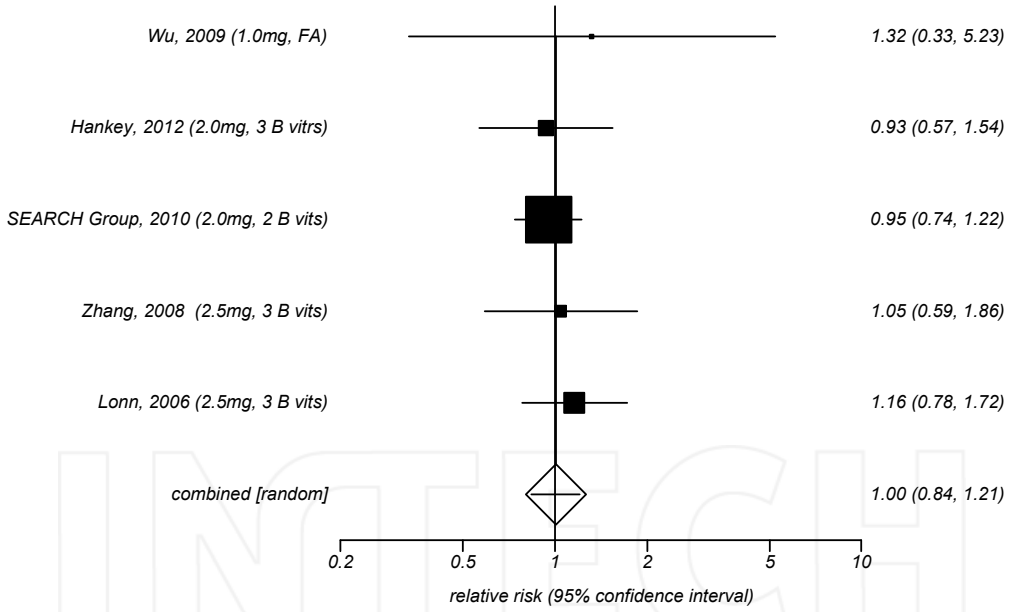

Figure 7. Meta-analysis of trials of the effect of folic acid on lung cancer incidence during the trial period, relative risk <1 favours folic acid (Studies ordered by increasing dose of folic acid, amount of folic acid given shown brackets, FA: folic acid administered without other B vitamins, 2 or 3 B: folic acid given with 1 or 2 other B vitamins)

Model	Description	Weighted relative risk (95% CI)	I^2 (95% CI)
0	Figure 8	0.99 (0.92-1.05)	11.4% (0%- 46.8%)
1	Figure 8 substituting the post-randomisation follow-up from the two Norwegian trials [43] for the in-trial results	1.00 (0.92-1.08)	28.9% (0%-57.0%)
2a	Studies using 0.4-1.0mg folic acid without other B vitamins [5, 52, 61, 67, 73]	0.64 (0.43-0.94)	14.4% (0%-69.0%)
2b	Studies using any dose of folic acid without other B vitamins [5, 52, 60, 61, 66, 67, 70, 73]	0.87 (0.53-1.41)	56.6% (0%-78.4%)
3a	Studies using 0.4-1mg folic acid with or without other B vitamins [5, 41, 42, 52, 61, 67, 73, 74, 77]	0.86 (0.64-1.15)	38.7% (0%-70.4%)
3b	Studies using 2.0-2.5mg folic acid with or without other B vitamins [53, 54, 58, 63, 64, 66, 75]	0.99 (0.94-1.05)	0% (0%-58.5%)
3c	Studies using 5.0 mg folic acid with or without other B vitamins [60, 62, 69, 70, 71, 72, 78]	1.15 (0.70-1.89)	22.3% (0% to 67.1%)

Table 6. Sensitivity analysis for all-cause mortality

3.2.5 Lung cancer incidence

Five studies (31,864 subjects) reported incident lung cancer data during the trial (Table 2) and two Norwegian cardiovascular trials have reported combined data for subjects in a follow-up that extended two years after the trial ceased.

Among the five trials with in-trial results (Figure 7) there was no effect on lung cancer incidence (RR=1.00; 95% CI: 0.84-1.21). I^2 was 0% (95% CI: 0-64.1%) indicating that variation among the results of the studies can be attributed to chance. Including the lung cancers reported in the post-trial follow-up of the two Norwegian studies increased the relative risk slightly (RR=1.04).

3.3 All-cause mortality

More studies have reported all-cause mortality than cancer outcomes (Table 2). Although Liem et al [60] report cardiovascular mortality in their paper their data are included because there were no non-cardiovascular deaths (A Liem, personal communication). The number of deaths used to analyse three trials [5, 52, 77] differ from some other reports owing to clarifications provided by the authors (L Mott, personal communication; R Logan, personal communication, J Walker, personal communication).

In 23 studies with 47,993 subjects, the overall risk of mortality is 0.99 (95% CI: 0.92-1.05; Figure 8). I^2 is 11.4% which indicates low heterogeneity among the studies

(Table 6) and that most of the variation in results among the studies can be attributed to chance. There is no indication of publication bias in the funnel plot.

Substituting the longer post-trial follow-up data from the two Norwegian studies for their in-trial results does not change the results of the overall analysis importantly (RR=1.00) (Table 6). When divided into dose categories, there appears to be a protective relationship with doses of 1mg folic acid or less reducing death risk, no relationship for 2-2.5mg folic acid and in increase in mortality in the studies using 5mg although the confidence intervals for the three groupings overlap (Table 6).

The studies in Table 6 were conducted in various countries with a range of folic acid fortification policies. Three studies were conducted in Australia and New Zealand after voluntary fortification with folic acid was introduced but before mandatory fortification was introduced in Australia. The lowest dose of folic acid used in these studies, 0.4mg/day, with vitamin B12, was given in the trial of Walker et al [77] conducted in Australia between October 2005 and September 2008. Flicker et al [64] recruited subjects in Western Australia between 2001-2004 and VITATOPS, which included subjects from Australia and New Zealand, was conducted between November 1998 and December 2008 [75].

3.4 Trials investigating folic acid and recurrence of colorectal adenoma

The relationship between the recurrence of colorectal adenoma (a lesion which may progress to colorectal cancer over time, in some people) and folic acid has been examined in six trials (Table 2). The methods used in three of these studies are not reported in great detail [55, 56, 70]

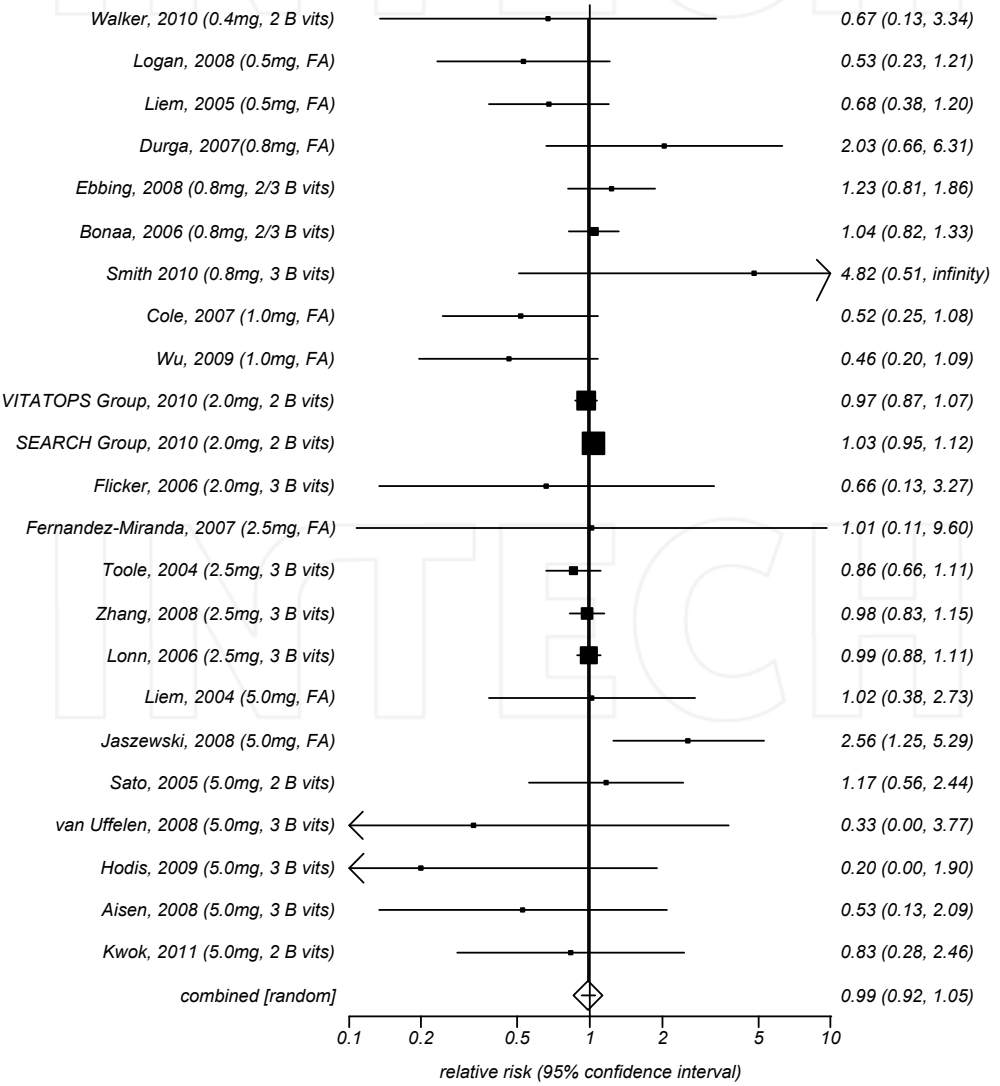

Figure 8. Meta-analysis of trials examining the effect of folic acid on all-cause mortality during the trial period, relative risk <1 favours folic acid (Studies ordered by increasing dose of folic acid, amount of folic acid given shown brackets, FA: folic acid administered without other B vitamins, 2 or 3 B: folic acid given with 1 or 2 other B vitamins)

In two trials [5, 55], the recurrence is reported separately for two time periods. In these studies, it is not clear whether individuals who experienced a recurrence in the first period also had a recurrence in the second period. Consequently the total number of individuals experiencing one or more recurrent colorectal adenomas during the follow-up cannot be determined from the data given in the papers. Additional adjustments made little difference to the results for the second period [79]. By contrast, Wu et al [73] do report the number of subjects who had a recurrence at any of their three follow-ups. Therefore the data for the first year from Paspatis et al [55] and the first three years from Cole et al [5] are used in the primary analysis (Figure 9). Count data for the trial of Jaszewski et al [70] was obtained from the authors (A Majumdar, personal communication).

The trial by Cole et al [5] has attracted particular attention because a longer follow-up in a subset of the subjects found an increase in adenoma recurrence (Table 7). There were two follow-up periods: more than 96% had a colonoscopy to identify recurrent adenoma in the first three years whereas fewer than 60% had a colonoscopy at the end of the second follow-up period, 4-6 years after randomisation. Owing to the high non-colonoscopy rate it is not clear whether the adenoma results of the second period can be generalised to other populations. (However, vital status and cancer incidence was assessed on virtually all those initially randomised at the end of the study; see other tables in this report).

Period of observation	Folic acid		Placebo		**RR (95% CI)**
	N	% with any adenoma	N	% with any adenoma	
Baseline to 1st colonoscopy	501	44.1	486	42.4	1.04 (0.9-1.2)
Baseline to 2nd colonoscopy	303	71.3	304	65.5	1.09 (0.98-1.2)
1st colonoscopy to 2nd colonoscopy#	303	41.9	304	37.2	1.13 (0.9-1.4)

includes subjects who had a first recurrent adenoma between baseline and 1st colonoscopy

Table 7. Recurrence of adenoma during different follow-up periods in the trial of Cole et al [5] in the whole population for the first follow-up period and the sub-population having colonoscopies in two follow-up periods

Figure 9 shows the combined results of these six trials. (The relative risk for the trial of Cole et al differs from that reported by the authors (Table 7) because data were extracted from all studies using the same method and so unadjusted results were calculated in the meta-analysis). Administration of 0.5-5 mg folic acid decreased the recurrence of any adenoma by 3% (RR=0.97, 95% CI: 0.83 to 1.14) over 1-7 years of follow-up in the trials. The combined relative risk for any adenoma in the three well reported studies, which lasted 3-7 years, was 1.03 (95% CI: 0.92 to 1.15), or an increase of 3% (Table 8). The relative risk for advanced adenoma in the same three studies was 1.11 (95% CI: 0.87 to 1.42) (Figure 10, Table 8).

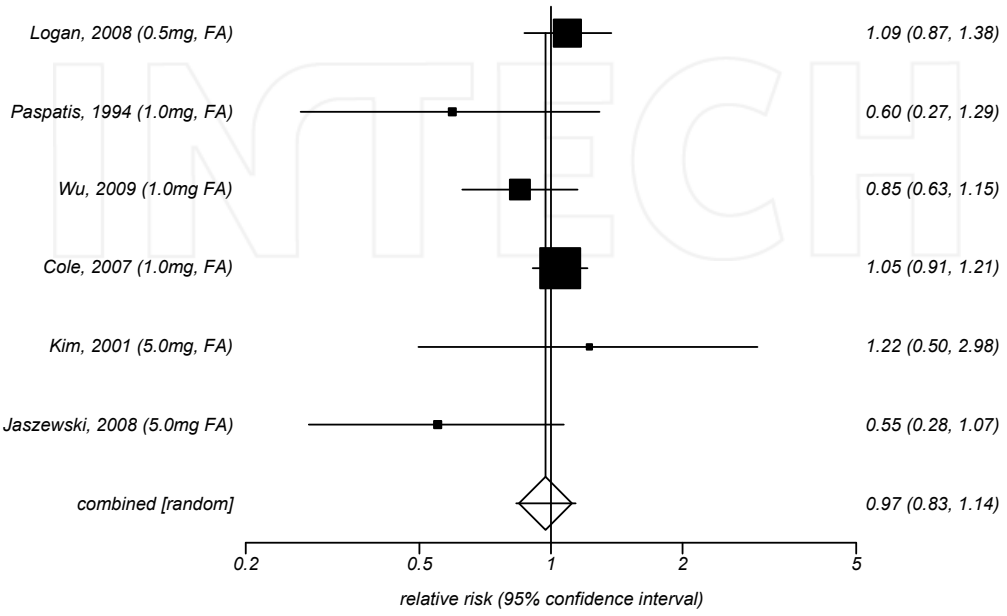

Figure 9. Meta-analysis of trials of folic acid and recurrence of colorectal adenoma, relative risk <1 favours folic acid (Studies ordered by increasing dose of folic acid, amount of folic acid given shown brackets, FA: folic acid administered without other B vitamins)

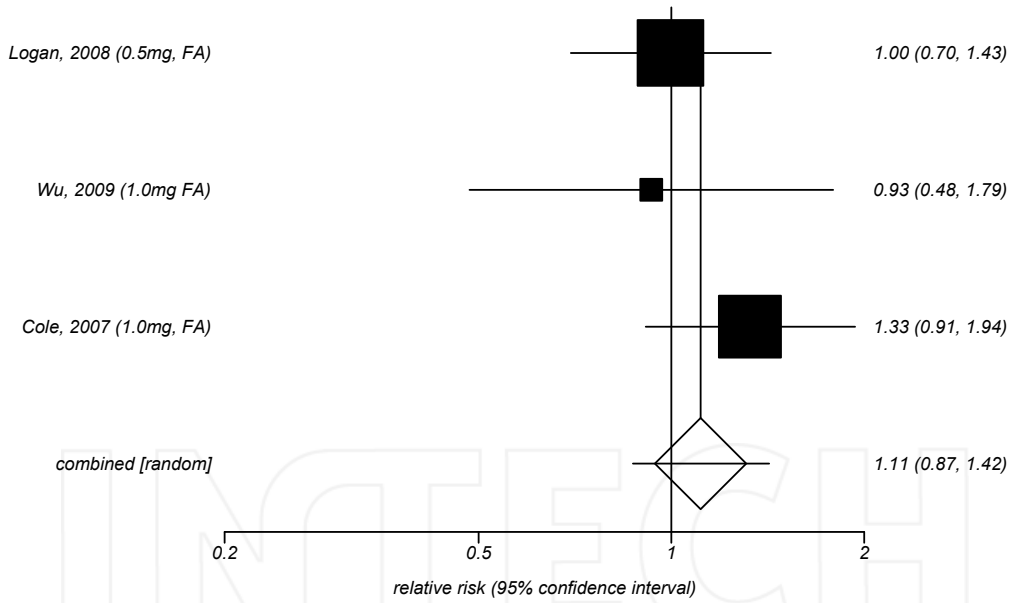

Figure 10. Meta-analysis of trials of folic acid and recurrence of advanced colorectal adenoma, relative risk <1 favours folic acid (Studies ordered by increasing dose of folic acid, amount of folic acid given shown brackets, FA: folic acid administered without other B vitamins)

Model	Description	Weighted relative risk (95% CI)	I^2 (95% CI)
0	Any adenoma: all studies (Figure 9)	0.97 (0.83-1.14)	26.8% (0%-70.6%)
1	Any adenoma: well-reported studies [5, 52, 73]	1.03 (0.92-1.15)	0% (0%-72.9%)
2	Advanced adenoma (Figure 10) (data given only in the well-reported studies [5, 52, 73])	1.11 (0.87-1.42)	0% (0%-72.9%)

Table 8. Sensitivity analysis of studies of recurrence of colorectal adenoma

First author (Reference) (folic acid/day)	N	Endpoint	RR (95% CI)
Song [80] (2.5mg)	1470	Any adenoma	1.00 (0.83-1.20)
		Advanced adenoma	1.06 (0.76-1.50)

Table 9. Results for a trial of the effect of folic acid on occurrence of any colorectal adenoma and advanced colorectal adenoma at any follow-up endoscopy, up to 7.3 years of follow-up [80]

In late 2012, Song et al [80] reported results from the WAFACS trial [53, 68] which has been included in all analyses above except prostate cancer (all participants in WAFACS were women, Table 2). This trial did not include an endoscopy as part of the trial procedures but took advantage of subject reports that the procedure had occurred during the trial to collect information about the occurrence of colorectal adenoma during the trial. As the trial was double-blind, the assignment to folic acid or placebo should not have influenced the subjects' decision to have an endoscopy. Although the results (Table 9) cannot be included in the meta-analysis above because subjects did not have all colorectal adenomas removed prior to the trial, they are consistent the finding of no effect on recurrence (Figure 9) and raise the possibility that the risk of recurrence of advanced adenoma might have been overestimated (Figure 10). The risk of 2 or more adenomas, which was reported for the first follow-up endoscopy only, was lower in the folic acid group than the placebo group (RR=0.93 (0.61-1.43) [80].

4. Discussion

Overall, there is no effect on total mortality (RR=0.99) in those taking up to 5mg folic acid/day for up to seven years. The relative risk was slightly elevated for total cancer (RR=1.04). There was a stronger elevation for prostate cancer (RR=1.16) and a decrease for breast cancer (RR=0.82). There was no effect on the incidence of colorectal or lung cancer (RR=1.00). The majority of participants in the large cardiovascular trials were men except for the study of Zhang et al [53]. Consequently total cancer incidence in these studies reflects conditions in men and does not necessarily reflect what would be seen in the general population. There was little effect on the recurrence of any colorectal adenoma (RR=0.97) although there was an increase in recurrence of advanced adenoma (RR=1.11) in those who had had a prior colorectal adenoma removed.

None of these results are statistically significant at the customary level ($p<0.05$). However, this has been a living meta-analysis conducted over some years and so the alpha to declare statistical significance should be corrected for multiple comparisons and repeated analysis over time. This correction would reduce the alpha for declaring statistical significance (e.g. <0.01 or lower). This should also be considered for any future updates.

The actual interventions used varied among the trials. Most trials are indirect tests of folic acid fortification in Australia because there is mandatory co-fortification of the same flour with thiamin [1] but not other nutrients. Some trials gave a combination of folic acid, vitamin B12 and vitamin B6, others used folic acid and vitamin B12 and yet others administered folic acid alone. Some studies were conducted in countries that had mandatory fortification with folic acid and others were not. Most of the data come from the large cardiovascular trials which used doses of 0.8-2.5mg/day (with co-administration of vitamin B12 at least) although the range was 0.4-20mg/day). These doses are about 10-fold higher than the mean additional folic acid intake intended with mandatory fortification in Australia. Vollset et al [17] found that the mean serum folate concentration in the large trials (Table 10) was 13.5 and 57.3nmol/L in the placebo and intervention (average 2mg folic acid/day) groups respectively. The impact of fortification in Australia has been commensurately lower. Brown et al [81] found mean serum folate concentrations of 17.7nmol/L and 23.1nmol/L in a large sample of Sydney blood donors in April 2009 and 2010 respectively.

There is no trial examining the effect of widespread fortification of food on cancer rates. Population-based time trend studies are complicated by changes in screening practices and diagnostic coding. For example, fecal occult blood testing is decreasing but endoscopy and use of colorectal test results is increasing in the US [82]. Mason et al [6] suggested that the three-year change in direction of colorectal cancer incidence between 1995-1998 in the US was due to mandatory fortification with folic acid which was optional from late 1996 and fully implemented in late 1998. There was a decline in colorectal cancer in both men and women in the 10 years prior and following this period [82]. The annual increase in the three year period was similar to the annual decline in the period before and after it. If this temporary increase is due to mandatory fortification with folic acid, then an effect should have been observed in the meta-analysis of trials reporting colorectal cancer (Figure 4), especially as they used a much greater dose of folic acid and most lasted longer than three years. Vollset et al [17] comment that, as there was no increase in colorectal cancer mortality in the US, the increase in incidence is more likely to be due to increased diagnosis rather than reflecting a true increase in incidence. A time trend study of hospital discharge rates for colorectal cancer from Chile [83] is difficult to interpret owing to non-correction of the time scale for missing years of data in the trend graph. This is further impeded because the missing data were for the years immediately prior and following the introduction of mandatory folic acid fortification in that country.

It is mandatory to report all cancers (except certain types of skin cancer) in all Australian states and territories. Data are available from 1982 onwards (http://www.aihw.gov.au/cancer/aacr/, accessed 5th July, 2013). There was little difference in the age-standardised incidence of bowel cancer between 1982-2006 in men or women, with the highest incidence occurring in 2000-1 [7]. However, the National Bowel Screening Program (http://www.cancerscreening.gov.au/internet/screening/publishing.nsf/Content/bowel-about, accessed 5th July, 2013) was introduced at a similar time to mandatory fortification with folic acid. This Program would be expected to increase apparent incidence of colorectal cancer due to increased diagnosis. Any attempt to attribute any changes in bowel cancer incidence to fortification would be further complicated by the reissue of test kits to certain participants in 2009 and ongoing expansion of the program to additional age groups scheduled for 2013 and 2015.

4.1 Comparison to other meta-analyses

The question of whether there is a relationship between folic acid and cancer has been examined in recent years by regulatory agencies [38, 39] and researchers. Table 10 compares previous meta-analyses of folic acid and cancer and Table 11 compares previous meta-analyses of folic acid and mortality to the results of the current review. Only two of the earlier meta-analyses have examined both cancer and all-cause mortality. Reviews are shown in order according to the final date of their literature search. None of the previous meta-analysts had goals exactly overlapping with the goal of the FSANZ analysis and their literature searches were completed at different dates. Several of the other meta-analyses have restricted their searches to find studies of homocysteine lowering or with cardiovascular outcomes or with at least 500 subjects. Some have included studies that were excluded in the current meta-analysis: studies conducted in patients with chronic renal failure, diabetic neuropathy or with transplants or which lasted for less than one year. Some have included the trial testing 5MTHF [13] which was excluded in the current analysis because 5MTHF is not a permitted alternative to folic acid for mandatory fortification in Australia [1, 12]. None of the other meta-analyses have included the trials testing the effect of folic acid on cognitive outcomes which were included in the current analysis (primarily the mortality meta-analysis). Another difference between meta-analyses is the decision about how to handle the two Norwegian studies (Table 3)

First Author, year (reference)	Inclusion criteria and search date	RR (95% CI)					Primary differences compared to present review, apart from differences due to search date
		Total cancer	Colorectal cancer	Breast cancer	Prostate cancer	Lung cancer	
Carroll, 2010 [19]	Trials of colorectal cancer or recurrence of colorectal adenoma, Search performed in June 2008	-	1.13 (0.77-1.64) N=3	-	-	-	
Marti-Carvajal, 2009 [23]	Trials of homocysteine lowering interventions with CVD outcomes lasting one year or longer, renal failure excluded. Search performed up till August/September 2008	1.06 (0.9-1.25) N=3	-	-	-	-	* VISP [58] which used a placebo containing 0.02mg folic acid was analysed in a separate stratum
Clarke, 2010 [59]	Double blind trials of folic acid on vascular disease, at least 1000 participants & at least one year follow-up & completed by the end of 2009.	1.05 (0.98-1.13) N=7	-	-	-	-	Included: * 1 study in renal patients
Wien 2012 [86], Pike 2011 [37]	>0.4mg folic acid/day, renal failure etc included Final search on 6 May 2010#	1.07 (1.00-1.14) N=10	1.00 (0.83-1.21) N=9	0.86 (0.64-1.14) N=3	1.24 (1.03-1.49) N=6	1.11 (0.92-1.33) N= 6	Included: * 1 study in renal patients * 1 trial in pregnant women with <1 year duration of folic acid use
Baggott, 2012 [85]	Final search performed in June 2010	1.21 (1.05-1.39) N=6	-	-	-	-	* Weighted analysis by study duration instead of the inverse variance * Excluded Zhang et al [53] because it included only women from a location with high baseline folate levels
Vollset 2013 [17]	Pooled analysis of trials with at least 500 subjects and minimum 1 year duration, completed before 2011 (extension of Clarke et al [59])	1.06 (0.99-1.13) N=13	1.07 (0.83-1.37) N=13	0.89 (0.66-1.20) N=13	1.15 (0.94-1.41) N=13	1.08 (0.86-1.35) N=13	Included: * 1 study in renal patients * 5MTHF trial *previously unreported results for a number of studies * post-trial follow-up for 2 trials

Marti-Cavajal, 2013 [22]	Extension of Marti-Cavajal et al [22, 23], final search February 2012	1.06 (0.98-1.13) N=12	-	-	-	-	Included * 5MTHF trial
Present review (in trial follow-up)	See elsewhere Final search performed 16 May 2013	1.04 (0.97-1.11) N=13	1.00 (0.82-1.23) N=8	0.82 (0.63-1.07) N=4	1.16 (0.85-1.60) N=5	1.00 (0.84-1.21) N=5	N/A

Table 10. Comparison of FSANZ results to other meta-analyses of trials of folic acid and cancer incidence

Author	**Inclusion criteria and search date**	**Total mortality RR (95% CI)**	**Primary differences compared to present review, apart from differences due to search date**
Bazzano 2006 [18]	Trials with CVD outcomes. Search performed up to July 2006	0.96 (0.88-1.04) N=10	Included: * 2 studies < 1 year * 3 studies in renal patients
Marti-Carvajal, 2009 [23]	See Table 10	1.0 (0.92-1.09) N=6	* VISP [58] which used a placebo containing 0.02mg folic acid was analysed in a separate stratum
Clarke, 2010 [59]	See Table 10	1.02 (0.97-1.08) N=7	Included: * 1 study in renal patients
Huang, 2012 [34]	Trials investigating cardiovascular outcomes, no restriction on duration; final search November 2010	0.99 (0.95-1.04) N=15	Included: * 1 study < 1 year * 5 studies in renal patients
Yang, 2012 [35]	Trials investigating cardiovascular outcomes, allowed multivitamins, minimum duration 6 months, searched 1966 to May 2012	1.00 (0.96-1.04) N=24	Included * 2 studies < 1 year * 5MTHF trial * 2 studies in transplant patients * 1 study in renal patients * 1 multivitamin study
Marti-Cavajal, 2013 [22]	See Table 10	1.01 (0.96-1.07) N=10	Included * 5MTHF trial Excludes VISP [58] (see Marti-Cavajal, 2009 above)
Present review	See elsewhere Final search performed 16 May 2013	0.99 (0.92-1.05) N=23	N/A

Table 11. Comparison of FSANZ results to other meta-analyses of trials of folic acid and all-cause mortality

Both were factorial designs testing folic acid and vitamin B12 together versus placebo and vitamin B6 versus placebo. Some have combined the B6 arm with the placebo arm in their meta-analysis whereas this arm was deleted in the current meta-analysis. As illustrated above, there is the further decision about whether to use the in-trial results [41, 42] or the longer post-trial follow-up [43] for some outcomes of these studies.

Some other meta-analyses have subdivided their analyses into countries which do and do not have mandatory fortification with folic acid. This is a somewhat artificial distinction because in some countries, widespread voluntary fortification and/or supplement use could lead to folic acid intakes that are as high that achieved by mandatory fortification in other countries [84].

Author (date)	Search date	Any adenoma recurrence RR (95% CI)	Advanced adenoma RR (95% CI)	Studies included in the any adenoma analysis, comments
Carroll, 2010 [19]	June 2008	0.93 (0.61-1.41) N=3	-	Cole, 2007 [5] Logan, 2008 [52] Jaszewski, 2008 [70]
Fife, 2011 [87]	July 2008	Short follow-up 1.07 (0.88-1.30) N=2 Long follow-up 1.22 (0.88-1.69) N=1	Short follow-up 1.14 (0.85-1.53) N=2 Long follow-up 1.35 (1.06-1.70) N=2	Cole, 2007 [5] Logan, 2008 [52] Includes the colorectal cancer data of Lonn et al [63] as advanced adenoma for the long follow-up but does not include colorectal cancers from Cole et al [5]
Figueiredo, 2010 [88]	Not given	0.98 (0.82-1.17) N=3	1.06 (0.81-1.39) N=3	Cole, 2007 [5] Logan, 2008 [52] Wu, 2009 [73] Number seen at follow-up used as the denominator to calculate risks
Wien 2012 [86], Pike, 2011 [37]	10 February, 2011	0.97 (0.83-1.14) N=6	1.17 (0.89-1.56) N=3	Paspatis, 1994 [55] Kim, 2001 [56] Cole, 2007 [5] Logan, 2008 [52] Jaszewski, 2008 [70] Wu, 2009 [73]
Present review	16 May 2012	0.97 (0.83-1.14) N=6	1.11 (0.87-1.42) N=3	Paspatis, 1994 [55] Kim, 2001 [56] Cole, 2007 [5] Logan, 2008 [52] Jaszewski, 2008 [70] Wu, 2009 [73]

Table 12. Comparison of FSANZ results to other meta-analyses of trials of folic acid and colorectal adenoma recurrence

Vollset et al [17] is an extension of Clarke et al [59]. These were pooled analyses in which the authors obtained original data from trial investigators and were able to ensure that all cancer definitions were consistent across studies. Consequently some of the numbers of outcomes reported by Vollset et al [17] differ from the original papers. They used the in-trial, not the post-trial extended, results for the two Norwegian studies and the Cole study, and results for another trial which had not been reported previously [48]. (As their appendices do not include trial-specific data for all the outcomes of interest, the data from the original papers was used in the current analysis for consistency.) Availability of original data also meant that Vollset et al [17] could calculate rate ratios using person-time data rather than risk ratios using the numbers randomised and this would explain some of the differences in their results compared to the current study. Despite these differences in methods compared to other meta-analysts, their results are very similar.

Previous meta-analyses of total cancer incidence report relative risks ranging between 1.04 and 1.07 except for Baggott et al [85] who report a relative risk of 1.21 (Table 10). This difference is due to their decision to weight their analysis according to the duration of the study [85] rather than the more customary inverse variance method used in other reviews and by FSANZ. If the six studies included by Baggott [85] are re-analysed using the inverse variance as the weighting, then the effect is similar to the other meta-analyses (RR=1.08, 95%CI: 1.0-1.17).

The first meta-analysis of colorectal cancer [19] included only three studies [53, 63, 57]. Subsequent meta-analyses with additional studies found lower relative risk. Similarly, the increasing number of studies has lowered the overall result for prostate cancer compared to that found by Wein et al [86].

The lack of an effect of folic acid on all-cause mortality has changed little over time despite the variation in the number of trials included across the different meta-analyses. All the previous meta-analysts have focused their search to find studies of homocysteine-lowering or reporting cardiovascular outcomes (Table 11). Consequently, they have excluded trials investigating the effects of folic acid on cognitive function or recurrent colorectal adenoma. However, the common set of large cardiovascular trials included by all recent meta-analysts is the main driver of the results.

The number of trials included in prior meta-analyses of the recurrence of colorectal adenoma is determined by the date of the literature search and whether the more obscure trials were found (Table 12). As would be expected from the similarity in results of most trials (Figure 9, Figure 10), there was little variation in the recurrence of any adenoma across the meta-analyses despite differences in which studies were included. An exception is the results for long followup of any and advanced adenoma by Fife et al [87]. Unlike other authors, Fife et al [87] classed colorectal cancer as a type of advanced adenoma. Although they counted colorectal cancers from Lonn et al [63] as adenoma in their analysis, they did not include the colorectal cancers reported by Cole et al [5] and Logan et al [52] despite including the adenoma data from these two trials.

Overall, the similarity in the results of the various meta-analyses is more striking than the differences. The similarity is due to the inclusion of a core set of large studies in most analyses. The decision of different authors to limit their search strategies in different ways has less influence because the remaining studies have smaller numbers of outcomes and so have lower weighting in the overall results.

4. Future data

There are a number of trials testing folic acid on a range of outcomes listed in trial registries. Given the number of subjects included in the meta-analyses to date and the low incidence of the cancers of greatest interest, future trials would need to have a large number of events and a relative risk that is substantially different from the overall relative risk in current meta-analyses to alter the overall result when their results are added to a meta-analysis. One such trial might be the China Stroke Primary Prevention Trial [NCT00794885] which is testing the addition of folic acid 0.8 mg daily to 10mg enalapril in 19,000 Chinese people with primary hypertension for five years. Future meta-analyses will also need to consider how to correct tests of significance for multiple testing over time.

5. Conclusion

This analysis of one aspect of the hazard characterisation of folic acid is part of a larger initiative to monitor the effects of mandatory fortification of wheat flour for bread making with folic acid in Australia. A number of randomised controlled trials have been conducted in humans to test the hypothesis that higher intakes of folic acid would have beneficial effects on heart disease or stroke, recurrence of colorectal adenoma and cognitive function. Data from these studies has been used to examine the effects of folic acid on all-cause mortality, cancer incidence and recurrence of colorectal adenoma. In trials using doses of folic acid of up to 5mg/day lasting for up to seven years, there is little or no effect. Other meta-analyses which have included studies of people with severe conditions such as chronic renal failure find similar results. The only results of note are a relative risk of 1.16 for prostate cancer, 0.82 for breast cancer and 1.11 for recurrence of advanced colorectal adenoma. None of these are significant at the conventional level, even without making an allowance for ongoing monitoring and re-analysis as new trial results were published. The two elevated relative risks are strongly influenced by the results of the same trial [5]. As there are relatively few trials reporting prostate cancer and advanced adenoma outcomes, it is not possible to determine whether this trial has extreme results by chance or not. There is no effect on all-cause mortality in trials which including a total of 48,000 people and lasting up to 7 years.

Declaration of interest

At the time of working on this analysis, all authors were employees of Food Standards Australia New Zealand, the agency which introduced mandatory folic acid fortification in Australia.

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Appendix

Search strategy and quality assessment of included trials

The following Medline search was performed using PubMed on 16 May, 2013: Search: "Folic Acid"[Mesh] OR "Vitamin B Complex"[Mesh] OR "folic acid"[All Fields] OR ("folic acid"[MeSH Terms] OR ("folic"[All Fields] AND "acid"[All Fields]) OR "folic acid"[All Fields] OR "folate"[All Fields]) OR "B-vitamins"[All Fields] OR "B vitamins"[All Fields] OR "homocysteine lowering"[All Fields] OR "homocysteine-lowering"[All Fields] AND "humans"[MeSH Terms] AND (Clinical Trial[ptyp] OR Randomized Controlled Trial[ptyp]) AND English[lang] AND "adult"[MeSH Terms] Filters: From 2001/01/01 to 2001/12/31

The search shown to the right was performed in CENTRAL on 16 May 2013 and then the search results were then trimmed to delete records dated 2000 and earlier.

Step 1	MeSH descriptor:[Folic Acid] explode all trees
Step 2	MeSH descriptor:[Vitamin B Complex] explode all trees
Step 3	folic acid
Step 4	folic and acid
Step 5	folate
Step 6	B-vitamins
Step 7	B vitamins
Step 8	homocysteine lowering
Step 9	homocysteine-lowering
Step 10	#1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9
Step 11	MeSH descriptor:[Infant, Newborn] explode all trees
Step 12	MeSH descriptor:[Infant] explode all trees
Step 13	MeSH descriptor:[Child] explode all trees
Step 14	MeSH descriptor:[Child, Preschool] explode all trees
Step 15	MeSH descriptor:[Adolescent] explode all trees
Step 16	#11 or #12 or #13 or #14 or #15
Step 17	#10 not #16

Author, Year (reference)	**Loss to followup for main study outcome**	**Concealed sequence generation**	**Placebo/ blinding**	**Main study outcome assessed blind?**
Paspatis, 1994 [55]	Not stated	?	not described	✓
Kim, 2001 [56]	15%	✓	matching	?
Zhu, 2003 [57]	2.3%	?	not blind – only the folic acid group received injections of vitamin B12	×
Toole, 2004 [58]	0.8%	✓	matching	✓
Liem, 2004 [60]	Not described	?	open-label	✓
Liem, 2005 [61]	0	?	open-label	✓
Sato, 2005 [62]	11.0%	✓	not described	✓
Lonn 2006 [63]	0.3%	✓	matching	✓
Bonaa 2006 [41]	6% of survivors	✓	matching	✓
Flicker, 2006 [64]	14.7%	✓	matching	✓
Cole, 2007 [5]	3.3% (at 3 years)	✓	matching	✓
Fernandez-Miranda, 2007 [66]	9.5%	✓	open label	?
Durga, 2007 [67]	0.6%	✓	matching	✓
Logan, 2008 [52]	9.2%	✓	matching	✓
Albert, 2008; Zhang, 2008 [68, 53]	7.4%	?	matching	✓
Ebbing 2008 [42]	7.0%	✓	matching	✓
Van Uffelen, 2008 [69]	19.0%	✓	matching	✓
Jaszewski, 2008 [70]	31.4%	?	not described	?
Aisen, 2008 [71]	15.9%	✓	matching	?
Hodis, 2009 [72]	10.7%	✓	matching	✓
Wu, 2009 [73]	29.3%	✓	similar appearance	✓

SEARCH Group, 2010 [54]	1%	✓	matching	✓
Smith, 2010 [74]	17.7%	✓	not described	?
VITATOPS Group, 2010; Hankey, 2012 [76, 75]	8.7% in placebo, 8.5% in vitamin group	✓	matching	✓
Walker, 2010 [77]	13.5%	✓	matching	?
Kwok, 2011 [78]	10.7%	?	matching	✓

✓ criterion met, ? unclear if criterion met/not stated; × criterion not met

Table A1. Quality summary of included trials