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EFSA meeting summary report

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FOLIC ACID: AN UPDATE ON SCIENTIFIC DEVELOPMENTS

21-22 January 2009, Uppsala, Sweden

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About EFSA

The European Food Safety Authority (EFSA) was established and funded by the European Community as an independent agency in 2002 following a series of food crises that caused the European public to voice concerns about food safety and the ability of regulatory authorities to fully protect consumers.

In close collaboration with national authorities and in open consultation with its stakeholders, EFSA provides objective scientific advice on all matters with a direct or indirect impact on food and feed safety, including animal health and welfare and plant protection. EFSA is also consulted on nutrition in relation to Community legislation.

EFSA's work falls into two areas: risk assessment and risk communication. In particular, EFSA's risk assessments provide risk managers (EU institutions with political accountability, i.e. the European Commission, European Parliament and Council) with a sound scientific basis for defining policy-driven legislative or regulatory measures required to ensure a high level of consumer protection with regard to food and feed safety.

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I INTRODUCTION

In 2008, the European Food Safety Authority (EFSA) established an EFSA Scientific Cooperation Working Group (ESCO WG) on the "Analysis of risks and benefits of fortification of food with folic acid", with the aim of sharing experiences and concerns regarding folic acid food fortification amongst Member States.

The ESCO WG on folic acid was asked to consider the following issues as part of their terms of reference:

- To review current practice in Member States regarding the level of voluntary fortification of foods and categories of foods to which the addition of folic acid is allowed.
- To consider new evidence regarding the risk of high intakes of folic acid and the need for a review of current guidance on safe upper levels of folic acid for all population groups.

EFSA and the Swedish National Food Administration organised a scientific meeting on "*Folic Acid: An Update on Scientific Developments*", in Uppsala, Sweden, on 21-22 January 2009. The aim of the meeting was to consider the evidence regarding folic acid and risk of cancer.

Over 60 scientific experts from the European Union (EU), Switzerland, the United States and Canada attended the meeting to assess the latest scientific evidence on the possible relationship between dietary intakes (including fortified foods and food supplements) of folate and folic acid, and cancer risks, including cancer of the colon, breast and prostate.

All the available scientific evidence concerning folate metabolism, animal and mechanistic studies, and human studies was reviewed and discussed. In group discussions, experts considered whether it was possible: to identify an association of folic acid intake with risk of cancer; the population groups concerned; dose-response relationships; the different dietary sources of folic acid; and whether the available data were sufficient to allow a quantitative risk assessment. Areas for further scientific research were also identified.

Since the completion of this report, further papers on folic acid and cancer risk have been published. Only papers and presentations presented at the meeting are considered in this report.

II BACKGROUND SESSIONS

Folate is a generic term for a naturally occurring family of B-group vitamins comprising an aromatic pteridine ring linked to p-aminobenzoic acid and one or more glutamate residues. It is found naturally in a variety of foods including green leafy vegetables, fruit, liver, and yeast. Folic acid is a synthetic form of folate which is widely used in supplements and for food fortification. Folic acid is more stable in foods and is better absorbed than natural folates.

Dietary folates are converted in the intestinal mucosa to 5-methyl tetrahydrofolic acid (5- MTHF) which is the form of folate present in the systemic circulation. Folic acid has to be reduced and methylated in the gut mucosa before it can be converted to 5-MTHF, the form found in the circulation. The capacity of the body to convert folic acid to 5-MTHF is limited and unmetabolised folic acid has been detected in the systemic circulation following folic acid supplementation (from both supplements and fortified foods) at oral doses above 260 µg (Kelly et al., 1997).

2.1 Benefits and potential risks

Randomised controlled trials have conclusively demonstrated that folic acid supplementation can prevent up to two-thirds of neural tube defects (NTDs) (MRC Vitamin Study Research Group, 1991). It might also reduce the risk of other congenital malformations such as orofacial clefts. The effectiveness of mandatory folic acid fortification programmes in the USA and Canada have resulted in significant declines in the occurrence of NTD affected pregnancies (Williams et al., 2005; De Wals et al., 2007). The percent declines range from 28% to 46% in the USA and Canada, respectively.

Findings from observational studies had also suggested that high intakes of folate (or high blood levels of folate) were associated with a lower risk of cardiovascular disease (CVD) and cancer, and less-age related cognitive decline. Randomised trials had not confirmed any such associations with CVD and cancer. Although limited data from randomised trials have generally not demonstrated any significant beneficial or adverse effects of folic acid on cognitive function, one randomised controlled trial (Durga et al., 2007) reported that folic acid supplementation had a beneficial effect on improving cognitive function in older adults with low folate status and without vitamin B_{12} deficiency.

High intakes of folic acid have also been associated with theoretical risks of adverse effects. Since high dosages of folic acid can correct the anaemia associated with vitamin B_{12} deficiency, there are concerns that high intakes of folic acid could delay the diagnosis of vitamin B_{12} deficiency by treating ("masking") the anaemia of vitamin B_{12} deficiency which could lead to irreversible neurological damage if treatment with vitamin B_{12} is not provided. However, current medical practice does not rely on the presence of anaemia for the detection of vitamin B_{12} deficiency, which frequently presents without anaemia.

While generally, observational studies have suggested that folic acid supplementation slows down the rate of cognitive decline with age, some have suggested that it may accelerate it.

Other postulated adverse effects of folic acid supplementation include reducing the efficacy of antifolate drugs such as methotrexate used in chemotherapy for cancer treatment and drugs used to treat epilepsy but this research question has been insufficiently studied. Concerns have also been raised about the presence of unmetabolised folic acid in the blood following folic acid at oral doses of 260 µg or greater (see page 10, Background sessions). However, the current available data are insufficient to adequately assess the long-term effects of exposure to unmetabolised folic acid.

There are also data suggesting the possibility that high folic acid intakes may be associated with increased risks of cancer; the evidence suggesting a potential link relates specifically to folic acid. There is no evidence to suggest that high intakes of natural folates found in foods are associated with increased cancer risk.

A possible role of folic acid in cancer development is supported by biologically plausible mechanisms. Folate is essential in biological methylation reactions and nucleotide synthesis and impairment of these processes are thought to be involved in cancer development. The evidence regarding folic acid and cancer risk is considered in section 2.5.

2.2 Current recommendations

Many countries in the EU recommend that women who might become pregnant should take folic acid supplements to reduce the risk of NTD occurrence, but public health campaigns promoting this advice have been unsuccessful in most Member States. Directive 2002/46/EC on the approximation of the laws of Member States relating to food supplements establishes harmonised rules for the labelling of food supplements and introduces specific rules on vitamins and minerals in food supplements in the EU.

Some countries in the EU have considered mandatory fortification of wheat flour or bread as a strategy to reduce the prevalence of NTDs. Mandatory wheat flour fortification is currently under review in the United Kingdom but has not been endorsed in Sweden or Italy. It has been recommended in Ireland but implementation has been deferred.

Voluntary fortification is permitted in most European countries. There is considerable variation across the EU in the levels of folic acid that have been added to foods on a voluntary basis, and variation in the categories of foods that are fortified. Recently the EU introduced new rules to regulate voluntary food fortification. These are set out in Regulation (EC) No 1925/2006 on the addition of vitamins and minerals and of certain other substances to foods. As part of the implementation of this regulation, work is currently ongoing on the setting of maximum amounts for the addition of vitamins and minerals. Maximum amounts will be set concurrently for vitamins and minerals in fortified foods and in food supplements (European Commission, 2006).

2.3 Recommended upper intake levels for folic acid in Europe

In 2000, the Scientific Committee on Food (SCF) set a tolerable upper intake level (UL) for folic acid of 1 mg/day for adults (SCF, 2000). ULs for adolescents and children were adjusted downwards on the basis of body weight. The UL is an estimate of the highest level of usual intake of a nutrient which carries no appreciable risk of adverse health effects. The UL was based on the risk of progression of neurological symptoms caused by the "masking" of the haematological symptoms of vitamin B_{12} deficiency.

2.4 Relation of dietary sources of folic acid to blood folate concentrations

There are two sources of folic acid: foods fortified with folic acid and supplements containing folic acid. Data from the National Health and Nutrition Examination Survey (NHANES) 2001-2004 of adults in the USA showed that higher intakes of folic acid and higher blood folate concentrations were primarily associated with use of supplements containing folic acid and were moderately associated with intake of voluntarily fortified foods containing folic acid; lower folic acid intake from mandatory fortification was not associated with these higher values (Yeung et al., 2008).

2.5 Folic acid and cancer risk

ANIMAL STUDIES

Animal models have suggested the possibility of a dual role of folic acid in cancer development, depending on the timing and dose of the intervention: high intakes may suppress development of early lesions in normal tissue but increase the progression of established neoplasms.

Data from animal studies suggest that animals maintained on folate deficient diets are at increased risk of colorectal cancer and that modest folic acid supplementation reduces this risk (Kim, 2004). However, in folate replete animals, and animals with established lesions, high doses of folic acid increase the risk of colorectal cancer (Kim, 2003).

HUMAN STUDIES

Ecological studies

In the USA, voluntary fortification of enriched grain products with folic acid was first authorised in March 1996 and compliance became mandatory from January 1998. In Canada, fortification of foods with folic acid was permitted in December 1996 and cereal grains, especially white flour, were mandated to be fortified with folic acid since November 1998.

Time trends in colorectal cancer incidence in the USA and Canada between 1986 and 2002 indicated an abrupt reversal in the downward trend in colorectal cancer incidence between 1996 and 1998 at around the time of the introduction of folic acid fortification. The downward trend later resumed with the incidence curve shifted upwards because of the temporary increase. Mason et al. (2007) hypothesised that folic acid fortification may have been responsible for the significant deviation from the pre 1996 trend resulting in an excess of about 4-6 additional cases of colorectal cancer cases per 100,000 individuals.

This type of ecological evidence cannot exclude the possibility that the observed fluctuations in colorectal cancer were due to improved screening programmes for colorectal cancer. While there was an increase in colorectal cancer incidence at around the time of the introduction of folic acid fortification, there was no corresponding increase in colorectal cancer mortality, which is consistent with the fluctuations being due to improved screening rather than increased incidence of cancer. However, cancer mortality may not be a useful endpoint in this context as an ecologic study can not take account of the effects on cancer mortality of new cancer treatments that became available in the 1990s.

Observational studies

Several epidemiological studies have explored associations of folate intake and blood folate concentrations with cancer, and in particular with colorectal or breast cancer.

Although the results are inconsistent, most studies of folate intake and colorectal cancer risk suggest a protective effect of high folate intakes on colorectal cancer risk. Studies of serum folate and colorectal cancer risk are inconclusive. Several studies using folate biomarkers are difficult to compare due to, for example, different analytical matrices (serum, plasma, or blood).

The available epidemiological studies of folate and breast cancer risk have reported that folate intake or folate status is unrelated with breast cancer risk, but some studies have suggested an increased risk of breast cancer associated with low folate intake combined with alcohol consumption. One observational study (Stolzenberg-Solomon et al., 2006) reported that folic acid supplements of 400µg or more per day may be associated with an increased risk of breast cancer in postmenopausal women (hazard ratio: 1.19; 95% CI, 1.01-1.41) compared with women consuming no folic acid supplements.

Since there is potential for differential effects of natural dietary folates obtained from food and folic acid from fortified foods and supplements it is important to clearly distinguish between the two. However, many epidemiological studies did not distinguish between intakes of natural folates from foods and folic acid from supplements and fortified foods. Some studies addressed this issue indirectly by examining the use of supplements; other studies considered intakes of natural food folates and total folate intake separately.

Findings from epidemiological studies come from observations that could be confounded by other dietary and non-dietary factors associated with cancer risk.

MTHFR gene variants and cancer risk

Genetic variability of a number of enzymes that are involved in folate metabolism can modify their activity and affect folate status. Several studies have investigated associations of polymorphic genes involved in folate metabolism with colorectal and breast cancer risk. Most studies have considered the MTHFR 677 C→T and 1→98 A&C polymorphisms, which are associated with high homocysteine levels in the setting of low folate status. Most, but not all studies, have reported reduced colorectal cancer risk associated with the MTHFR 677TT variant. The MT→FR 1298 A&C polymorphism has been less extensively studied, and results have been inconsistent (Sharp & Little, 2004; Hubner & Houlston, 2006; Huang et al., 2007).

Genetic variability in folate metabolism is still inadequately characterised and the ability to jointly investigate multiple factors in a biological pathway is very limited.

Randomised controlled trials

Data from randomised controlled trials on the effects of folic acid intakes on breast, prostate and other cancers are limited. One study (Charles et al., 2004), which followed up approximately 3000 women that had participated in a folic acid supplementation trial during pregnancy reported an increased risk of all cancer and a trend for an increased breast cancer risk in women who had been supplemented with 5 g/d of folic acid. However this study was not designed to test the hypothesis that folic acid supplementation has an effect on cancer risk and the study design and statistical analysis may not have been appropriate. A trial (Cole et al., 2007) that examined the efficacy of folic acid (1 mg/day) for prevention of recurrent colorectal adenomas (n=1021) reported that folic acid supplementation was associated with a significantly increased risk of prostate cancer. However, the authors noted that this could be a spurious finding given the number of adverse events evaluated. This trial is described in further detail on page 16, Colorectal adenoma prevention trials.

Two categories of randomised controlled trials have provided evidence on effects of folic acid on risk of cancer and in particular on colorectal cancer: (i) those which have investigated the effects of folic acid supplementation for the prevention of new recurrent colorectal adenomas in individuals with a previous history of colorectal adenomas and (ii) those which have investigated the effect of B-vitamins (including folic acid) on CVD risk, which also collected data on cancer outcomes.

Colorectal adenoma prevention trials

Four small randomised controlled trials (Paspatis and Karmanolis, 1994; Cole et al., 2007; Jaszewski et al., 2008; Logan et al., 2008) and one unpublished US trial (E. Giovannucci 2009 personal communication) have assessed the effect of folic acid supplementation on the risk of colorectal adenoma recurrence in individuals with a prior history of colorectal adenomas. Only the trial by Cole et al. (2007) had duration of more than 3-4 years.

Paspatis and Karamanolis (1994) reported that folic acid supplementation (1 mg/day for 2 years; n=60) decreased colorectal adenoma risk compared with placebo, although the differences were not statistically significant; Jaszewski et al. (2008) reported that folic acid supplementation (5 mg/day for 3 years; n=93) significantly reduced adenoma recurrence compared with the placebo group. The results from these two small trials suggested that folic acid supplementation reduced the risk of colorectal adenoma. The results of these small trials need to be treated with caution as they are likely to be statistically underpowered.

Cole et al. (2007) investigated the effect of folic acid supplementation (1 mg/d; n=1021) with or without aspirin for up to 8 years. This trial reported that folic acid supplementation did not prevent the development of colorectal adenomas. There was no difference in the incidence of at least 1 colorectal adenoma between the placebo group and the folic acid groups after 3 years (RR, 1.04; CI, 0.90-1.20; p=0.58) or after 6 years (RR, 1.13; CI, 0.93-1.37; p=0.23). However, during subsequent treatment/follow-up in a sub-group analysis of this trial (n=607) there was a significantly greater incidence of advanced lesions in the folic acid group compared to the placebo group (RR, 1.67; CI, 1.00-2.80; p=0.05) and significantly more people in the folic acid group with 3 or more adenomas (RR, 2.23; CI, 1.23-4.35).

Results of the trial by Cole et al. (2007) suggested that folic acid at doses in excess of 1 mg/day may increase the risk of developing multiple/advanced adenomas after a few years' delay and consequently increase the risk of colorectal cancer.

The trial by Logan et al. (2008) reported that folic acid supplementation (0.5 mg/day for 3 years; n=853) did not have a significant effect on adenoma recurrence (RR, 1.07; 95% CI, 0.85-1.34). The unpublished US trial (E.Giovannucci, personal communication, 2009) also found no effect of folic acid supplementation (1mg/day for 3 years; n=692) on colorectal adenoma recurrence.

Of the three larger trials (n=700 to 1000) participants received 0.5mg/day of folic acid in one study (Logan et al., 2008) and 1 mg/day in the other two studies (Cole et al., 2007; unpublished trial). Only the trial by Cole et al. (2007) followed participants for more than 3 years and increased risks were observed in the longer follow-up (6-8 years). The trial by Logan et al. (2008) and the unpublished trial both had short follow-up periods (3-4 years); risk ratios from these trials are consistent with those reported by Cole et al. (2007) during their first follow-up (3-4 years). A meta-analysis (n=2652) of the results from these 3 trials (Cole et al., 2007; Logan et al., 2008; unpublished trial) found no evidence of any significant effects of folic acid supplementation on any cancer in this population (unpublished results). This meta-analysis was limited to the shorter follow-up time frame of 3-4 years.

CVD prevention trials

A number of intervention trials have investigated B-vitamin supplementation (including folic acid) for prevention of cardiovascular disease (CVD) in people with a prior history of CVD or renal disease. These trials also examined effects of folic acid supplementation on overall risk of cancer, cancer at specific sites, and mortality from cancer.

Few of the individual trials of B-vitamin supplements for prevention of vascular diseases had adequate statistical power to assess the effects of B-vitamins on CVD or on cancer. The B-Vitamin Treatment Trialists' Collaboration (BVTT) was set up as a prospective meta-analysis of results from all the B-vitamin trials in order to provide more reliable evidence for the effects of B vitamins on vascular and non-vascular outcomes (unpublished results).

The preliminary results of the BVTT meta-analysis of 8 of the trials, involving 37,485 participants, found no significant beneficial or adverse effects of B-vitamin supplementation (folic acid dose of 0.8-40mg/day for a median duration of 5 years) on vascular events, all-cause mortality, cancer, or cancer in any of the pre-specified sub-groups or at any specific sites (including colorectal, lung, prostate or breast cancer (unpublished results). The interpretation of these results is limited by the short duration of follow-up in comparison to the longer periods of time over which cancers usually develop.

Results from a sub-group of two of the B-vitamin trials from Norway (NORVIT & WENBIT) involving 6837 participants with an additional three years of follow-up after the end of the intervention period were due to be presented in June 2009 at the International Homocysteine Conference in Prague (http://www.homocysteine2009. org/).

III REPORTS FROM DISCUSSION GROUPS

3.1 Discussion Group 1: Folic acid and colorectal cancer risk

The available evidence on the associations of folic acid with cancer was considered hierarchically.

ANIMAL STUDIES

Although animal studies are useful for exploring potential mechanisms, caution should be exercised in their interpretation and extrapolation to humans. For example, the doses of folic acid used in animal studies are 4 to 10 times higher than the expected intakes from folic acid food fortification.

HUMAN STUDIES

Ecological evidence

This type of evidence is useful for generating hypotheses but should be treated with caution because of a number of inherent limitations.

A number of points were raised in relation to the study by Mason et al. (2007), including:

- Uncertainty regarding the precise timing of the increase in the population exposure to folic acid in relation to the upturn in colorectal cancer incidence.
- The plausibility of an immediate cancer effect, although this finding is consistent with a possible very late and immediate progression of established adenomas to colorectal cancer.
- Improvements in screening for colorectal cancer in the USA occurred at around the same time as the introduction of folic acid fortification and this could have accounted for the increase in colorectal cancer incidence. Sudden increases in cancer incidence can be caused by a change in screening practice or data collection (case ascertainment, definition, or diagnostic practice). Although this is supported by the fact that there was no subsequent increase in colorectal cancer mortality, the introduction of new chemotherapeutic agents in this time period may have had positive effects on cancer mortality rates.

It was agreed that, as an ecological study, the paper by Mason et al. (2007) had a number of limitations. However, the paper had raised issues about the safety of folic acid and had also highlighted the importance of monitoring trends in colorectal incidence for countries that decide to introduce mandatory fortification with folic acid in the future.

Observational studies

Although the results are inconsistent, most observational studies have shown a protective effect of higher intakes of total folate on colorectal cancer risk compared to those with the lowest folate intakes. Most studies investigated total dietary folate and did not distinguish between natural folates and folic acid.

Epidemiological data on folate (natural folates and folic acid contained in supplements and fortified foods) and cancer risk were reviewed by the World Cancer Research Fund (WCRF/AICR, 2007). The WCRF concluded that there is limited evidence suggesting a protective effect of folate against colorectal cancer (based on papers published before 2006). The report noted, however, uncertainty because of potential confounding and effect modification (particularly with intake of dietary fibre). The WCRF report did not distinguish between folic acid from supplements/fortified foods and natural folates.

It is not possible to reach conclusions about folate and potential colorectal cancer risk from observational data because of problems with assessment of dietary folate intake, potential confounding with other factors that may affect cancer risk and effect modification by other factors that could interfere in 1-carbon metabolism (particularly B vitamins or other methyl donors). Associations between folic acid and potential cancer risk in epidemiological studies may also differ due to pre-existing supplement use or voluntary fortification status in the studied populations.

Randomised controlled trials

Of the five randomised controlled trials which assessed the effect of folic acid supplementation (0.5-1mg/day) on risk of recurrence of colorectal adenomas in people with a prior history of colorectal adenoma (see pages 16-17, Colorectal adenoma prevention trials), none reported adverse effects within 3 years of folic acid supplementation. Only one randomised controlled trial (Cole et al., 2007) reported data on follow-up of more than 3 years; this trial reported that during the later treatment/follow-up, folic acid supplementation (1mg/d) was associated with more multiple, advanced, and larger (unpublished information) adenomas compared with the placebo group. It was agreed that results from this study raise concerns about long-term exposure to folic acid.

The BVTT meta-analysis showed no evidence of any significant effect of folic acid supplementation on overall risk of cancer (Unpublished). There were extensive discussions on the power of this meta-analysis to detect an association between folic acid and cancer risk. It was agreed that an adequately powered meta-analysis for site-specific cancers such as colorectal cancer would not be possible because of the very large numbers of people that would be required and it was therefore unlikely that this question could be resolved in the near future. It was also agreed that the current data involved relatively short follow-up time periods in comparison to the time usually required for the development of cancers.

It was noted that cancer endpoints from 3 further B-vitamin trials would be included in the meta-analysis in 2009 and 2011 and that 2 Norwegian studies (NORVIT and WENBIT) were expected to report follow-up cancer outcomes in 2009. Since Norway does not allow foods to be fortified with folic acid, background exposure to folic acid would have been very low in these trials. Prolonged follow-up of participants in such trials after the cessation of folic acid supplementation may provide useful information on possible long-term effects of folic acid on cancer risk.

The general consensus was that the findings from the B-vitamin treatment trials did not support or refute the suggestion that high folic acid intakes increase colorectal cancer. The levels of folic acid intake associated with potential risk are considered in page 22, Intake levels and cancer risk.

Population groups and cancer risk

Population groups potentially at greater risk of developing colorectal cancer with folic acid supplementation may include individuals with cancer, undetected cancer, or premalignant colorectal adenomas. Older people, who are at increased risk of developing colorectal adenomas may also be at increased risk.

The effects of folic acid on treatment efficacy of commonly used chemotherapeutic drugs (such as methotrexate and 5-fluorouracil) have been insufficiently studied.

Intake levels and cancer risk

The difficulty of assessing a threshold for a possible carcinogenic effect of folic acid, based on interpretation of the cancer studies in humans, was recognised.

The possibility of using the amount of folic acid that would cause the appearance of free folic acid in the circulation as a threshold for intake was discussed. However, it was noted that there was insufficient evidence to assess possible risks associated with unmetabolised folic acid in the circulation. Since folate metabolism is under polygenetic control it would be difficult to factor genetic considerations into any reconsideration of the UL.

It was agreed that people should not consume more than the current UL of 1 mg/day of folic acid. Although the UL is based on limited supporting evidence, it could be used as a general guidance value in order to prevent potential adverse effects of excess intakes of folic acid. It was not possible to identify whether there was a dose response relationship or a threshold for the effects of folic acid on potential colorectal cancer risk.

It is also important to distinguish between different sources of folate, i.e. natural food folates and folic acid from fortified foods and from supplements. Data from the USA (NHANES) have shown that the population group of ≥ 60 years of age had the highest folic acid intakes with the largest amounts deriving from supplements. In this population group, which is at highest risk for colorectal cancer, lower dosage mandatory fortification was not likely to have influenced serum folate levels.

3.2 Discussion Group 2: Folic acid and other cancers (breast, prostate, pancreatic, oesophageal)

CONSIDERATION OF THE EVIDENCE

Data from animal studies regarding the relationship between folic acid and breast cancer are limited.

Time trend data from the USA do not show temporal changes in the incidence of breast and prostate cancer following voluntary and mandatory fortification of enriched grain products with folic acid (1996-1998). In Canada, there was a significant increase in the incidence of prostate cancer after 1996 (voluntary fortification was introduced in December 1996).

A prospective cohort study has suggested a potential harmful effect of folic acid intake (≥ 400µg/d) on breast cancer risk (Stolzenberg-Solomon et al., 2006) (see page 14, Observational studies). The WCRF report concluded that the epidemiological data for an association between folate and breast cancer was too inconsistent or limited to allow conclusions to be reached and that there was limited evidence that foods containing folate protect against pancreatic and oesophageal cancer.

It was noted that the existing evidence is inadequate to make a judgement on the possible association between folic acid and breast cancer risk and that breast cancer is a multifactorial and complex disease which makes assessment of any folic acid-cancer association very difficult. It was agreed that issues that required further consideration included:

- Interactions between folate and alcohol intake
- Age at menarche and menopause
- Form of folate (natural vs folic acid)
- Interaction of folate with other nutrients
- Dose
- Other risk factors
- Genetic background.

FOLIC ACID FOOD FORTIFICATION

A range of foods are voluntarily fortified[1] with folic acid at variable levels. This makes it difficult for individuals and risk managers to assess the actual intakes of folic acid. Modelling work undertaken in the UK (SACN, 2006) suggests that mandatory folic acid fortification of flour together with restriction of folic acid from all voluntary sources would result in a more even distribution of folic acid intakes across the population.

POPULATION GROUPS AND CANCER RISK

Population groups that might be vulnerable to folic acid supplementation were not discussed as food fortification would have an impact on the whole population.

INTAKE LEVELS AND CANCER RISK

It was agreed that it was not possible to determine whether there was a dose-response or threshold level associated with possible risk of breast, prostate, pancreatic or oesophageal cancer. However, the consensus was that intakes should not exceed the UL.

[1] *Voluntary folic acid food fortification is regulated under the provisions of Regulation (EC) No. 1925/2006 on the addition of vitamins and minerals and certain other substances to foods.*

IV PLENARY DISCUSSION AND CONCLUSIONS

4.1 Final comments and conclusions

Divergent views were expressed during the discussion and there was disagreement between experts regarding the interpretation of the trial evidence and the UL of 1mg/d. Some considered that the available evidence did not support an association of high intakes of folic acid with possible cancer risk or the UL of 1 mg/day which is based on limited data. The following general conclusions reflect the consensus of participants.

The beneficial effect of folic acid in reducing the risk of NTDs is well established. Women who might become pregnant are the target population for this benefit. Others with low folate intakes would also benefit from folic acid fortification. Suggestions for additional benefits, including reductions in CVD, cancer occurrence, and cognitive decline, have also been made; evidence for these benefits is not supported by randomised controlled trials.

Evidence from animal studies, trend data for colorectal cancer incidence, and a randomised controlled trial have raised concerns of a possible association between high intakes of folic acid and promotion of cancer development and progression. While the totality of the randomised trial evidence from the CVD trials does not suggest that folic acid intakes are associated with increased cancer risk, these trials probably did not have sufficient power to detect overall cancer risk or site-specific cancer risk and their duration of follow-up may have been too short to detect cancer risk.

There are currently insufficient data to allow a full quantitative risk assessment of folic acid and cancer or to determine whether there is a dose-response relationship or a threshold level of folic acid intake associated with potential colorectal cancer risk

The current evidence does not show an association between high folic acid intakes and cancer risk but neither do they confidently exclude a risk. The uncertainties in relation to cancer risk highlight the importance of ensuring monitoring systems are set up for assessment of folic acid intake and status and NTD and cancer incidence in countries that decide to introduce mandatory fortification.

Targeted generation of additional data and knowledge, both epidemiological and animal/mechanistic, might be important in informing the risk/benefit assessment of folic acid in the future.

Intakes of folic acid should not exceed the established UL of 1mg/day (SCF, 2000). However, the UL is based on limited data and may need to be revised when further data become available.

Setting maximum safe levels for the amount of folic acid that can be added to foods voluntarily fortified with folic acid and supplements will be important in ensuring that consumption of foods fortified with folic acid and folic acid supplements does not lead to intakes above the UL.

4.2 Further research

Further research in the following areas may be helpful in informing future risk assessments on the possible association between high intakes of folic acid and cancer risk:

Continued long-term follow-up (5-10+ years) for cancer risk in participants in folic acid supplementation trials after the cessation of the trials.

An update of the B-Vitamin Treatment Trialists' meta-analysis to assess the effects on risk of any cancer and on site-specific cancers after completion of the 3 ongoing B-vitamin trials that are due to report in the next 18-24 months.

Future studies need to take better account of total folate and total folic acid exposure (natural food folate and folic acid from voluntary and mandatory fortified foods and supplements) and folate status (measured by best/recommended assays, including measurement of different folate forms and unmetabolised folic acid).

Further experimental studies on the pharmacokinetics of folic acid in animals and humans (including folate metabolism in adenomas).

Modelling of population effects of food folates and folic acid intakes from voluntary and mandatory fortification and from supplements.

Animal studies on the effect of folic acid supplementation on precancerous-resected lesions.

In vitro and in vivo studies on proliferation effects.

Monitoring possible effects of unmetabolised folic acid on health outcomes.

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ANNEXES

ANNEX 1: PROGRAMME OF THE CONFERENCE

Overall Chairs: Alan REILLY and Leif BUSK
Overall Rapporteur: Åke BRUCE

21 January 2008

09.00 - 12.30	**Session 1: Introductory Plenary Session**	Chair: Leif BUSK,
09.00	Welcome address	Inger ANDERSSON Alan REILLY
09.10	Folate metabolism; overview of risks and benefits of dietary folic acid	John SCOTT
09.50	Epidemiological studies versus clinical trials of micronutrients in cancer prevention: why do the results seldom agree?	Lars HOLMBERG
10.10	*Questions*	
10.30	Coffee	
11.00	Animal and mechanistic studies on folic acid and cancer	Young-In KIM
11.20	*Questions*	
11.30	Overview of the experience following fortification with folic acid on cancer risks	Stein Emil VOLLSET
11.50	*Questions*	
12.00	Assessment of studies thought to suggest associations between folic acid and cancers of the colon and breast	Nicholas WALD
12.20	*Questions*	
12.30	Lunch	

13.30 - 17.00	**Session 2:** **Folic acid and cancer**	Chair: Ada KNAAP
13:30	Folic acid and colon cancer: outcome of clinical trials	John BARON
13:50	*Questions*	
14:00	Folate polymorphisms and colorectal cancer risk	Cornelia ULRICH
14:20	*Questions*	
14:30	Folic acid and cancer - trial evidence	Robert CLARKE
15:00	*Questions*	
15.10	Coffee	
15:30	Folate/folic acid and breast/prostate cancer risk	Anders EKBOM
15:50	*Questions*	
16.00	Relation of dietary sources of folic acid to blood folate concentrations for the assessment of cancer risk	Robert J. BERRY
16.20	*Questions*	
16.30	Commentary on folic acid and cancer	Alan JACKSON
16.50	*Questions*	
17:00 - 18:30	**Session 3:** **Discussion in break-out groups**	
	DG 1 Folic acid and colon cancer	Chair: Göran HALLMANS Rapporteur: Alison TEDSTONE
	DG 2 Folic acid and other –breast and prostate- cancers	Chair: Alfonso LAMPEN Rapporteur: Mary FLYNN

22 January 2008

09.00 - 10.00	**Session 3: continued**	
10.00 - 13.00	**Session 4: Final Plenary Session**	Chair: Alan REILLY
10.00	Interventions with folic acid: WHO perspective for evidence-based guidelines	Juan Pablo PENA-ROSAS
10.20	*Questions*	
11.00	Report back from DG 1	Alison TEDSTONE
11.20	*Discussion*	
11.50	Report back from DG 2	Mary FLYNN
12.10	*Discussion*	
12.40	Concluding remarks and end of meeting	Alan REILLY Leif BUSK

ANNEX 2: PARTICIPANTS AT THE CONFERENCE

Name	Affiliation	Country/Unit
Judith Amberg-Müller	Federal Office of Public Health FOPH	Switzerland
Göran Annerén	Department of Clinical Genetics, Uppsala Universlty	Sweden
John Baron	Dartmouth-Hitchcock Medical Center	USA
R.J. Berry	Centers for Disease Control and Prevention	USA
Anna Maria Castellazzi	Policlinico San Matteo	Italy
J.M. Jacqueline Castenmiller	Food and Consumer Product Safety Authority (VWA)	The Netherlands
Robert Clarke	University of Oxford	United Kingdom
Marta Ebbing	University Hospital, Haukeland	Norway
Anders Ekbom	Karolinska Institute	Sweden
Mary-Ann Flynn	Food Safety Authority of Ireland	Ireland
Paul Haggarty	Rowett Institute of Nutrition and Health, University of Aberdeen	United Kingdom
Göran Hallmans	University of Umeå	Sweden
Helmut Heseker	Universität Paderborn	Germany
Lars Holmberg	"King's College London, Division of Cancer Studies, Cancer Epidemiology Unit, Research Oncology"	United Kingdom
Alan Jackson	Southampton General Hospital	United Kingdom
Ingegard Johansson	University of Umeå	Sweden
Ellen Kampman	Wageningen University, Division of Human Nutrition	The Netherlands
Tim Key	Cancer Epidemiology Unit, University of Oxford	United Kingdom
Young-In Kim	University of Toronto	Canada
Ada Knaap	Scientific Committee of EFSA	The Netherlands

Name	Affiliation	Country/Unit
Berthold Koletzko	Dr. von Hauner Children's Hospital, Ludwig-Maximilian-University of Munich - German Society of Paediatrics	Germany
Alfonso Lampen	Bundesinstitut für Risikobewertung	Germany
Alberto Mantovani	Istituto Superiore di Sanità	Italy
Iréne Margaritis	Direction de l'évaluation des risques nutritionnels et sanitaires, AFSSA	France
Joel B. Mason	Tufts University	USA
Torsten Mossberg	The National Board of Health and Welfare / Socialstyrelsen	Sweden
Juan-Pablo Pena-Rosas	World Health Organisation	Switzerland
David Phillips	Institute of Cancer Research	United Kingdom
Hilary Powers	University of Sheffield	United Kingdom
Oliver Racz	Safarik University	Slovak Republic
Lone Banke Rasmussen	National Food Institute	Denmark
Sheela Reddy	Department of Health	United Kingdom
Helga Margareta Refsum	University of Oslo	Norway
Alan Reilly	Food Safety Authority of Ireland	Ireland
John Scott	Trinity College Dublin	Ireland
Mamta Singh	Food Standards Agency	United Kingdom
A. David Smith	"University of Oxford, Department of Physiology, Anatomy & Genetics"	United Kingdom
Maria Szeitzné Szabó	Hungarian Food Safety Office (MEBiH)	Hungary
Alison Tedstone	Food Standards Agency	United Kingdom
Anne Tjønneland	Danish Cancer Society, Institute of Cancer Epidemiology	Denmark
Cornelia M Ulrich	Fred Hutchinson Cancer Research Center	USA

Name	Affiliation	Country/Unit
Henk Van Kranen	RIVM - National Institute for Public Health and the Environment	The Netherlands
Stein Emil Vollset	University of Bergen	Norway
Nicholas Wald	Queen Mary University London	United Kingdom
Rianne Weggemans	Health Council of the Netherlands	The Netherlands
Anke Weissenborn	Bundesinstitut für Risikobewertung	Germany

Livsmedelsverket (Swedish National Food Administration) Staff

Lilianne Abramsson Zetterberg	Livsmedelsverket / National Food Administration	Sweden
Inger Andersson	Livsmedelsverket / National Food Administration	Sweden
Wulf Becker	Livsmedelsverket / National Food Administration	Sweden
Ulf Bohman	Livsmedelsverket / National Food Administration	Sweden
Åke Bruce	Livsmedelsverket / National Food Administration	Sweden
Leif Busk	Livsmedelsverket / National Food Administration	Sweden
Eva Corp	Livsmedelsverket / National Food Administration	Sweden
Katarina Hörnfeldt Olson	Livsmedelsverket / National Food Administration	Sweden
Ulla-Kaisa Koivisto Hursti	Livsmedelsverket / National Food Administration	Sweden
Irene Mattisson	Livsmedelsverket / National Food Administration	Sweden

EFSA Staff

Bernhard Berger	European Food Safety Authority	Italy
Lucia De Luca	European Food Safety Authority	Italy
Anne-Laure Gassin	European Food Safety Authority	Italy
Saadia Noorani	European Food Safety Authority	Italy
Ilias Papatryfon	European Food Safety Authority	Italy
Ariane Titz	European Food Safety Authority	Italy

Organising Committee Members

Alan Reilly	Food Safety Authority Ireland (FSAI)	Ireland
Leif Busk	National Food Administration (SLV)	Sweden
Alfonso Lampen	Federal Institute for Risk Assessment (BfR)	Germany
Alison Tedstone	Food Standards Agency (FSA)	United Kingdom
Bernhard Berger	European Food Safety Authority (EFSA)	Italy
Ilias Papatryfon	European Food Safety Authority (EFSA)	Italy

Meeting Secretariat

Saba Giovanacci	European Food Safety Authority (EFSA)	Italy
Hanna Figoluszka	European Food Safety Authority (EFSA)	Italy
Ann Almlöf	National Food Administration (SLV)	Sweden

ANNEX 3: PRESENTATIONS OF SPEAKERS

Folate metabolism: overview of risks and benefits of folic acid fortification

JOHN M. SCOTT
School of Biochemistry & Immunology
Trinity College - Dublin

Abstract:

Improved folate status either through food folate or synthetic folic acid, has two proven and several other probable and/or possible benefits. The two proven benefits are prevention of NTDs and deficiency/anaemia. Probable benefits are reduction in stroke. Possible benefits are reduction in: heart disease; occurrence of certain cancers; cognitive decline; Alzheimer's disease; osteoporosis: The above benefits would flow either from optimum purine/pyrimidine and with it DNA biosynthesis or from provision of adequate s-adenosylmethionine (SAM), with optimum methylation at the appropriate sites on proteins, lipids or preformed DNA.

It is now recognised that attainment of sufficient folate status to achieve the above is not attainable through most even apparently good diets. This is due in part to the poor bioavailability of natural folates but more particularly, to their innate chemical instability.

By contrast, the synthetic form of the vitamin folic acid, is very bio-available and extremely stable in stored food and during cooking. Given the above, the practice of using synthetic folic acid as supplements (tablets) or by the mandatory or voluntary addition of folic acid to food to fortify the diet is very commonplace. Which of the three approaches is used varies widely in practice in different countries.

The drive for fortification is driven partially by commercial interest but also by public health concerns that adequate status cannot be achieved with food folates alone. This is almost universally recognised as necessary to prevent even a proportion of NTD affected births.

The use of folic acid has the benefit of bioavailability, stability and price. However, it is not used without giving rise to some concern. The most prominent concern is that it prevents the timely diagnosis of vitamin B_{12} deficiency. This issue depends upon the fact that folic acid above a certain level appears in the circulation and enters cells in a way that is not under normal metabolic control. In addition, it directly stimulates DNA biosynthesis and cell division. When this happens in B_{12} deficiency it masks the emergence of the usual anaemia, allowing the other effect of B_{12} deficiency, namely neuropathy, to proceed undiagnosed to where it may be irreversible. A more recent concern is that a similar mechanism may be at play in cancer. Thus, while folate/folic acid may prevent the occurrence of new cancers, it may accelerate the growth of pre-existing cancers. These latter concerns are based largely on animal models. More recently observations of apparent increases in colon cancer post fortification in US/Canada have been published. Of greater concern is that recurring polyps in subjects on high dose folic acid were more numerous and had worse pathology than placebo treated peers. Both with respect to masking of anaemia and accelerated cancer growth, greater risk exists in the face of increasing levels of unmetabolised folic acid is in the circulation. Such folic acid does not arise at low intakes but the body's ability to convert it to natural folate is easily saturated by moderate levels of intake.

Presentation:

Benefits and risks of folic acid/folate

Benefits

Certain	-	Anaemia and Deficiency
	-	Neural Tube Defects
Probable	-	Stroke
	-	Heart Disease
Possible	-	Cancer Occurrence
	-	Cognitive Decline
	-	Alzheimer's Disease
	-	Other Birth Defects (eg. OFCs)

Risks

Certain	-	Mask Pernicious Anaemia
Probable	-	Accelerates Existing Tumour
Possible	-	Accelerates cognitive decline
	-	Impairs immune function
	-	Interferes with Methotroxate Therapy

Benefits

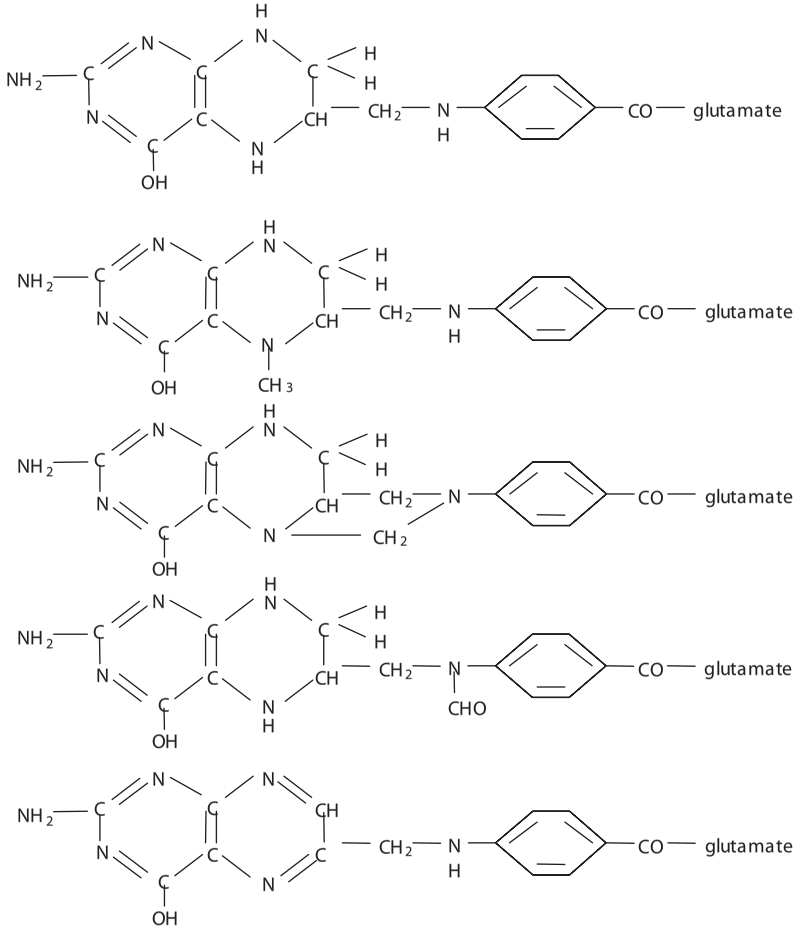
Certain	-	Anaemia and Deficiency
	-	Neural Tube Defects
Probable	-	Stroke
Possible	-	Heart Disease
	-	Cancer Occurrence
	-	Cognitive Decline
	-	Alzheimer's Disease
	-	Other Birth Defects (eg. OFCs)

Risks

Certain	-	Mask Pernicious Anaemia
Probable	-	Accelerates Existing Tumour
Possible	-	Accelerates cognitive decline
	-	Impairs immune function
	-	Interferes with Methotroxate Therapy

Terminology

- folic acid:
  - synthetic form (supplements, fortified foods)
  - a monoglutamate
  - no attachments to basic molecule
- folates:
  - natural forms (plant and animal tissues)
  - polyglutamates
  - usually have a one-carbon attachment



Biologically Active Folates

Tetrahydrofolate

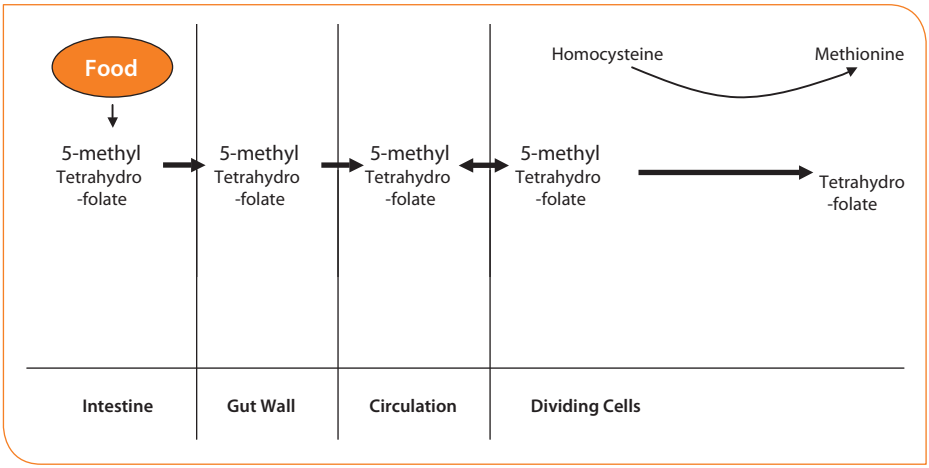
5-Methyltetrahydrofolate

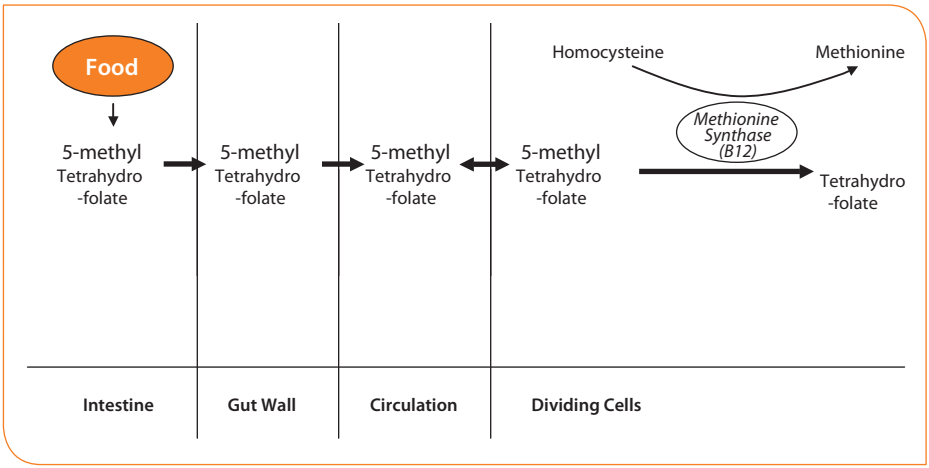
5,10-Methylenetetrahydrofolate

10-Formyltetrahydrofolate

Synthetic Vitamin Precursor

Folic acid


Food
5-methyl Tetrahydro -folate
5-methyl Tetrahydro -folate
5-methyl Tetrahydro -folate
5-methyl Tetrahydro -folate
Homocysteine
Methionine
Tetrahydro -folate
Intestine
Gut Wall
Circulation
Dividing Cells


Food
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Methionine Synthase (B12)
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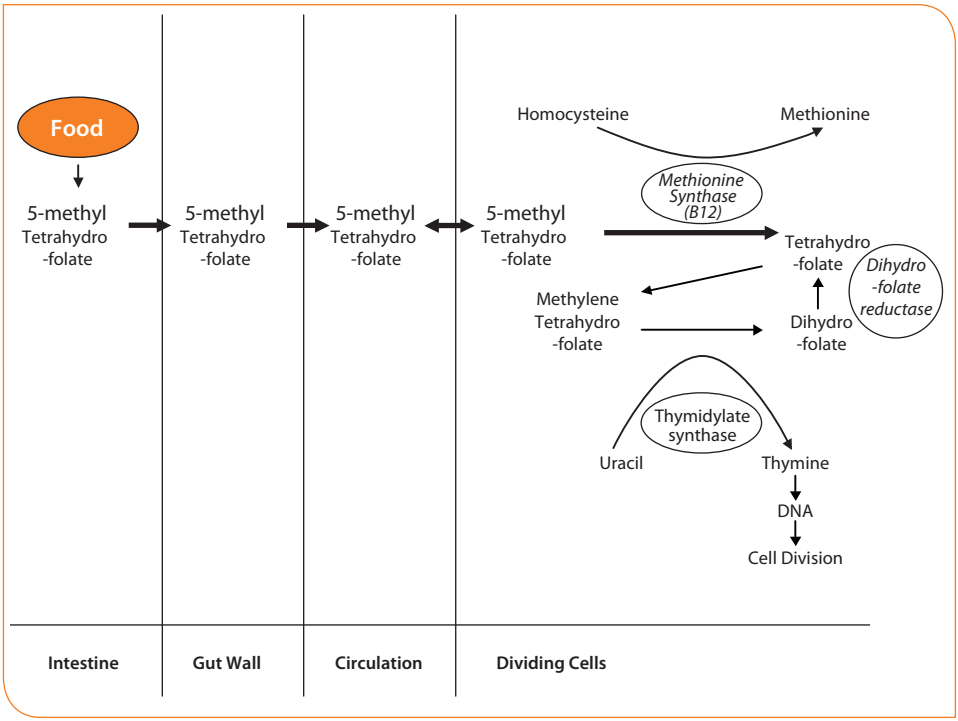
Intestine
Gut Wall
Circulation
Dividing Cells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Methionine
Methionine
Synthase
(B12)
Tetrahydro
-folate
Dihydro
-folate
reductase
Methylene
Tetrahydro
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Cell Division
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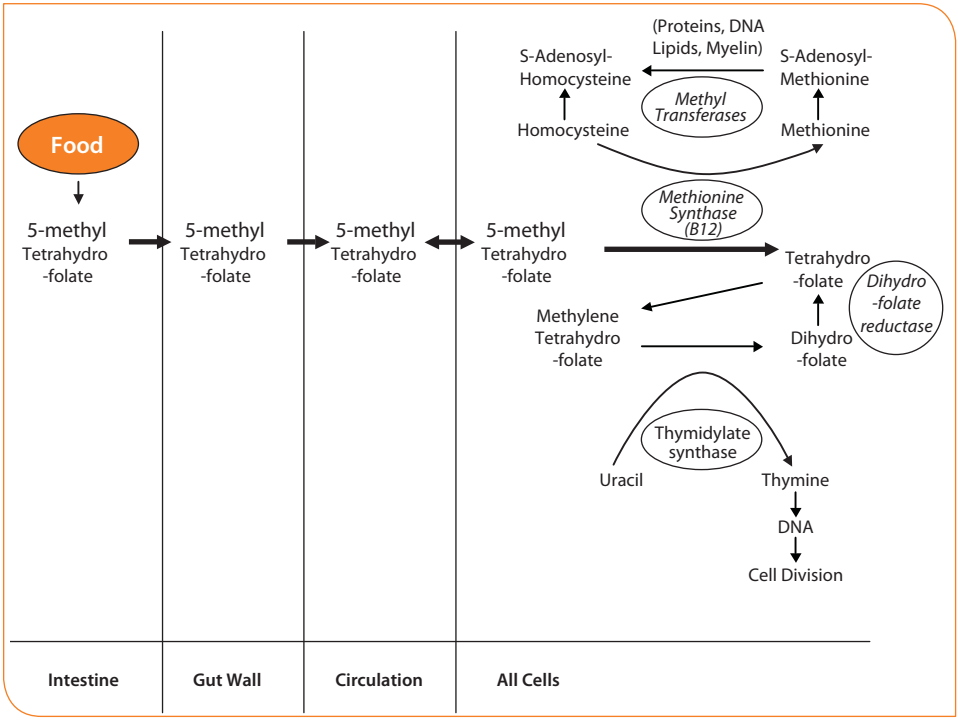
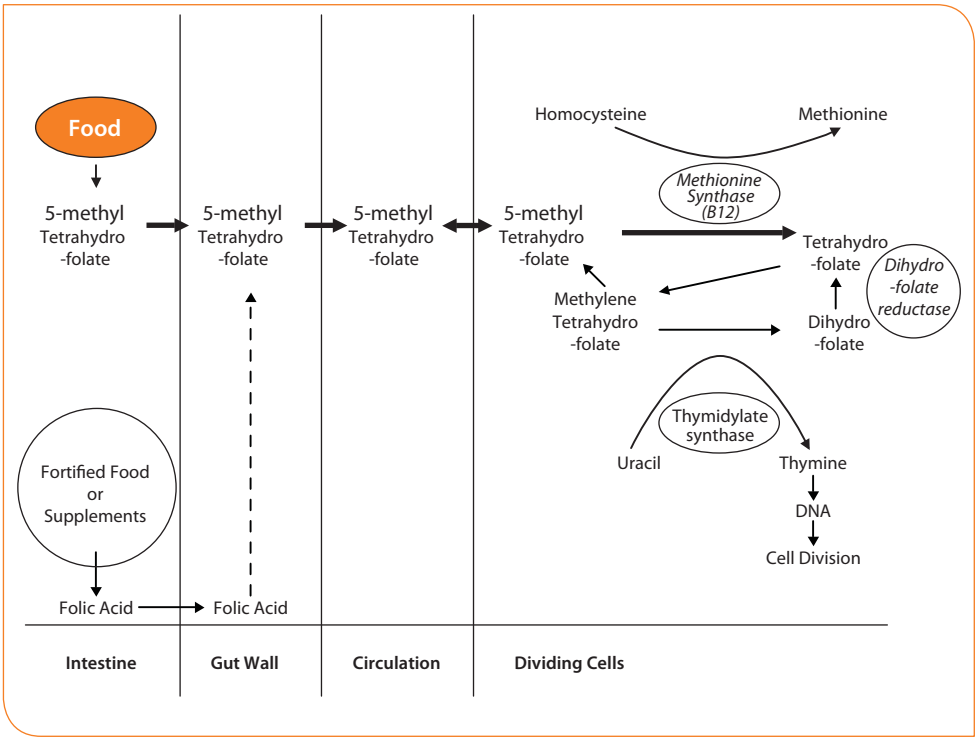
Dividing Cells


Food
5-methyl Tetrahydro -folate
5-methyl Tetrahydro -folate
5-methyl Tetrahydro -folate
5-methyl Tetrahydro -folate
(Proteins, DNA Lipids, Myelin)
S-Adenosyl- Homocysteine
S-Adenosyl- Methionine
Methyl Transferases
Homocysteine
Methionine
Methionine Synthase (B12)
Tetrahydro -folate
Dihydro -folate reductase
Methylene Tetrahydro -folate
Dihydro -folate
Thymidylate synthase
Uracil
Thymine
DNA
Cell Division
Intestine
Gut Wall
Circulation
All Cells


Food
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Methionine
Synthase
(B12)
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-folate
Dihydro
-folate
reductase
Methylene
Tetrahydro
-folate
Dihydro
-folate
Thymidylate
synthase
Uracil
Thymine
DNA
Cell Division
Fortified Food
or
Supplements
Folic Acid
Folic Acid
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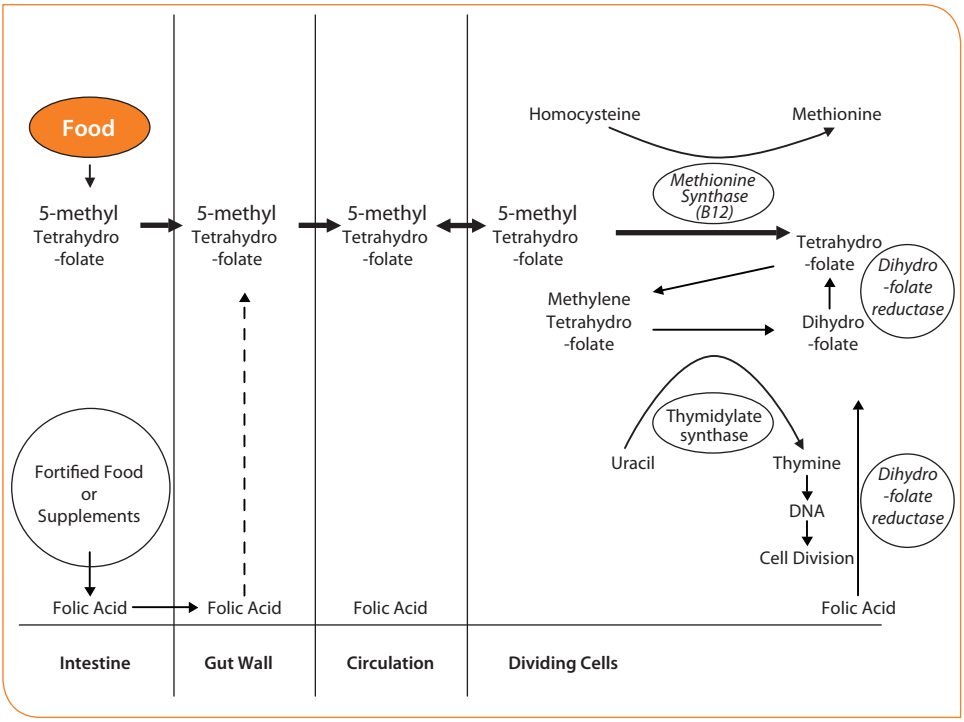
Food
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Homocysteine
Methionine
Methionine Synthase (B12)
Tetrahydro -folate
Dihydro -folate reductase
Methylene Tetrahydro -folate
Dihydro -folate
Thymidylate synthase
Uracil
Thymine
DNA
Cell Division
Dihydro -folate reductase
Fortified Food or Supplements
Folic Acid
Folic Acid
Folic Acid
Folic Acid
Intestine
Gut Wall
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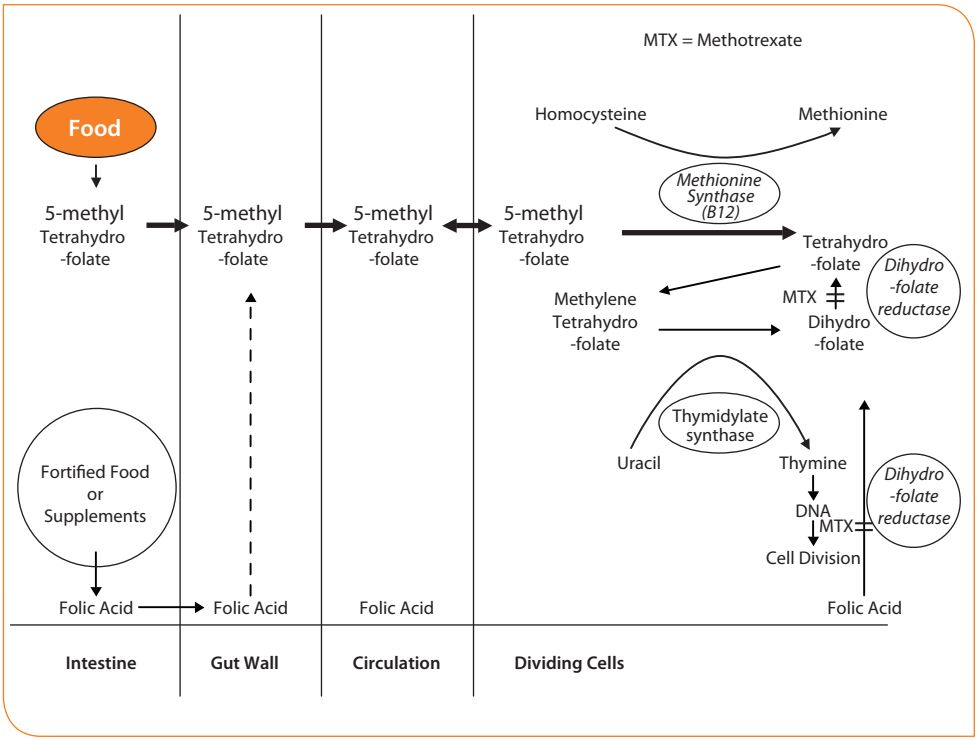
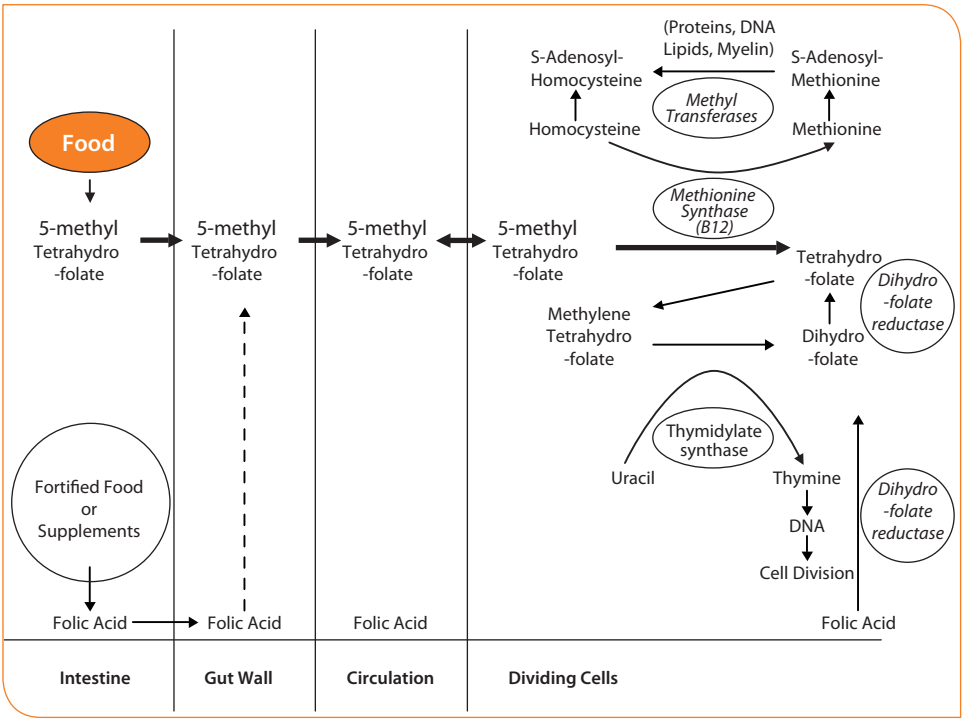

MTX = Methotrexate
Food
5-methyl Tetrahydro -folate
5-methyl Tetrahydro -folate
5-methyl Tetrahydro -folate
5-methyl Tetrahydro -folate
Homocysteine
Methionine
Methionine Synthase (B12)
Tetrahydro -folate
Dihydro -folate reductase
MTX
Dihydro -folate
Methylene Tetrahydro -folate
Thymidylate synthase
Uracil
Thymine
DNA
MTX
Cell Division
Dihydro -folate reductase
Fortified Food or Supplements
Folic Acid
Folic Acid
Folic Acid
Folic Acid
Intestine
Gut Wall
Circulation
Dividing Cells


Food
5-methyl Tetrahydro -folate
5-methyl Tetrahydro -folate
5-methyl Tetrahydro -folate
5-methyl Tetrahydro -folate
(Proteins, DNA Lipids, Myelin)
S-Adenosyl-Homocysteine
S-Adenosyl-Methionine
Methyl Transferases
Homocysteine
Methionine
Methionine Synthase (B12)
Tetrahydro -folate
Dihydro -folate reductase
Methylene Tetrahydro -folate
Dihydro -folate
Thymidylate synthase
Uracil
Thymine
DNA
Cell Division
Dihydro -folate reductase
Folic Acid
Fortified Food or Supplements
Folic Acid
Folic Acid
Folic Acid
Intestine
Gut Wall
Circulation
Dividing Cells

Kelly P, McPartlin J, Goggins M, Weir DG, Scott JM.
Unmetabolized folic acid in serum: acute studies in subjects consuming fortified food and supplements. Am J Clin Nutr. 1997 Jun;65(6):1790-5.

Young and elderly volunteers on a 5-d regimen of fortified ready-to-eat-cereal and bread in addition to their normal diet **had a threshold intake of 266 µg folic acid per meal at which unaltered folic acid appeared in the serum**.

Subjects given folic acid in either isotonic saline, milk, or white bread also had a threshold > 200 micrograms.

Sweeney MR, McPartlin J, Weir DG, Daly L, Scott JM.
Postprandial serum folic acid response to multiple doses of folic acid in fortified bread. Br J Nutr. 2006 Jan;95(1):145-51.

Healthy folate replete adults were pre-saturated with 400 µg folic acid supplements daily for 14-weeks.

Measurable unmetabolised folic acid was present in serum of 6/19 subjects after the 14-week supplementation period.

Subjects then consumed folic acid fortified bread daily over one week as follows:

400 µg: (2x 200 µg slices)	**Folic acid detected**
200 µg: (2x 100 µg slices)	**No folic acid detected**
100 µg: (2x 50 µg slices)	**No folic acid detected**

Sweeney MR, McPartlin J, Scott J.
Folic acid fortification and public health: report on threshold doses above which unmetabolised folic acid appear in serum. BMC Public Health. 2007 Mar 22;7(147):41.

This study investigated the consequences of consuming baked bread preparations containing 1 mg folic acid (UL) as follows:

1. a single dose of 1000 microg,
2. two doses of 500 microg,
3. three doses of 333 microg,
4. five doses of 200 microg,
5. ten doses of 100 microg.

Folic acid appeared in all subjects.

Contrary to expectation, folic acid was more persistent in serum after repetitive intake

Sweeney MR, McPartlin J, Weir DG, Daly S, Pentieva K, Daly L, Scott JM.
Evidence of unmetabolised folic acid in cord blood of newborn and serum of 4-day-old infants. Br J Nutr. 2005 Nov;94(5):727-30.

Folic acid in the circulation

Sweeney, et al (in press)
Sample of 50 blood donors (unfasted) 49 positive
Level 4.9% of total folate.
Samples of 20 women (fasting) and cords at caesarean section
18 out of 20 had a mean level of folic acid (2.5% total folate).
Folic acid present in 17 out of 20 cords

Benefits and risks of folic acid/folate

Benefits

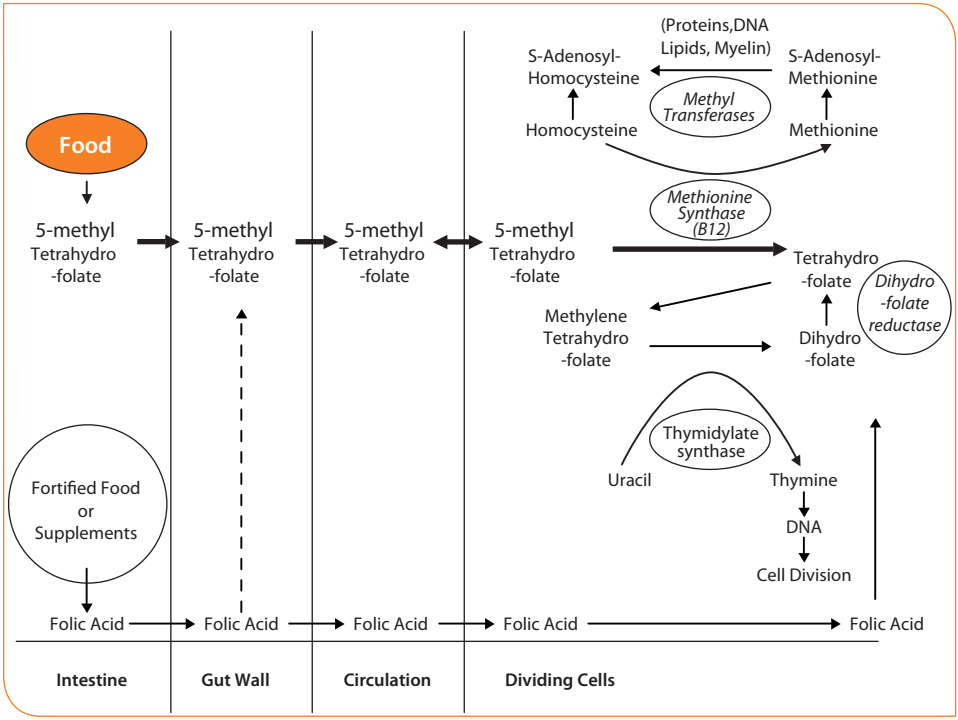
Certain	-	Anaemia and Deficiency
	-	Neural Tube Defects
Probable	-	Stroke
Possible	-	Heart Disease
	-	Cancer Occurrence
	-	Cognitive Decline
	-	Alzheimer's Disease
	-	Other Birth Defects (eg. OFCs)

Risks

Certain	-	Mask Pernicious Anaemia
Probable	-	Accelerates Existing Tumour
Possible	-	Accelerates cognitive decline
	-	Impairs immune function
	-	Interferes with Methotroxate Therapy

Trends in serum folate status of older adults

Serum Folate	1998 Older adults N=124	2002 Older adults N=101	2006 Older adults N=109	2007 Older adults N=462
≤2 ng/mL (4.5 nmol/L) **Deficient**	4.8%	3.0%	0%	0.7%
2-3 ng/mL (4.5-6.7 nmol/L) **Possible deficiency**	5.6%	1.0%	0%	1.8%
3-10 ng/mL (6.8-22.6 nmol/L) **Low-Adequate**	62.9%	41.6%	40.4%	31.5%
10-20 ng/mL (22.7-45 nmol/L) **Adequate-High**	16.1%	33.7%	33.9%	31.7%
>20 ng/mL (45 nmol/L) **High**	10.5%	20.8%	25.7%	34.3%



Summary

Current diets do not ensure optimum folate status for most. Correcting this by increasing intake of food folates is not a realistic option, leading to the use of supplements or fortification of food either mandatory or voluntary. For reasons of stability and bioavailability, the synthetic form of the vitamin, folic acid is used. Depending upon the level this prevents anaemia, reduces NTD births and may have other benefits. The capacity to convert folic acid to the natural cellular forms is limited with relatively low intake giving rise to the appearance of this unnatural form to cells. At sufficient levels, this masks the diagnosis of vitamin B_{12}. Of greater concern is that it may accelerate the growth of pre-existing cancers.

Epidemiological studies vs RCT:s of micronutrients in cancer prevention Why do the results seldom agree?

LARS HOLMBERG
King's College London

Abstract:

Several micronutrients that seemed promising as preventing cancer in observational research or mechanistic animal or cell culture studies have been taken to randomized clinical trials. The majority of these trials have failed to show preventive effects, some of them have even suggested harmful effects. Many possible explanations for these discrepancies in results have been forwarded. The explanations have amongst others included that timing of the intervention is crucial, that there may be non-linear relationships between dose and effect, that the interaction with other nutrients and life-style factors is severely complicating any intervention that is not overly complicated, that the clinical trials have not had long enough exposure or follow-up times, that the base-line level of exposure and/or the genetic polymorphisms present in populations are critical and not have been considered enough.

A broader methodological question is if traditionally designed observational studies of intended positive effects are inherently so difficult to do in valid way that their design needs to be reconsidered. Furthermore, sometimes laboratory studies that show any relationship between cellular processes involved in cancer etiology and micronutrients have been interpreted as supporting a plausible biological mechanism although hypotheses about a causal chain of events have not been tested.

It is major intellectual and methodological challenge to tackle all the mentioned problems. The approach will have to be multidisciplinary, multiprofessional and international. Maybe, despite the gloomy picture currently painted in the randomized clinical trials, chemoprevention with micronutrients will survive as a promising weapon against cancer, but in a new context of understanding and practice after such an effort.

Presentation:

A common scenario

- Observational studies show promising results
- Biologically tentative mechanisms are found
- Exploratory studies show positive effects on intermediate endpoints
- (Secondary results from RCT:s promising)
- The final hypothesis test in a large RCT fails

Recent trials

- Vit C, E and Beta Carotene in 8,171 women;
  Lin et al in JNCI 2009;101: 14 – 23
- Selenium and Vit E in 35,533 men;
  Lippman et al in JAMA. 2009;301(1):(doi:10.1001/jama.2008.864)
- Vit C and E in 14,641 men;
  Gaziano et al in JAMA. 2009;301(1):52-62

Timing

- During initiation of a cancer: early or late in this phase?
- During progression: works on biological or clinical progression?
- Interferes with the metastatic process?
- How long during respective phase?
- Implications for trial duration

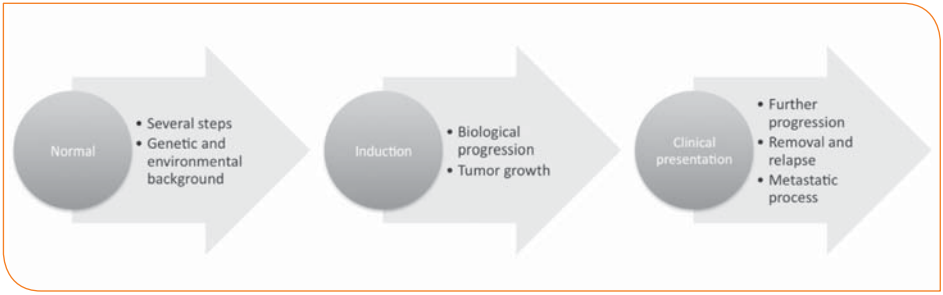
Doses and pharmacological form

- Strong individual variation in effective dose?
- Non-linear relation between dose and effect?
- Every-day or intermittent administration?
- Form of administration essential?

Modification of effect and interactions

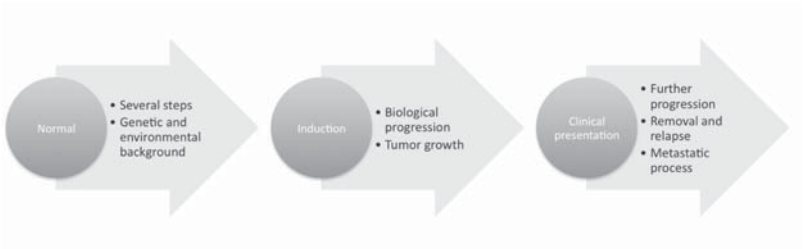
- Different effects given the baseline level
- Interaction with other nutrients
- … or with other life-style factors, e.g. smoking
- Changes of exposures over time
  - And of their effects during different phases of the the natural history of the cancer
- Genetic polymorphism
  - Interactions with all the above, e.g. with baseline levels

An extremely complex and long winded story…



What do we need to know about the population?

Where do we intervene with which dose?



Should we consider the whole fruit/vegetable?

Which is the relevant endpoint?

Null trials or those with unexpected outcomes should not, however, be viewed as failures; they have and will continue to shed light on the causes of cancer and help us discover the means for its prevention.

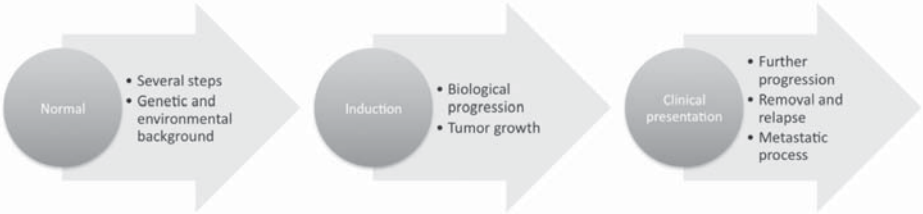
D Albanes JNCI editorial January 7 2009

A more serious question - is it all wrong?

- Observational studies of intended positive effects are inherently invalid
- Study results of unwanted side effects have been more robust
  E.g. when HRT for cardiovascular protection was tested in RCT:s, cancer risks were very similar to those in observational settings

- In a complex biological system far beyond our understanding we can always find tentative "biological mechanisms"
- Mechanistic studies undertaken after the idea is formed

There are many possibilities to find a relation between a naturally occurring component of our diet and a mechanism involved in cell growth control.



- Intermediate endpoints are seldom to trust
- A very strong relation between the intermediate endpoint and final outcome necessary
- Micronutrient intake is only a marker of a selection mechanism or confounding factor
- Naïve belief that only one molecule fixes a major problem

Can we reconcile these two perspectives?

- A rigorous approach to epidemiological study design
  - Selection bias
  - Timing and dose
- Strict criteria for causal inference in mechanistic studies
- Constantly be prepared to revisit the basic underlying assumptions

Not the first time in medicine…

- Immunology and cancer
- Super-radical surgery for cancer with regional metastases
- Both have "survived" a radical re-orientation and are now seen and used in new contexts

Animal and mechanistic studies on folic acid and cancer

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Abstract:

Folate is a water-soluble B vitamin that is present naturally in foods whereas folic acid is the synthetic form of this vitamin that is used in supplements and in fortified foods. The sole biochemical function known for folate is mediating the transfer of one-carbon units involved in nucleotide synthesis and biological methylation reactions. In this role, folate may play an important role in cancer development and progression. Indeed, epidemiologic studies suggest that folate intake (dietary and supplemental) and blood folate levels are inversely associated with the risk of several malignancies including cancer of the colorectum, oropharynx, esophagus, stomach, pancreas, lungs, cervix, ovary, and breast and neuroblastoma and leukemia. The best epidemiologic evidence for the inverse association between folate status and cancer risk exists for colorectal cancer and its precursor, adenoma. Collectively, epidemiologic studies suggest a 20-40% reduction in the risk of colorectal cancer and adenomas in individuals with the highest folate intake compared with those with lowest intake. The role of folate in colorectal carcinogenesis has been further strengthened by the observations that genetic polymorphisms in the folate metabolic pathway modify colorectal cancer risk.

However, animal studies suggest that folate possesses dual modulatory effects on colorectal carcinogenesis depending on the stage of cell transformation at the time of folate intervention as well as the dose of folate supplementation. Folate deficiency has an inhibitory effect whereas folate supplementation has a promoting effect on the progression of established colorectal (pre)neoplasms. In contrast, folate deficiency in normal colorectal mucosa appears to predispose it to neoplastic transformation, and modest levels of folic acid supplementation suppress, whereas supraphysiologic supplemental doses enhance, the development of cancer in normal colorectal mucosa.

Although small human intervention trials have suggested potential beneficial effects of folic acid supplementation on biomarkers of colorectal cancer, more recent large intervention trials do not support these earlier observations. In the Asprin-Folate Polyp Prevention Study, folic acid supplementation (1 mg/d for up to 6 years) increased the risk of developing advanced lesions with a high malignant potential (OR=1.67; 95% CI, 1.00-2.80) and of developing multiple (>2) adenomas (OR=2.32; 95% CI, 1.23-4.35) compared with placebo in 1021 subjects with previously resected adenomas (JAMA 2007; 297: 2351). One explanation for this observation is that folic acid supplementation might have promoted the progression of already existing, undiagnosed microscopic preneoplastic lesions in these predisposed patients. Another secondary finding from this trial was that

the risk of cancers other than colorectal cancer was significantly increased in the folic acid supplemented group (P=0.02) largely due to an excess of prostate cancer in the folic acid group (P=0.01) (JAMA 2007; 297: 2351 and J Natl Cancer Inst 2009; 101: 432).

Several potential mechanisms relating to the role of folate in one-carbon transfer reactions and consequent DNA synthesis and epigenetic regulations exist to support the differential effects of folate on the development and progression of colorectal cancer in the normal colorectum and in established colorectal (pre)neoplastic foci. In normal colorectum, folate deficiency may enhance the development of colorectal cancer via DNA stand breaks, impaired DNA repair, increased mutagenesis, and genomic DNA hypomethylation, while folic acid supplementation may prevent the development of colorectal cancer by ensuring DNA stability and integrity, optimal DNA repair, decreased mutagenesis, and prevention of aberrant DNA methylation. In established colorectal cancer and adenomas, folate deficiency causes ineffective DNA synthesis leading to inhibition of tumor growth and progression (similar to chemotherapies using antifolates) and may reverse promoter CpG island methylation of tumor suppressor and other anticancer genes involved in colorectal carcinogenesis, thereby reactivating these genes and leading to inhibition of tumor progression. In established colorectal cancer or adenomas, folic acid supplementation may promote tumor progression by providing nucleotide precursors for proliferation and growth of neoplastic cells and de novo methylation of promoter CpG islands of tumor suppressor and anticancer genes leading to gene inactivation.

Population-based folic acid fortification, intended to prevent neural tube defects, and folic acid supplementation (consumed by up to 40% of the North American population), long presumed to be purely beneficial and believed to provide several health benefits, may have unintended deleterious influences on the development and progression of colorectal cancer. Given the incidence and mortality of colorectal cancer and the prevalence of adenomas (present in up to 50% of the population), whether or not folic acid fortification and supplementation promote the progression of (pre)neoplastic foci to colorectal cancer is a legitimate significant public health concern. In this regard, a recent study that examined a temporal trend of colorectal cancer incidence in the USA and Canada post fortification has reported that concurrent with folic acid fortification, the US and Canada experienced abrupt reversals of the downward trend in colorectal cancer incidence that the two countries had enjoyed in the preceding decades (Cancer Epidemiol Biomarkers Prev 2007; 16: 1325). Because of the lack of complete control of potential

confounding factors, this observation does not prove a causal link between folic acid fortification and increased rates of colorectal cancer in North America post fortification. Nevertheless, this observation provides a highly provocative impetus for further discussion, debate and research aimed at elucidating potential deleterious effects of folic acid fortification and supplementation on cancer risk.

There is an emerging body of evidence that suggests that folic acid fortification and periconceptional maternal use of folic acid supplementation may prevent the development of "new" cancers in a site-specific manner (e.g., pediatric neuroblastoma and acute lymphocytic leukemia). Folic acid fortification and periconceptional supplementation may therefore reduce the risk of certain childhood cancers in the offspring. Furthermore, folic acid supplementation may prevent the development of "new" cancers in normal tissues. However, folic acid supplementation and fortification may promote the progression of already, existing, undiagnosed preneoplastic and neoplastic lesions. At present, based on the lack of compelling supportive evidence and on the potential tumor-promoting effect, routine folic acid supplementation should not be recommended as a chemopreventive measure against colorectal cancer and other cancers. Furthermore, the potential tumor-promoting effect of the dramatically increased folate intake resulting from mandatory folic acid fortification and folic acid supplementation in the USA and Canada should be carefully monitored. More specifically for colorectal cancer, folic acid supplementation should not be given to individuals with previous colorectal adenomas because their colons may already be predisposed to neoplastic transformation and to those suspected of harboring precursor lesions of colorectal cancer in the colorectum. This of course applies to the large segment of the North American population as it has been estimated that ~25-50% of people by 50 years of age in the US (>60 million) and Canada (>10 million) harbor asymptomatic colorectal adenomas, and the prevalence increases with age. This translates to 16 to 32 millions of the Americans ≥50 years of age who might be susceptible to the tumor promoting effect of folic acid supplementation. Even a greater number of the North Americans might be harboring aberrant crypt foci (the probable earliest precursor of colorectal cancer) or microscopic adenomas in the colon and folic acid supplementation may accelerate the progression of these early precursor lesions to colorectal adenomas and cancer.

Presentation:

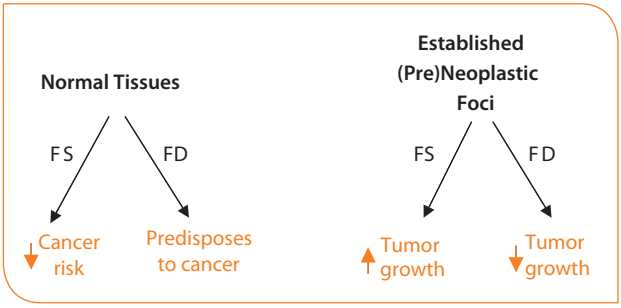
Folate deficiency and cancer risk

- **Colorectum**
- Lung, Oropharynx
- Breast
- Pancreas
- Prostate
- Cervix, Ovary
- Esophagus/Stomach
- Leukemia
- Neuroblastoma

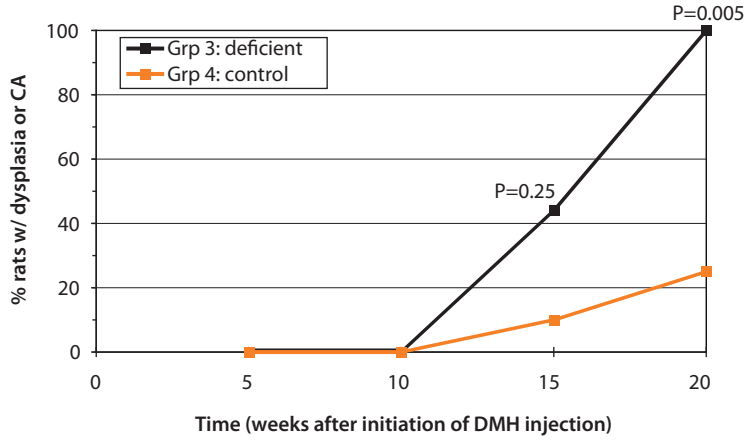
Folate protects against cancer?

Animal studies: dual effects of folate

Double-edged sword



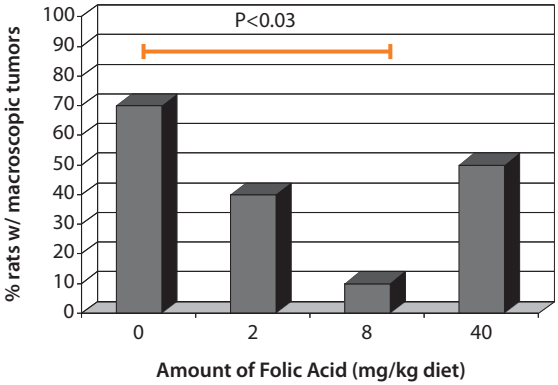
Incidence of Colorectal Dysplasia or CA



Cancer Res 1992; 52: 5002

Dose-response experiment

Incidence of macroscopic tumors



Gut 1996; 39: 732

Folate & CRC: other chemical models

Study	Model	Dose of FA	Duration of Diet	End point	Outcome
Carcin 2000	F344 rats AOM	Hi vs. Very Hi	30 wks	CA	↑ Incidence ↑ Multiplicity
AACR 1996	F344 rats AOM	2000 ppm	52 wks	CA	Ø Incidence ↑ Size ↑ Multiplicity
CEBP 1996	F344 rats AOM	2.5 g/kg 5.0 g/kg	2 wks	ACF	↑ (64%)

Carcinogenesis vol.25 no.8 pp.1507–1515, 2004
doi:10.1093/carcin/bgh137

Both suboptimal and elevated vitamin intake increase intestinal neoplasia and alter crypt fission in the *Apc*$^{Min/+}$ mouse

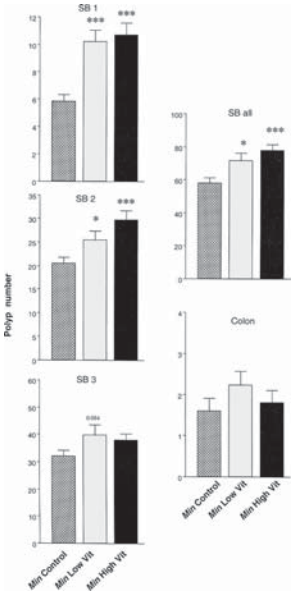


Table I. Vitamin doses in the three diets

Vitamin	Name	Control	Low vitamin diet	High vitamin diet
A	Retinol (IU)	5000	1666.7	10 000.0
B1	Thiamine	5.1	1.7	25.5
B2	Riboflavin	4.9	1.6	24.0
B3	Niacin	10.0	3.3	50.0
B5	Pantothenol	10.6	3.5	53.0
B6	Pyridoxine	5.0	1.67	25.0
B12	Cobolamine	0.013	0.004	0.065
	Folate	1.0	0.33	2.0
	Biotin	0.2	0.07	1.0
C	Ascorbic acid	19.6	6.50	98.0
D	Cholecalciferol (IU)	1000.0	333.3	5000.0
E	Tocopherol	50.0	16.7	250.0
K		4.8	1.60	24.0
	Selenium	0.08	0.03	0.40

All doses in mg/IU.

Low Dietary Folate Initiates Intestinal Tumors in Mice, with Altered Expression of G_2-M Checkpoint Regulators *Polo-Like Kinase 1* and *Cell Division Cycle 25c*

Erin Knock, Liyuan Deng, Qing Wu, Daniel Leclerc, Xiao-ling Wang, and Rima Rozen

Departments of Human Genetics and Pediatrics, McGill University Health Centre-Montreal Children's Hospital, Montreal, Quebec, Canada

Table 1. Tumor incidence in intestine of mice on control diet or folate-deficient diet with and without a null allele in *Mthfr*

No. mice examined for tumors	Diet		Total mice	% Mice with tumor
	Control	Folate deficient		
Mthfr$^{+/+}$	31 (0)	16 (2)	47	12.5
Mthfr$^{+/-}$	26 (0)	64 (18)	90	28.1*
Total mice	57 (0)	80 (20)	137	
% Mice with tumor	0	25†		
Histologic examination of intestine	**Total examined**	**Adenoma**	**Adenocarcinoma**	**Polyp**
Control diet	7	0	0	1
Folate-deficient diet	12	2	3	3

Control: 2 mg FA/kg diet + 1% SSFZ
FD: 0.3 mg FA/kg diet + 1% SSFZ
Duration: 12-14 months

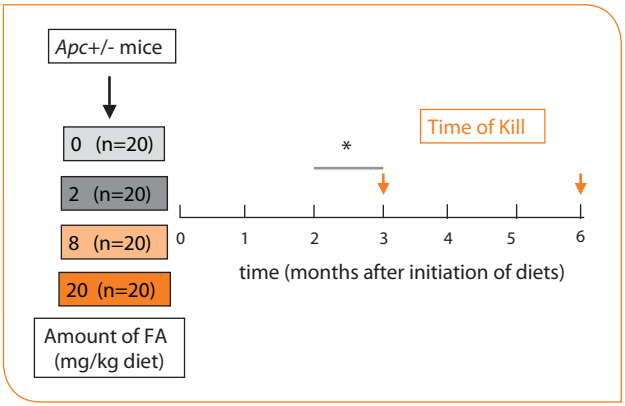
Cancer Res 2006; 66: 10349

Folate & CRC

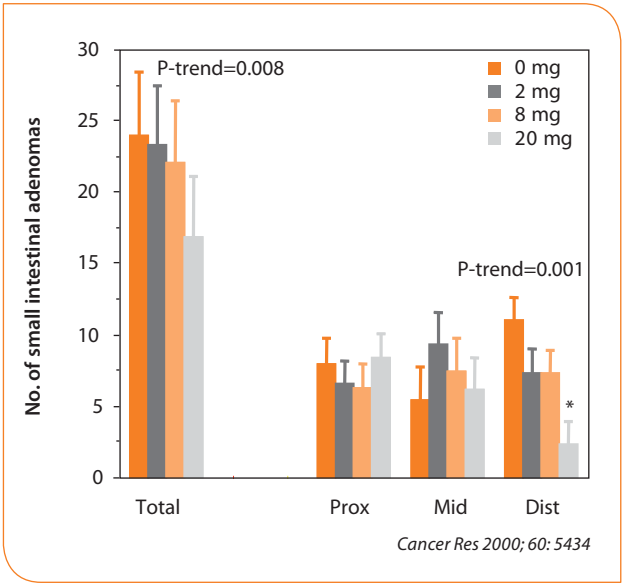
Animal models

- Folate modulates colorectal carcinogenesis over a wide range of dietary intakes.
  - Folate deficiency of a moderate degree enhances colorectal carcinogenesis.
  - Modest levels of FA supplementation above the basal dietary requirement suppress colorectal carcinogenesis.
  - Supraphysiologic levels of FA supplementation may enhance colorectal carcinogenesis.

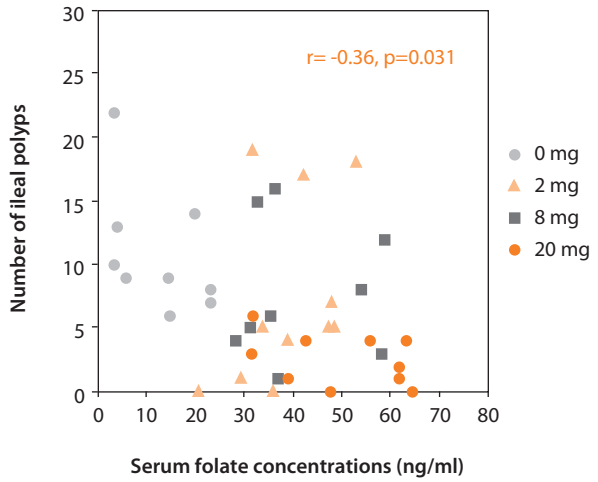
Effects of folic acid on intestinal tumorigenesis in MIN (Apc+/-) mice



Apc+/-: *small intestinal adenomas 3 months*

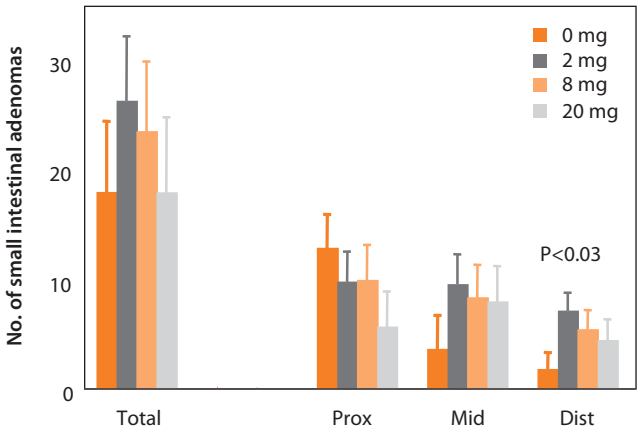


Correlation between serum folate concentrations and illeal adenomas at 3 months



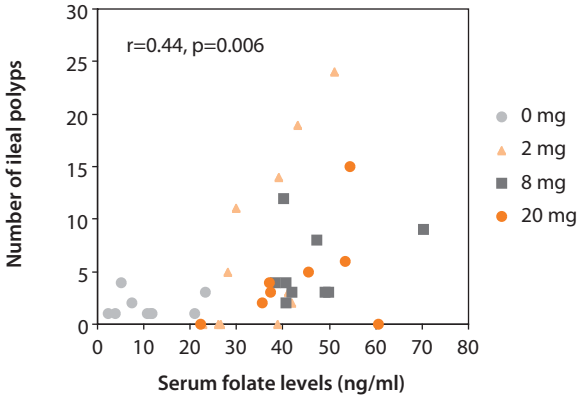
Cancer Res 2000; 60: 5434

Apc+/-: small intestinal adenomas 6 months



Cancer Res 2000; 60: 5434

Correlation between serum folate concentrations and ileal adenomas at 6 months



Cancer Res 2000; 60: 5434

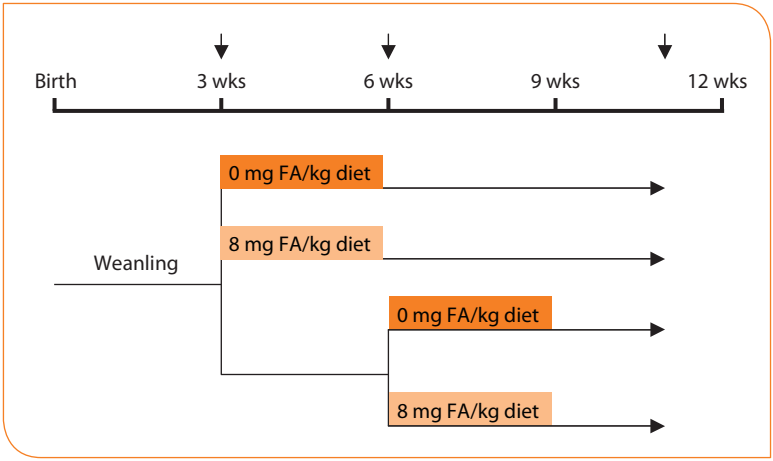
Apc+/- *x* Msh2-/- *mouse*

- Accelerated intestinal tumorigenesis
  - 350 small intestinal polyps (adenomas)
  - 8 colonic adenomas (no adenocarcinomas)
  - 55 ACF
  - by 80 days of age, at which time they become moribund and die (anemia or bowel obstruction)
- Average time required for a nascent tumor to develop into a macroscopically visible adenoma
  - small intestine: 42 days
  - colon: 27 days

Cancer Res 1996; 56: 2922

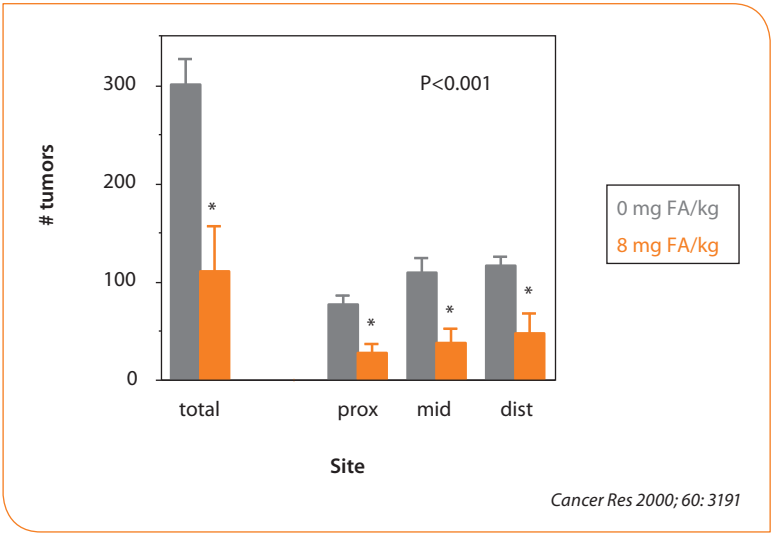
Effect of timing of folate intervention

Apc+/-Msh2-/- Murine Model



Apc+/-Msh2-/-: *small intestinal adenomas*

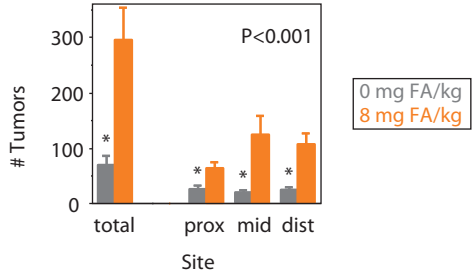
Folate intervention @ 3 weeks



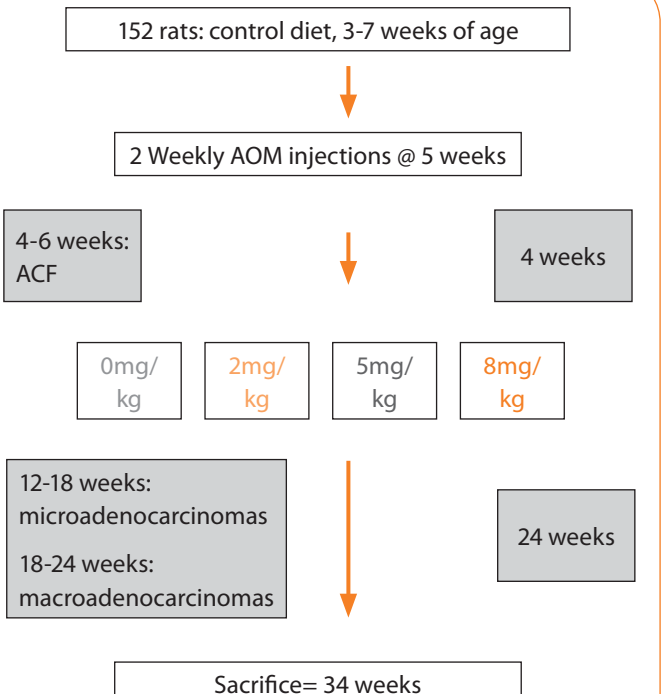
Cancer Res 2000; 60: 3191

Apc+/-Msh2-/-: *small intestinal adenomas*

Folate intervention @ 6 weeks

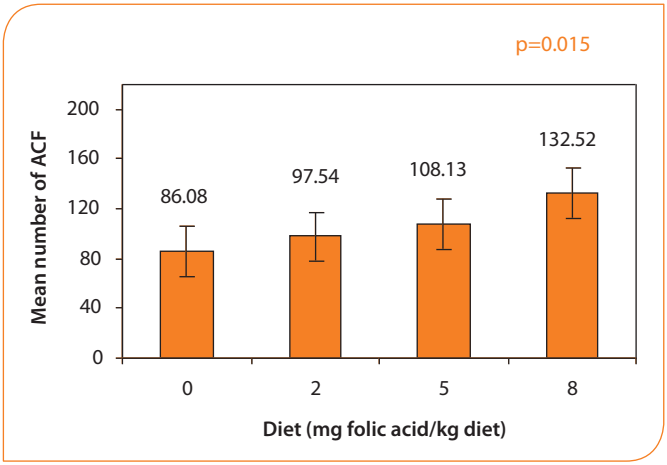


Cancer Res 2000; 60: 3191

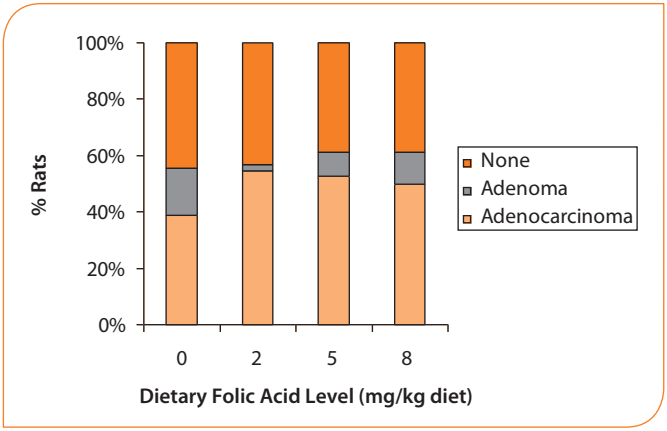


AACR 2007

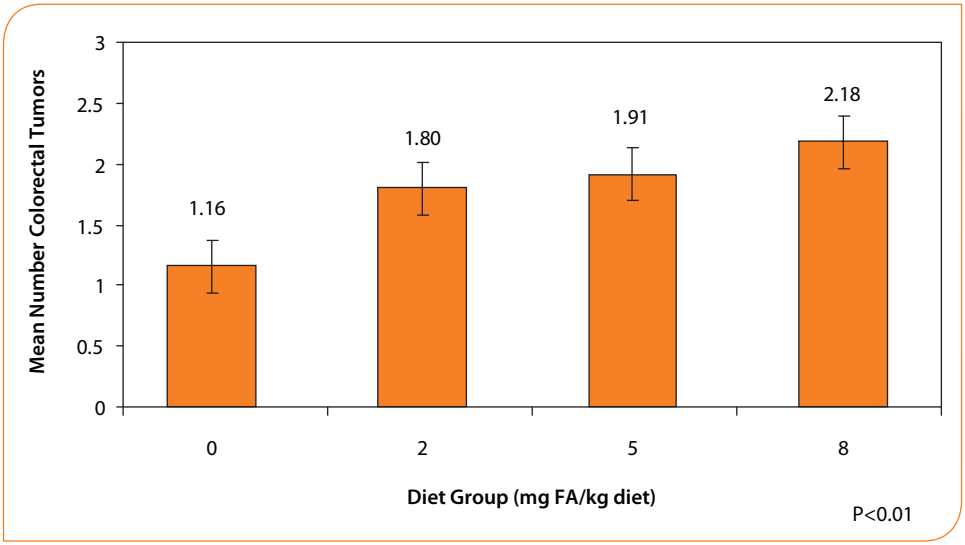
Effect of dietary folic acid on the mean number of ACF



The effect of FA supplementation on incidence of colorectal tumors

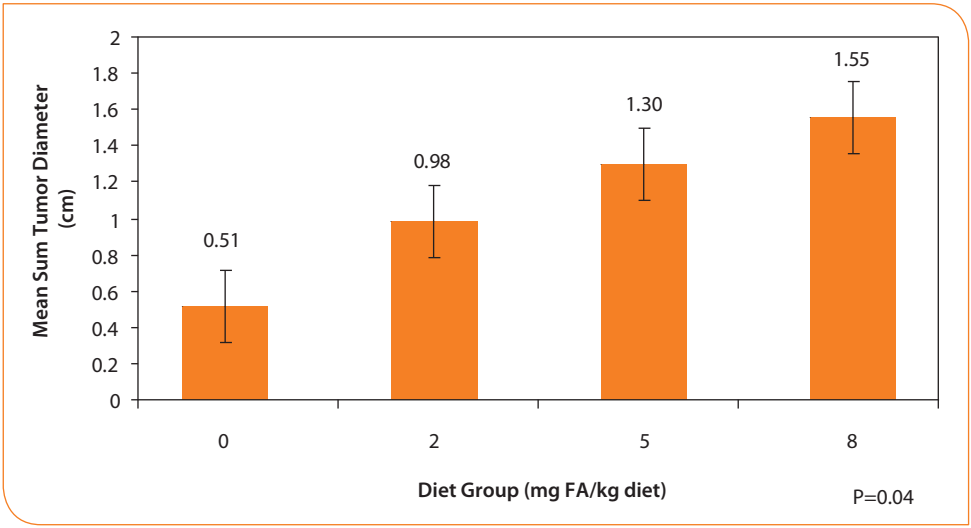


The effect of FA supplementation on tumor multiplicity (AdenoCA)



	# of adenoCA	# of all neoplasms
Dietary Folic Acid	**r=0.316** **p=0.006**	**r=0.322** **p=0.002**
Plasma Folate	r= -0.196 p= 0.382	r= 0.202 p= 0.103
Plasma Hcyst	**r= -0.289** **p= 0.017**	**r= -0.315** **p= 0.005**

The effect of FA supplementation on tumor burden



	Sum of tumor diameter
Dietary Folic Acid	r=0.346 p=0.001
Plasma Folate	r=0.329 p=0.008
Plasma Hcyst	r= -0.417 p<0.001

The Asprin Folate Polyp Prevention Study

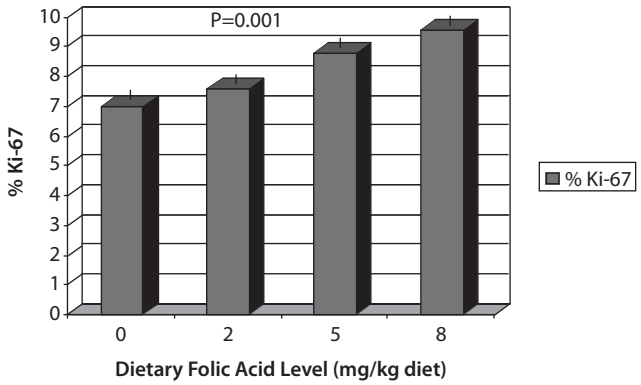
Table 3. Risk of Adenoma After Randomization in the Intention-to-Treat Population*

	First Follow-up Interval				Second Follow-up Interval			
	No. (%) of Participants				No. (%) of Participants			
End Point	Placebo (n = 486)	Folic Acid (n = 501)	Unadjusted RR (95% CI)	*P* Value	Placebo (n = 304)	Folic Acid (n = 303)	Unadjusted RR (95% CI)	*P* Value
Any adenoma	206 (42.4)	221 (44.1)	1.04 (0.90-1.20)	.58	113 (37.2)	127 (41.9)	1.13 (0.93-1.37)	.23
Advanced lesion	42 (8.6)	57 (11.4)	1.32 (0.90-1.92)	.15	21 (6.9)	35 (11.6)	1.67 (1.00-2.80)	.05
No. of adenomas								
1-2	168 (34.6)	174 (34.7)	1.00 (0.85-1.19)	.66	100 (32.9)	97 (32.0)	0.97 (0.77-1.22)	.02
≥3	38 (7.8)	47 (9.4)	1.20 (0.80-1.81)		13 (4.3)	30 (9.9)	2.32 (1.23-4.35)	

Abbreviations: CI, confidence interval; RR, risk ratio.
*The intention-to-treat population consisted of all randomized participants with a follow-up examination, including those participants who discontinued randomized supplementation. First follow-up interval included the initial 3-year protocol, and the second follow-up interval was 3 or 5 years later. *P* values are based on χ^2 tests. For number of adenomas, *P* values are global for the 3 categories (0, 1-2, and ≥3 adenomas), and separate RRs are shown to summarize the effect of folic acid on each adenoma-multiplicity category.

Cole, B. F. et al. JAMA 2007;297:2351-2359

The effect of FA supplementation on rectal epithelial proliferation



	Rectal Epithelial Proliferation (%Ki-67)
Dietary Folic Acid	r=0.39 p<0.001
Plasma Folate	r=0.34 p<0.001
Plasma Hcyst	r= -0.37 p<0.001
# of Colorectal Tumors	r=0.23 p=0.034
# of Colorectal AdenoCA	r=0.28 p=0.02

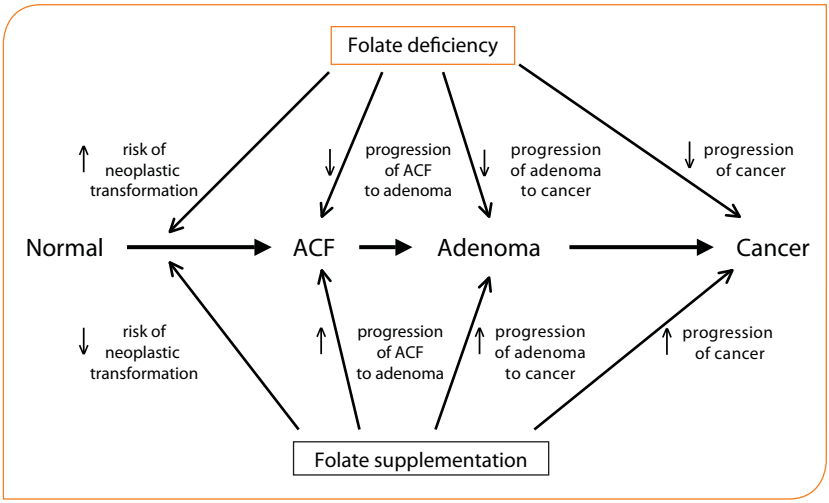
Folate and CRC

What have we learned from animal models?

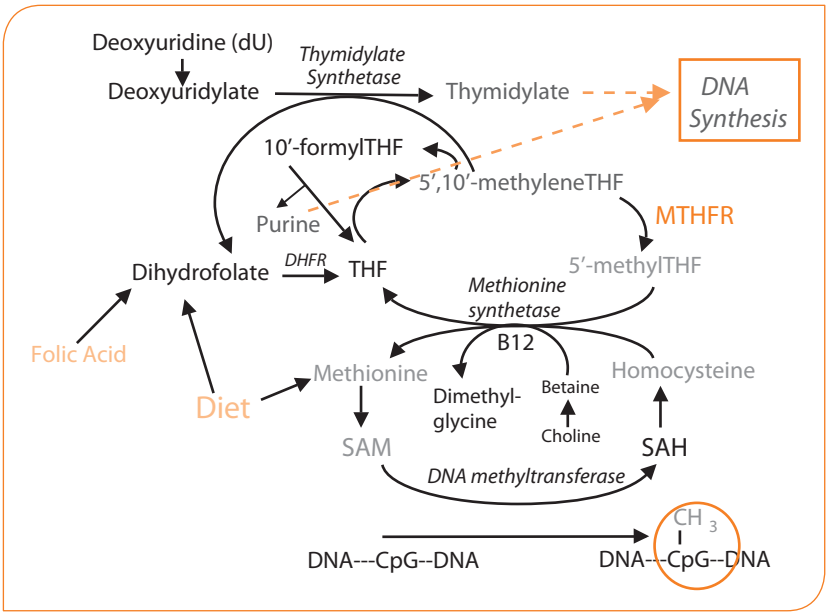
- Mild-moderate folate deficiency enhances colorectal carcinogensis in predisposed animals
- Modest levels of FA supplementation (4-10x) above the BDR suppress colorectal carcinogenesis if started before the establishment of neoplastic foci
- Folate status has an opposite effect on established neoplastic foci
- Adverse effect of supraphygiologic levels of FA supplementation

Effects of folate status on cancer

- Inhibition of growth of transplanted cancers in folate-deficient rats
- Inhibition of growth of virally-induced cancers by folate deprivation
- Delay in the development of nerve sheath tumors in transgenic mice by folate depletion
- Folate supplementation induces accelerated relapse and progression of leukemia



Biological mechanisms



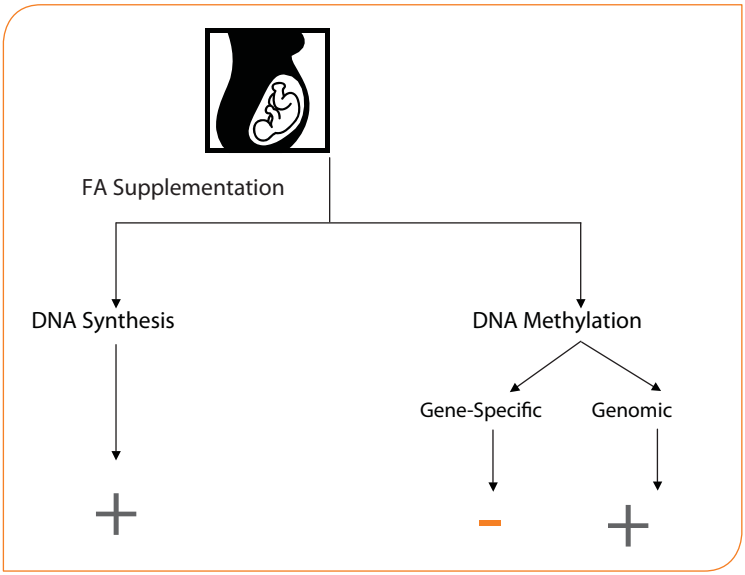
Effects of folate on normal colonic epithelial cells

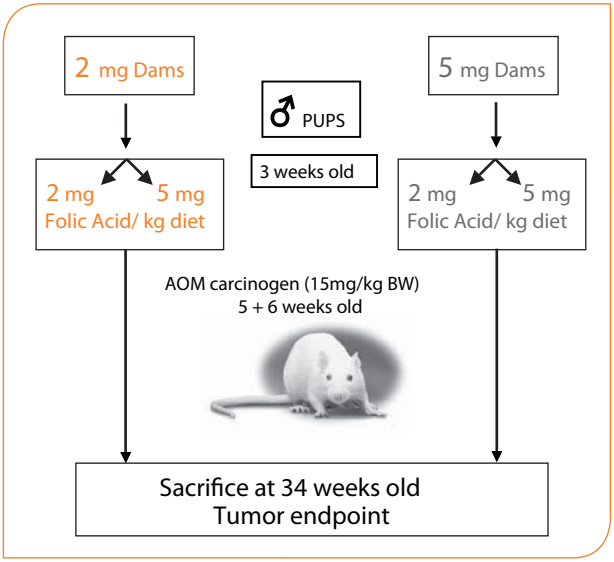
- Folate deficiency increases CRC risk
  - DNA strand breaks
  - Impaired DNA repair
  - Increased mutagenesis
  - **Genomic DNA hypomethylation?**
- FA supplementation decreases CRC risk
  - DNA stability & integrity
  - Optimal DNA repair
  - Decreased mutagenesis
  - **Prevention of aberrant DNA methylation?**

Effects of folate on colonic neoplastic cells

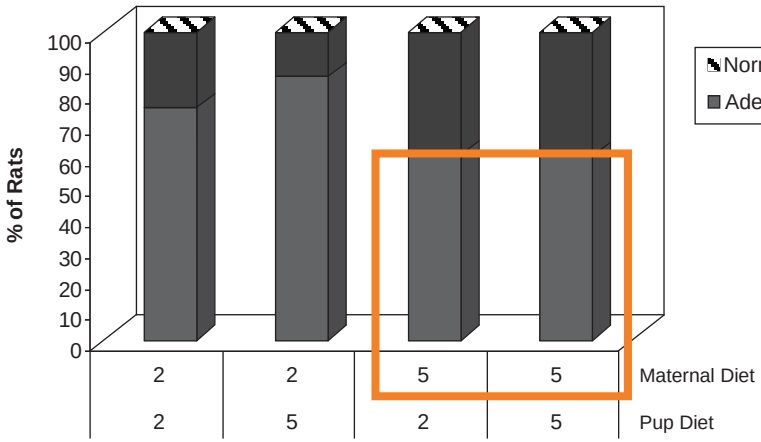
- Folate deficiency inhibits tumor progression
  - Ineffective DNA synthesis leading to inhibition of tumor growth & progression
  - **Reversal of promoter CpG islands hypermethylation?**
- FA supplementation promotes tumor progression
  - Provision of nucleotide precursors for proliferation & growth of neoplastic cells
  - **De novo methylation of promoter CpG islands of tumor suppressor genes leading to gene inactivation?**
  - Hypermutability of 5mC in CpG?

Effects of maternal FA supplementation on cancer risk



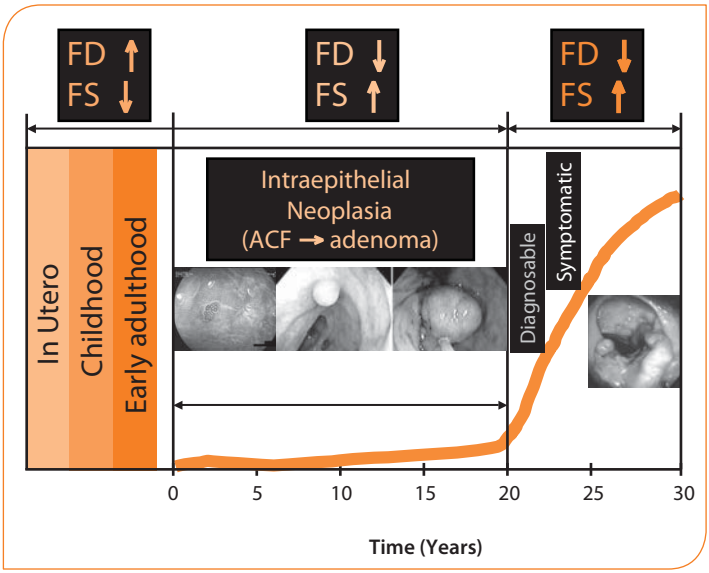


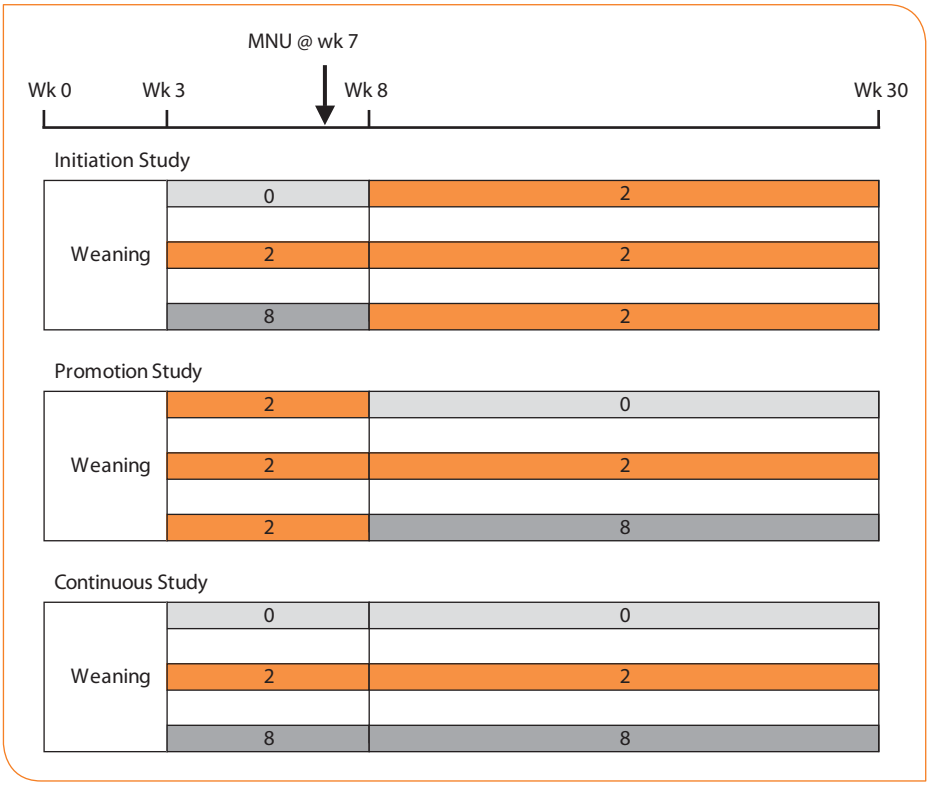
CRC incidence



OR =0.36 (p=0.003; 95%CI 0.18-0.71)

AACR 2008




MNU @ wk 7
Wk 0
Wk 3
Wk 8
Wk 30
Initiation Study
Weaning
0
2
2
2
8
2
Promotion Study
Weaning
2
0
2
2
2
8
Continuous Study
Weaning
0
0
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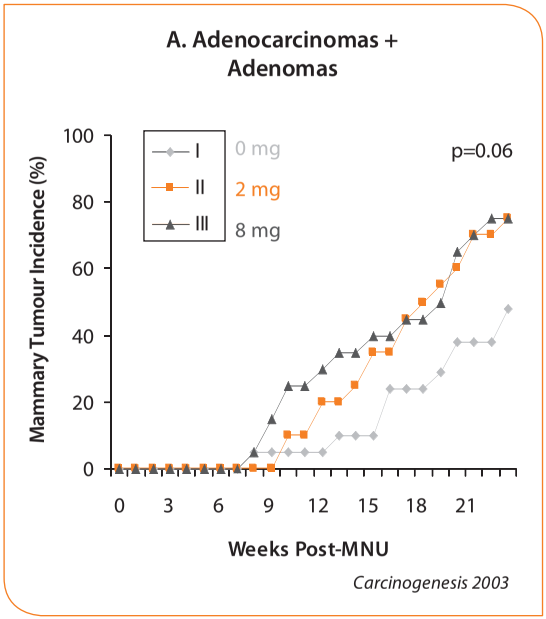
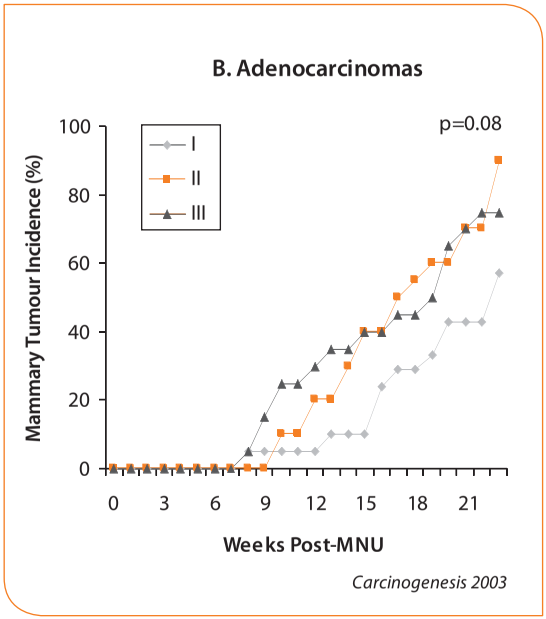
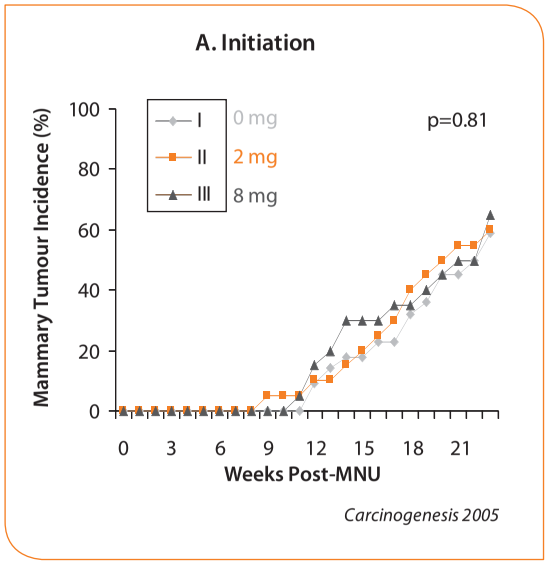
8
8

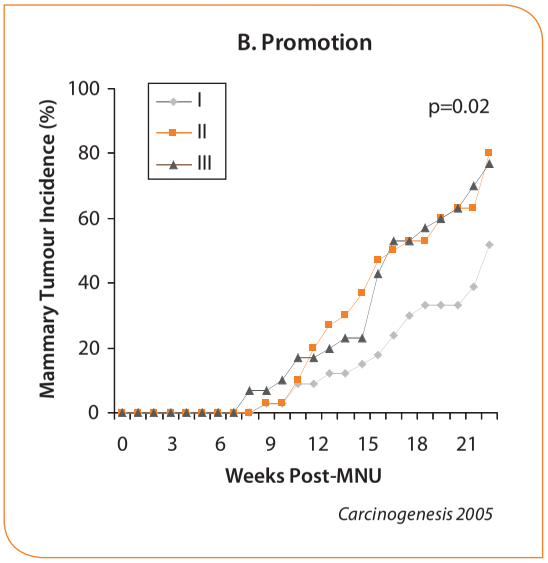
Effect of dietary folate on MNU-induced mammary tumorigenesis in rats

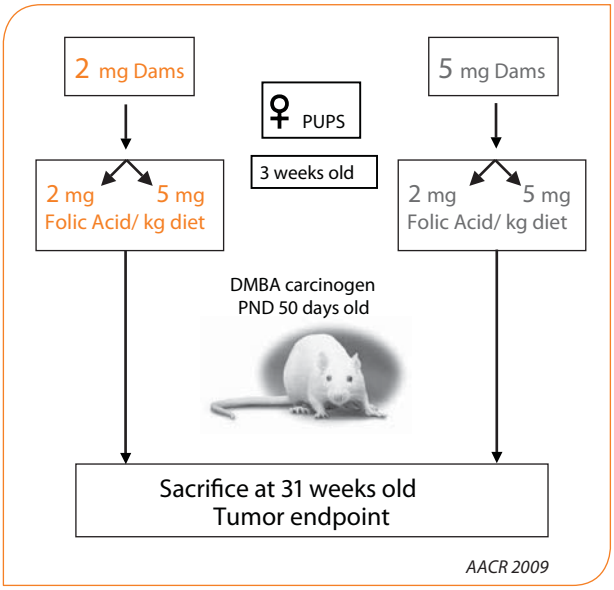




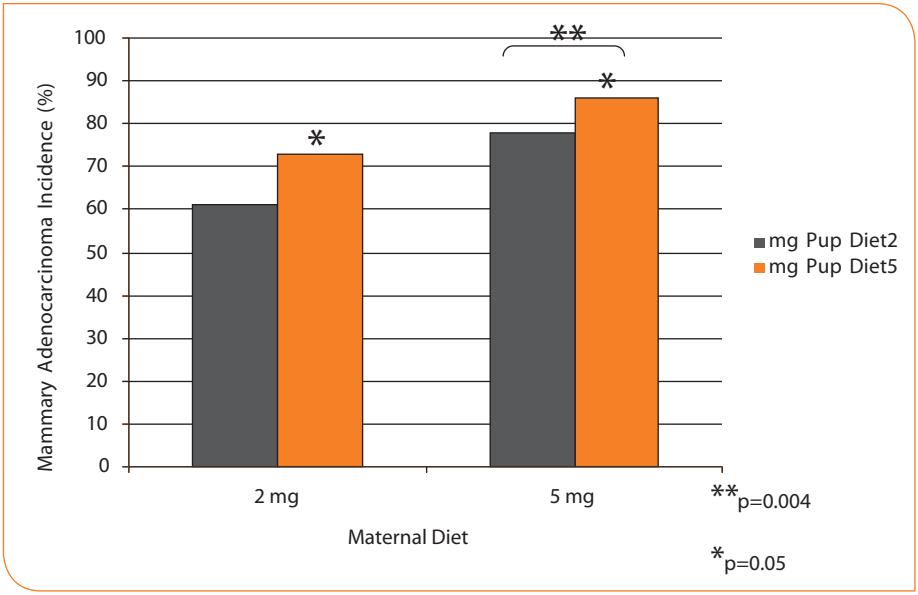
Effects of dietary folate on MNU-induced mammary adenocarcinomas in rats

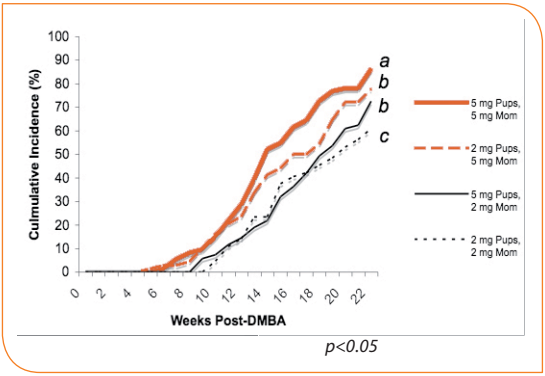






Mammary tumor incidence





FA supplementation and cancer prevention

- Timing of intervention
- Safe & effective dose range
- Site-specific effect
- Optimal preparation:
  folate vs. folic acid
- Applicability of animal data to humans

The DMH & AOM models

1,2-Dimethylhydrazine

↓

Azomethane

↓

Azoxymethane

↓

Methylazoxymethanol

↓

Methyldiazonium

↓

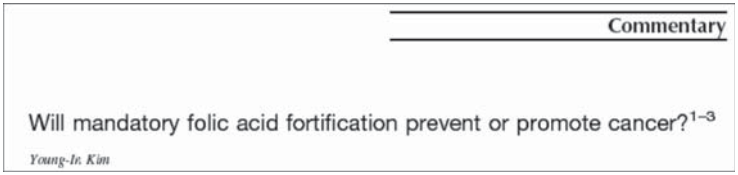
Carbonium

Comparability with human CRC

- distal > proximal
- histology
- adenoma - adenocarcinoma sequence
- molecular alterations (β-catenin, K-ras, C-myc)
- aberrant crypt foci (ACF)

Differences

- - high dosages of genotoxic chemicals
- - variable susceptibility
- - Apc and p53 alterations rare or absent
- - unique CRC susceptibility genes
- - flat foci of dysplasia



Commentary

Will mandatory folic acid fortification prevent or promote cancer?[1–3]

Young-In Kim

Am J Clin Nutr 2004; 80: 1123-8

- May prevent the development of new cancers
- May promote the progression of existing (pre) neoplastic lesions

460-377 BC Hippocrates' Primum non nocere, "First do no harm"

Acknowledgements

- University of Toronto
  - Jacquelin Song
  - Gillian Lindzon
  - Karen Sie
  - Anna Ly
  - Kyoung-Jin Sohn
  - Lillian Thompson
  - Steve Gallinger
  - Tak Mak
  - Alan Medline
  - Richard Renlund
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  - Ruth Croxford
- Tufts University
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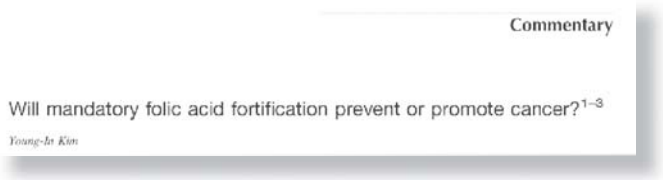
  - Jacob Selhub
  - Irwin Rosenberg
  - Sang Woon Choi
- Funding:
  - CIHR, NCIC, AICR

Overview of the experience following fortification with folic acid on cancer risks

STEIN EMIL VOLLSET
Norwegian Institute of Public Health and University of Bergen, Norway

American Journal of Clinical Nutrition 2004

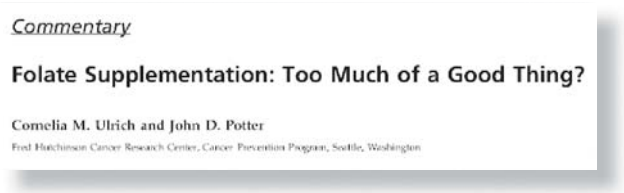


Commentary

Will mandatory folic acid fortification prevent or promote cancer?[1–3]

Young-In Kim

Cancer Epidemiology Biomarkers and Prevention 2006



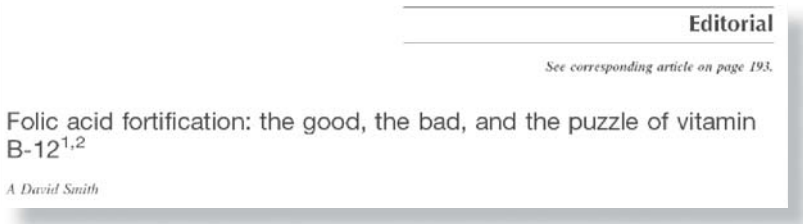
Commentary

Folate Supplementation: Too Much of a Good Thing?

Cornelia M. Ulrich and John D. Potter

Fred Hutchinson Cancer Research Center, Cancer Prevention Program, Seattle, Washington

American Journal of Clinical Nutrition 2007



Editorial

See corresponding article on page 193.

Folic acid fortification: the good, the bad, and the puzzle of vitamin B-12[1,2]

A David Smith

2007: 1.8 billion persons worldwide with access to fortified wheat flour

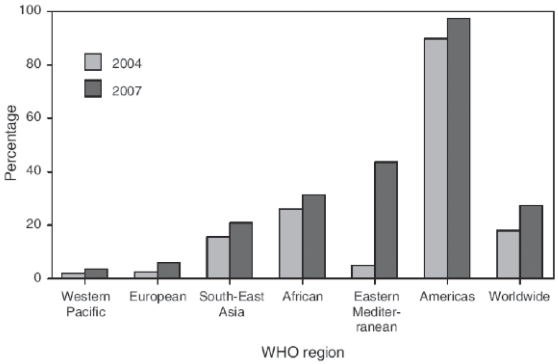
TABLE. Estimated number and percentage of persons and women who had access to fortified wheat flour and of newborns whose mothers had access to fortified wheat flour during pregnancy — worldwide, 2004 and 2007

Category	Total population	2004		2007		Change from 2004 to 2007	
		No.*	(%)	No.*	(%)	No.	(%)
Total persons	**6,512,822†**	**1,271,363**	**(19.5)**	**1,810,659**	**(27.8)**	**539,297**	**(8.3)**
Women aged 15–60 yrs	**2,142,225†**	410,091	(19.1)	577,461	(27.0)	167,370	(7.8)
Newborns whose mothers had access	**133,804§**	27,052	(20.2)	41,060	(30.7)	14,007	(10.5)

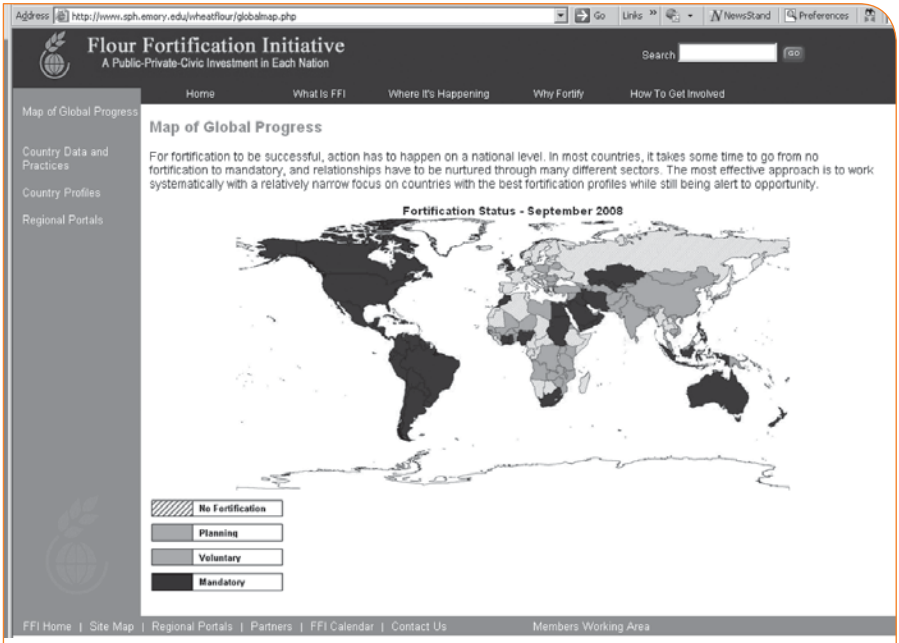
* In thousands. Calculated from data from the Flour Fortification Initiative, available at http://www.sph.emory.edu/wheatflour.
† In thousands, mid-2006 estimate. From U.S. Central Intelligence Agency, available at http://www.cia.gov.
§ In thousands. From United Nations International Children's Emergency Fund (UNICEF) birth rate estimates, available at http://www.unicef.org.

MMWR January 2008; www.cdc.gov/mmwr

FIGURE. Percentage of wheat flour processed in roller mills that was fortified — worldwide and by World Health Organization (WHO) region, 2004 and 2007



MMWR January 2008; www.cdc.gov/mmwr



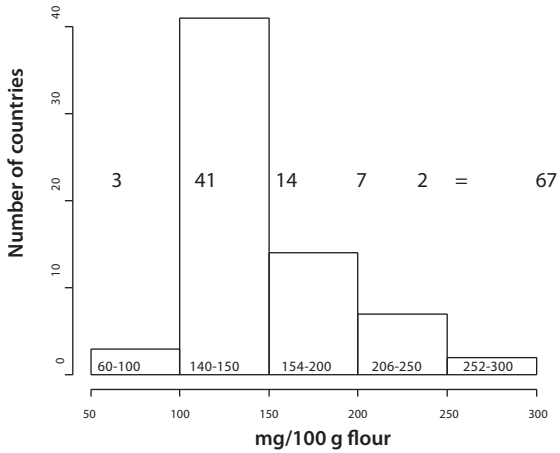
Source: Flour fortification initiative, www.sph.emory.edu/wheatflour

Folic acid fortification time line

Jamaica Guatemala	Ecuador El Salvador **USA**	**Canada** Belize **Chile** Costa Rica Honduras Panama Lesotho	Bolivia Colombia Paraguay Oman	Malawi	Bangladesh Saudi Arabia United Arab Emirates	Kuwait Bahrain Indonesia	Argentina Nicaragua Azerbaijan Jordan Kyrgyzstan Mongolia Qatar **South Africa**	Uzbekistan	Brazil Dominican Republic Afghanistan Syria Tajikistan	Fiji Uruguay Yemen	Congo Cote d'Ivoire Georgia India Kazakhstan Palestine Sudan Turkmenistan Uganda	Egypt Iran Morocco Pakistan	Barbados Cuba Grenada Guyana Haiti Mexico Puerto Rico Saint Vincent Ghana Israel Romania Ukraine New Zealand	Australia
pre-1996	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009

Source: Flour fortification initiative, www.sph.emory.edu/wheatflour

Folic acid flour fortification dose



Source: Flour fortification initiative, www.sph.emory.edu/wheatflour

Hypothesis

A Temporal Association between Folic Acid Fortification and an Increase in Colorectal Cancer Rates May Be Illuminating Important Biological Principles: A Hypothesis

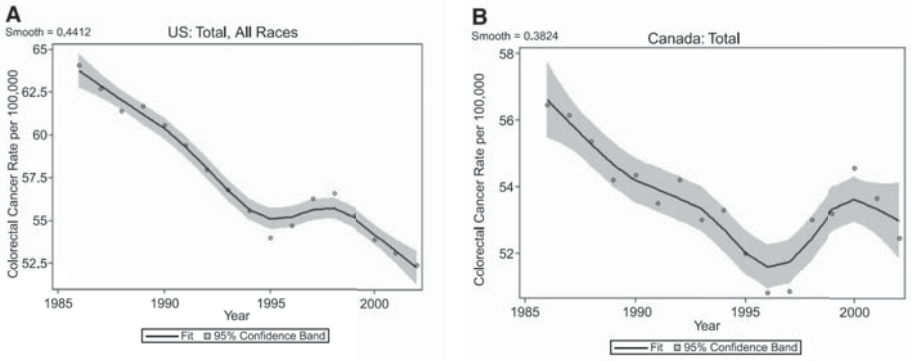
Joel B. Mason,[1,2] Aaron Dickstein,[2] Paul F. Jacques,[1] Paul Haggarty,[3] Jacob Selhub,[1] Gerard Dallal,[1] and Irwin H. Rosenberg[1,2]

[1]Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University; [2]Tufts University School of Medicine, Boston, Massachusetts; and [3]Rowett Research Institute, University of Aberdeen, Aberdeen, United Kingdom

Cancer Epidemiol Biomarkers Prev 2007;16(7). July 2007

Colorectal cancer incidence

A Temporal Association between Folic Acid Fortification and an Increase in Colorectal Cancer Rates May Be Illuminating Important Biological Principles: A Hypothesis



Mason et al. Cancer Epidemiol Biomarkers Prev 2007

Screening for colorectal cancer –endoscopy of colon and/or rectum

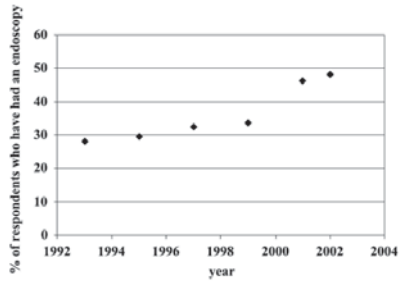
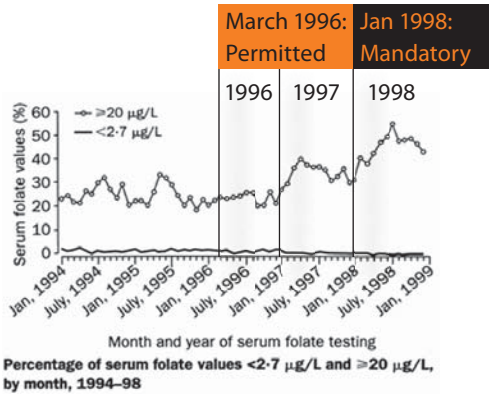


Figure 3. Endoscopy rates of the colon and/or rectum in the United States from 1993 to 2003. Endoscopy rates of the colon and/or rectum from 1993 to 2003 based on the Centers for Disease Control and Prevention Behavioral Risk Factor Surveillance System Surveys (32). A positive response was one in which a subject reported that they had undergone the procedure.

Mason et al. Cancer Epidemiol Biomarkers Prev 2007

Southern California, prevalence of high folate values 1994-1999



Percentage of serum folate values <2·7 µg/L and ≥20 µg/L, by month, 1994–98

Lawrence. Lancet 1999

Canada, serum folate 1996-2003 in elderly women

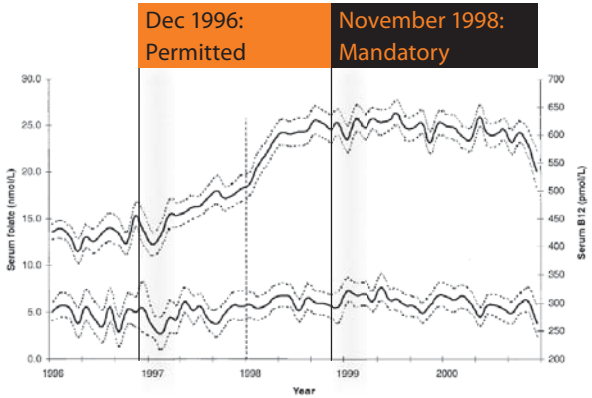
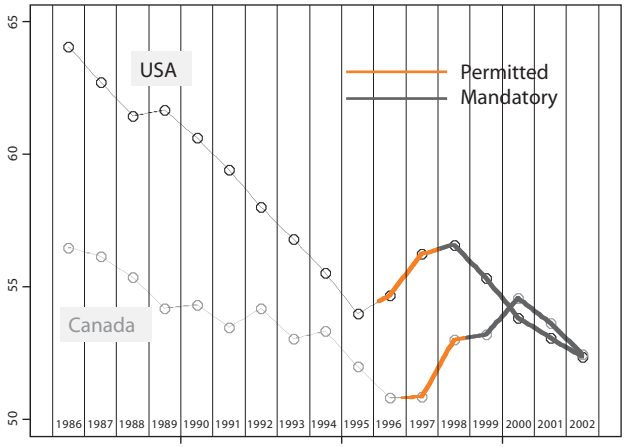


Fig. 1. Change in serum folate (top) and vitamin B12 (bottom) concentrations among Canadian women aged 65 years and older, before and after folic acid food fortification (vertical dashed line). Data are presented as means and 95% confidence limits.

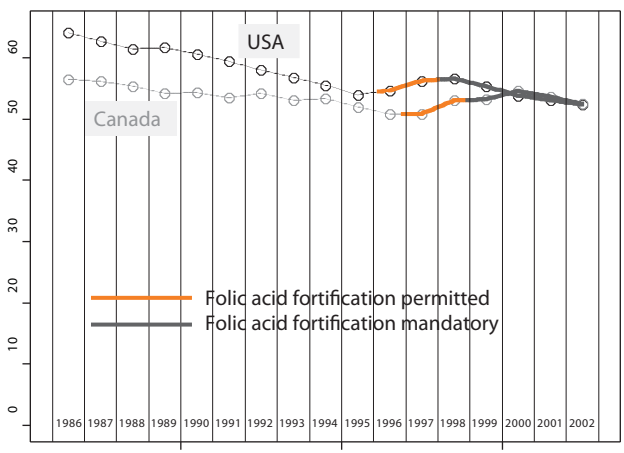
Ray et al. Clin Biochem 2003

"Mason-data": colorectal cancer incidence (per 100,000) USA and Canada 1986-2002



Redrawn after Figure in Mason et al 2007

"Mason-data": colorectal cancer incidence (per 100,000) USA and Canada 1986-2002



Redrawn after Figure in Mason et al 2007

Discussion points - Lancet

- Lancet-discussion (December 2007, April 2008)
  - Bayston et al, Dec 2007 – raise issues of timing of folate fortification, timing of screening increase, mortality.
  - and concludes the data should noy cause concern
  - Exchange of discussion points Mason et al & Bayston et al (April 2008)
- UK Scientific Advisory Committee on Nutrition (SACN) meets 21 January 2008 to discuss folic acid and CRC risk

SACN discussion - 2008

- Significance/non-significance of trend-change
- Discussion of changes in incidence/mortality patterns after breast and cervical cancer screening
- With CRC screening one would expect short term increase in incidence (earlier stage detection) and long term decrease
- In 1995 US Preventive Services Task Force reversed position and endorsed fecal occult blood testing and sigmoidoscopy for CRC screening. Could explain incidence increase.
- Questioned the IMMEDIATE response of CRC incidence on folate fortification
- Conclusion: no certain explanation for increase in CRC incidence around time of introduction of folate fortification in USA and Canada: either increased rates of colonoscopy or higher intakes of folic acid may have been responisble

Additional points

- Sudden cancer incidence change likely to be caused by change in screening practice or data collection (case ascertainment, definition, diagnostic practice)
- Data from other countries that have introduced folate fortification should be considered
- Data on cancer mortality, not only incidence
- Data on other cancers: prostate, breast, lung, hematological, all cancers combined

Chile, colorectal cancer mortality 1990-2003

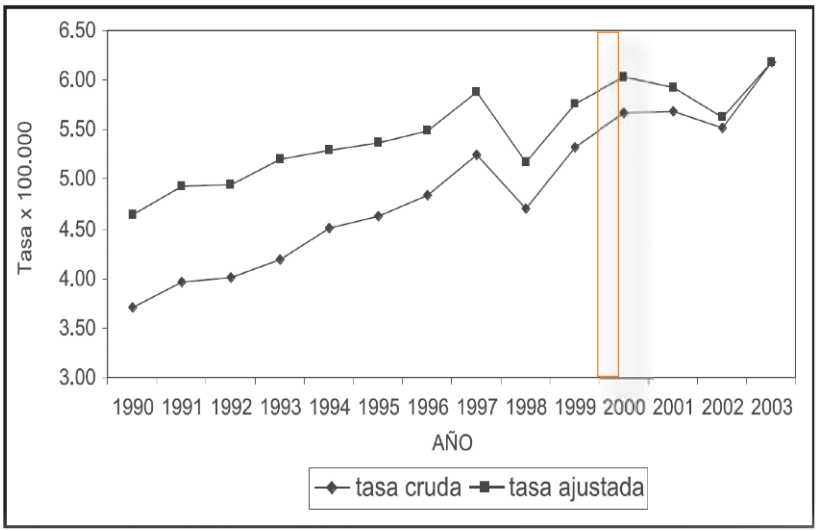
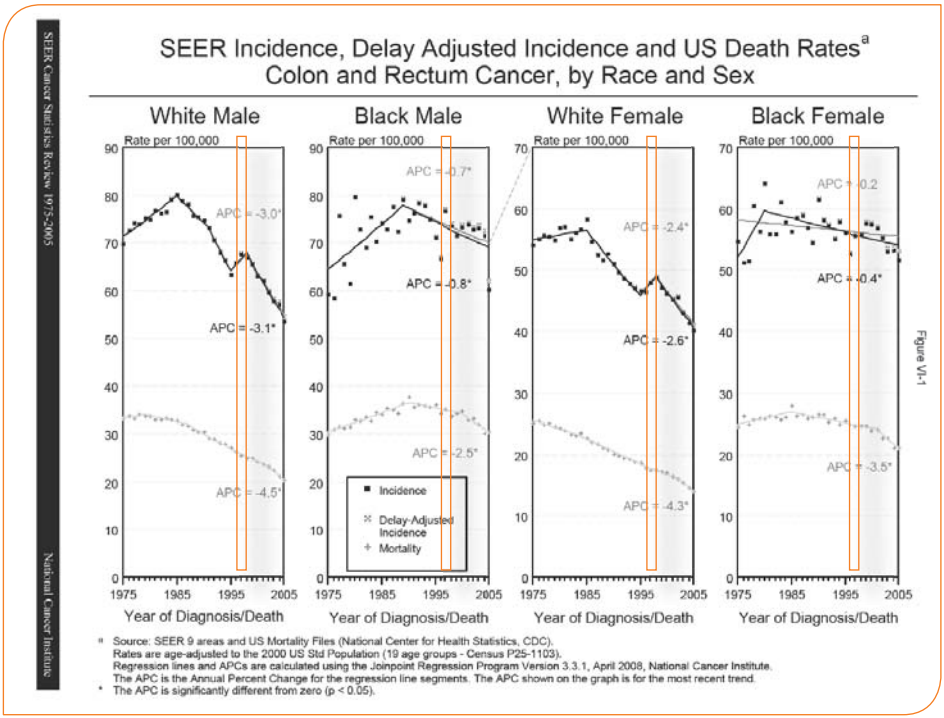


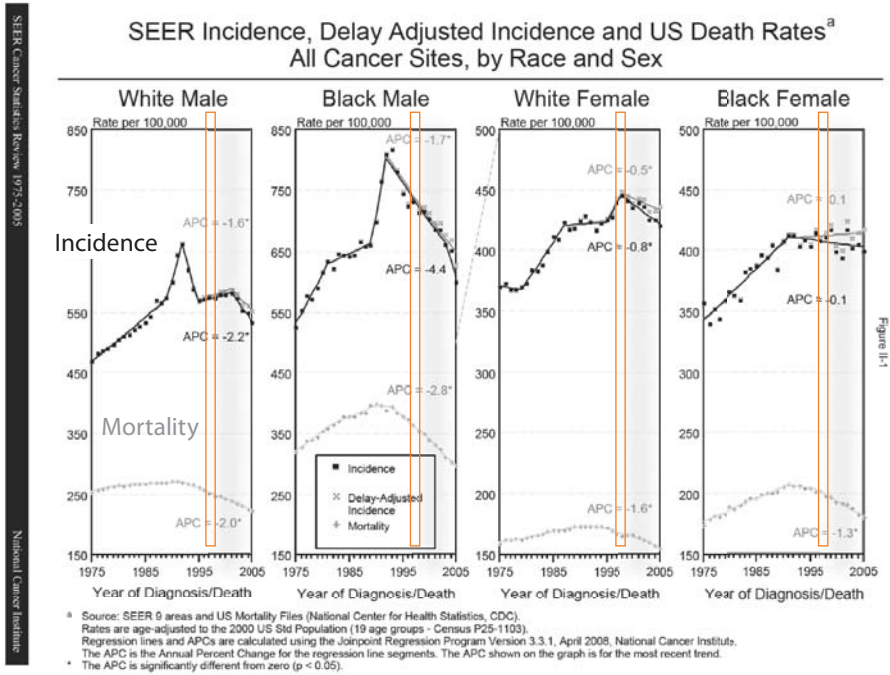
FIGURA 1. Tasas de mortalidad general por cáncer de colon, crudas y ajustadas por edad. Chile 1990-2003.

Folate fortification (0.22 mg/100g)
From January 2000,
Effective April 2000

U.S. SEER data: colorectal cancer 1975-2005



SEER data: all cancer sites 1975-2005



SEER data: prostate cancer 1975-2005

SEER Cancer Statistics Review 1975-2005

National Cancer Institute

SEER Incidence, Delay Adjusted Incidence and US Death Rates[a]
Prostate Cancer, by Race

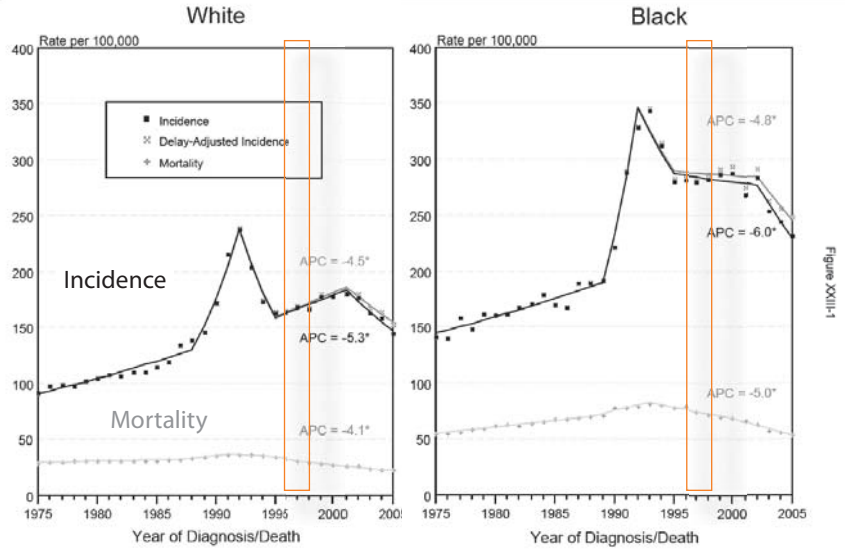
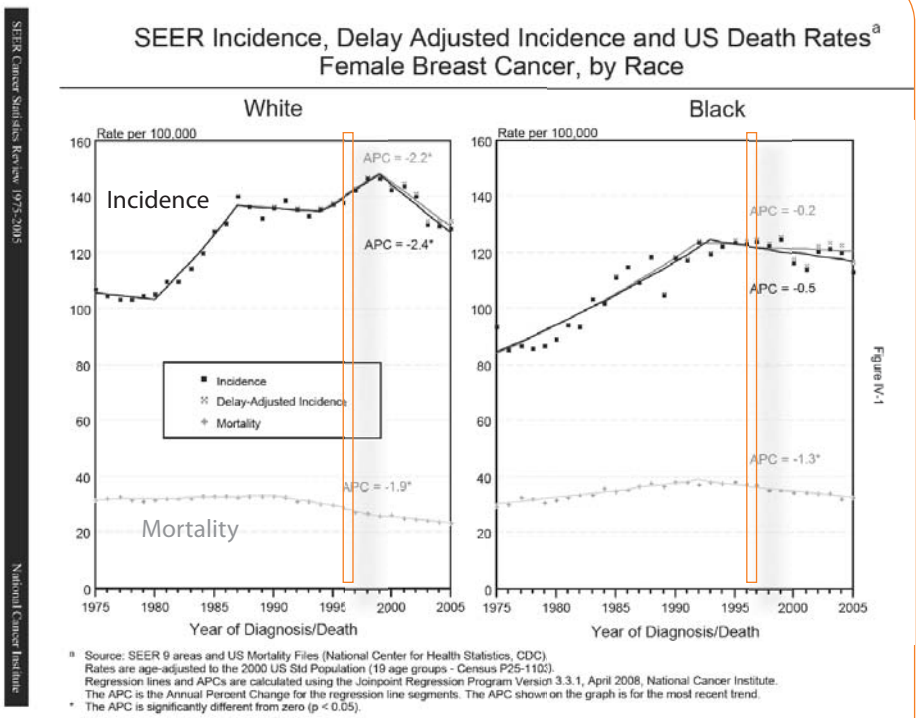


Figure XXIII-1

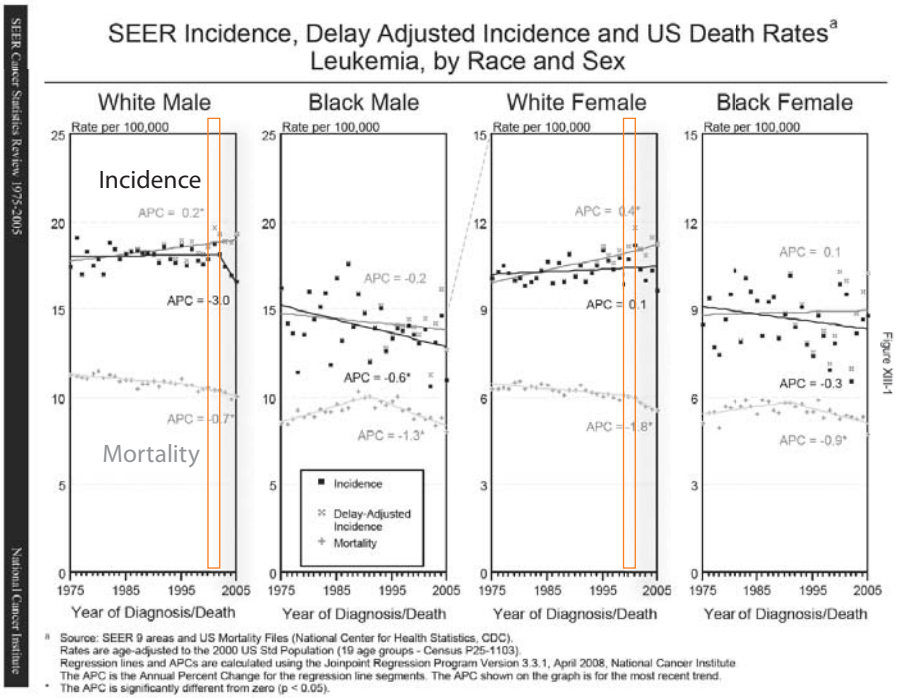
[a] Source: SEER 9 areas and US Mortality Files (National Center for Health Statistics, CDC).
Rates are age-adjusted to the 2000 US Std Population (19 age groups - Census P25-1103).
Regression lines and APCs are calculated using the Joinpoint Regression Program Version 3.3.1, April 2008, National Cancer Institute.
The APC is the Annual Percent Change for the regression line segments The APC shown on the graph is for the most recent trend.
* The APC is significantly different from zero ($p < 0.05$).

SEER data: breast cancer 1975-2005



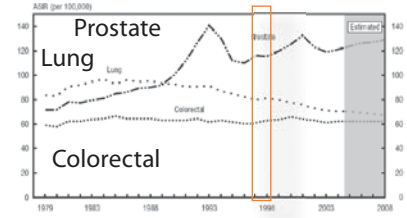
[a] Source: SEER 9 areas and US Mortality Files (National Center for Health Statistics, CDC).
Rates are age-adjusted to the 2000 US Std Population (19 age groups - Census P25-1130).
Regression lines and APCs are calculated using the Joinpoint Regression Program Version 3.3.1, April 2008, National Cancer Institute.
The APC is the Annual Percent Change for the regression line segments. The APC shown on the graph is for the most recent trend.
* The APC is significantly different from zero ($p < 0.05$).

SEER data: leukemia 1975-2005

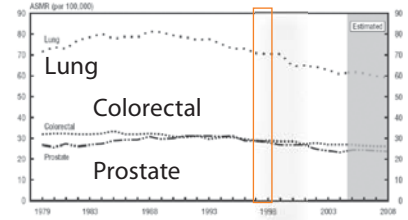


[a] Source: SEER 9 areas and US Mortality Files (National Center for Health Statistics, CDC).
Rates are age-adjusted to the 2000 US Std Population (19 age groups - Census P25-1103).
Regression lines and APCs are calculated using the Joinpoint Regression Program Version 3.3.1, April 2008, National Cancer Institute
The APC is the Annual Percent Change for the regression line segments. The APC shown on the graph is for the most recent trend.
* The APC is significantly different from zero ($p < 0.05$).

Canada 1979-2008, men

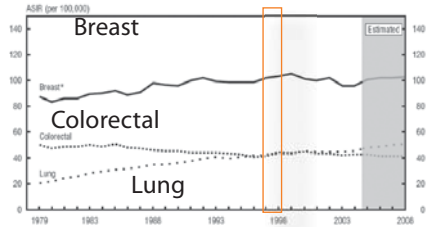


Incidence

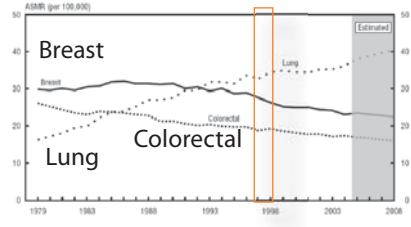


Mortality

Canada 1979-2008, women



Incidence



Mortality

Conclusion

- There is only limited evidence to suggest that folate fortification increase colorectal cancer risk
- In my opininon the evidence is weak for claiming that folate fortification is responsible for the transient increase in CRC incidence in the US and Canada.
- Screening practice or other factors (e.g, data collection) are likely to have played a role
- "Effect" was immediate which may not be plausible (if true similar effect should be observed in randomized trials – covered by other speakers)
- Careful analyses of trends in other countries should be performed
- Many (most) of the countries where folate fortification has been introduced lack good data on cancer incidence.
- Recently introduced folate fortification programs in New Zealand and Australia will provide possibilities for similar trend analyses in the coming 3-5 years
- Whatever is observed in national trend statistics need to be supplemented by data from more controlled designs – trial data and trial metaanalyses

Assessment of studies thought to suggest associations between folic acid and cancers of the colon and breast

NICHOLAS WALD
Wolfson Institute of Preventive Medicine
Barts & The London School of Medicine and Dentistry
Queen Mary University of London

Abstract:

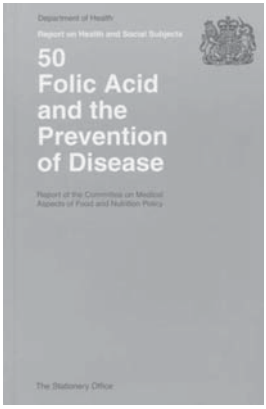
Two studies have been reported as suggesting a link between folic acid intake and an increased incidence of colon adenomas. One study has been reported as suggesting an association between the use of folic acid in pregnancy and breast cancer some 35 years later. These studies are examined in detail to see whether there are associations to be explained, and examine whether the concerns that have been raised are warranted. The conclusion is that there is no evidence that folic acid increases the risk of colon adenomas or breast cancer.

Presentation:

THE LANCET
Vol 338 Saturday 20 July 1991 No 8760

Prevention of neural tube defects: Results of the Medical Research Council Vitamin Study

MRC VITAMIN STUDY RESEARCH GROUP



"On scientific, medical and public health grounds, the committee concluded that universal folic acid fortification of flour at 240 µg/100 g in food products as consumed would have a significant effect in preventing NTD-affected conceptions and births without resulting in unacceptably high intakes in any group in the population."

COMA Report, 2000

Countries with mandatory fortification of flour with folic acid in place or agreed

Argentina	El Salvador	Mexico	Surinam
Australia	Fiji	Morocco	Trinidad and Tobago
Bahrain	Ghana	New Zealand	Turkmenistan
Barbados	Grenada	Nicaragua	Uruguay
Belize	Guadalupe	Oman	USA
Bolivia	Guatemala	Palestine, Occupied Territory	Venezuela
Brazil	Guyana	Panama	Yemen
Canada	Haiti	Paraguay	
Chile	Honduras	Peru	
Colombia	Indonesia	Puerto Rico	
Costa Rica	Iran	Qatar	
Cote d'Ivoire	Iraq	Saudi Arabia	
Cuba	Jamaica	South Africa	
Dominican Republic	Jordan	St. Vincent	
Ecuador	Kuwait	Sudan	

52 Countries

No EU country has fortified

NEWS HEADLINES > **REGULATION**

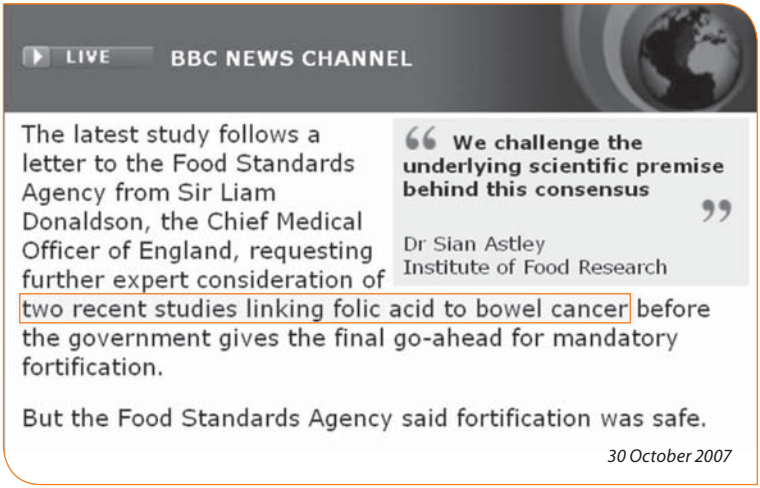
FSA finally agrees to recommend folic acid fortification

By Jess Halliday, 18-May-2007

The board of the UK's Food Standards Agency yesterday agreed unanimously to recommend mandatory fortification of some foods with folic acid, but whether it is bread or flour is still up for debate.

The decision, which was made at an open board meeting in London, comes as no great surprise since the FSA's Scientific Advisory Committee on Nutrition (SACN) gave a positive recommendation at the end of last year. The agency subsequently launched its final consultation, and received around 200 responses from stakeholders.

18 May 2007



LIVE BBC NEWS CHANNEL

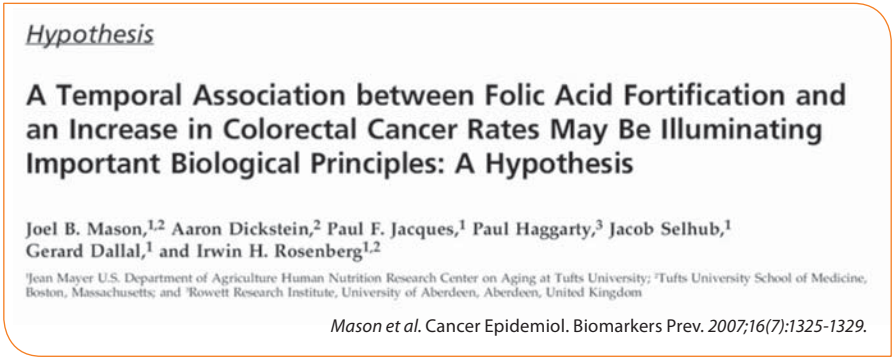
The latest study follows a letter to the Food Standards Agency from Sir Liam Donaldson, the Chief Medical Officer of England, requesting further expert consideration of two recent studies linking folic acid to bowel cancer before the government gives the final go-ahead for mandatory fortification.

> **"We challenge the underlying scientific premise behind this consensus"**
>
> Dr Sian Astley
> Institute of Food Research

But the Food Standards Agency said fortification was safe.

30 October 2007

Time trends relating to folic acid fortification and incidence of colorectal cancer in the USA and Canada



Hypothesis

A Temporal Association between Folic Acid Fortification and an Increase in Colorectal Cancer Rates May Be Illuminating Important Biological Principles: A Hypothesis

Joel B. Mason,[1,2] Aaron Dickstein,[2] Paul F. Jacques,[1] Paul Haggarty,[3] Jacob Selhub,[1] Gerard Dallal,[1] and Irwin H. Rosenberg[1,2]

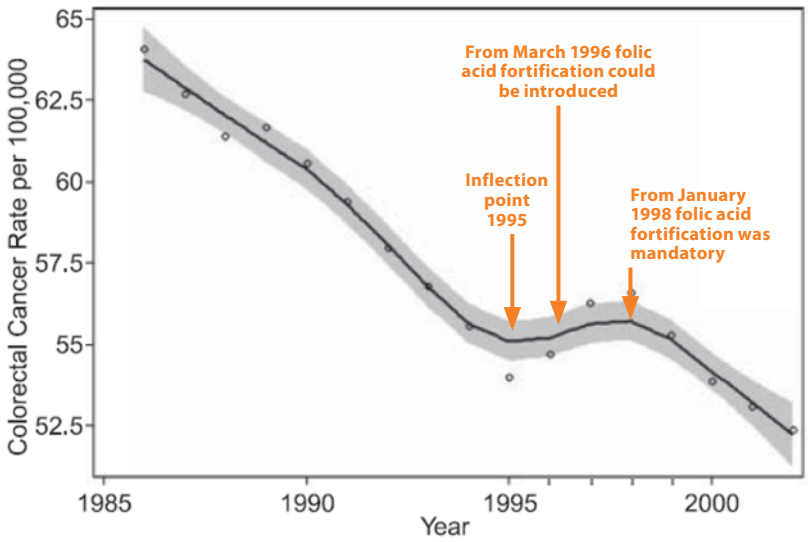
[1]Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University; [2]Tufts University School of Medicine, Boston, Massachusetts; and [3]Rowett Research Institute, University of Aberdeen, Aberdeen, United Kingdom

Mason et al. Cancer Epidemiol. Biomarkers Prev. *2007;16(7):1325-1329.*

Comments on the Mason et al hypothesis paper

- Bayston, Russell, Wald, and Hoffbrand. *Lancet* 2007;370:2004.
- Mason, Cole, Baron, Kim and Smith. *Lancet* 2008;371:1335.
- Bayston, Russell, Wald, and Hoffbrand. *Lancet* 2008;371:1335-1336.

Colorectal Cancer Incidence in USA 1986-2002



Plot taken from: Mason et al. Cancer Epidemiol. Biomarkers Prev. *2007;16(7):1325-1329.*

Trends in serum folate after food fortification in California

Year	No. of tests	% Test results < 2.7 ng/ml
1994	14493	1.3
1995	14750	1.3
1996	17642	1.3
1997	22805	0.6
1998	22662	0.3

Lawrence et al, Lancet, *1999, vol 354 no 9182, p. 915*

Year	No. of tests	% Test results < 2.7 ng/ml
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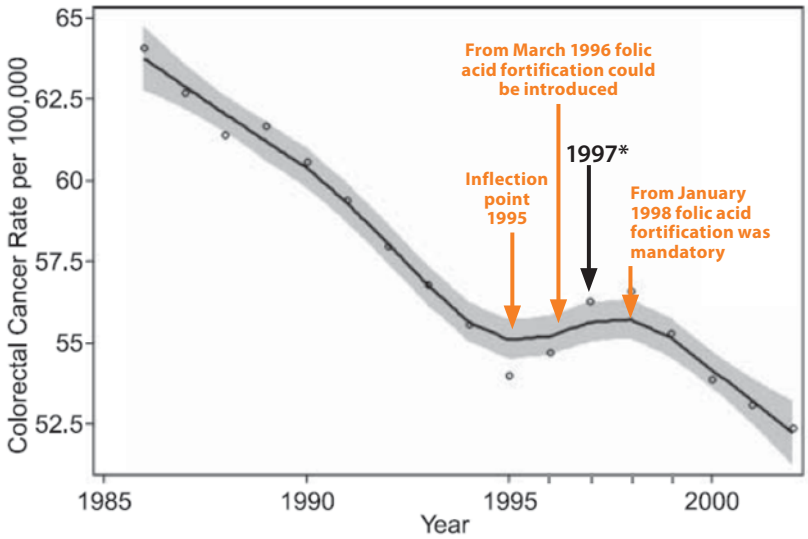
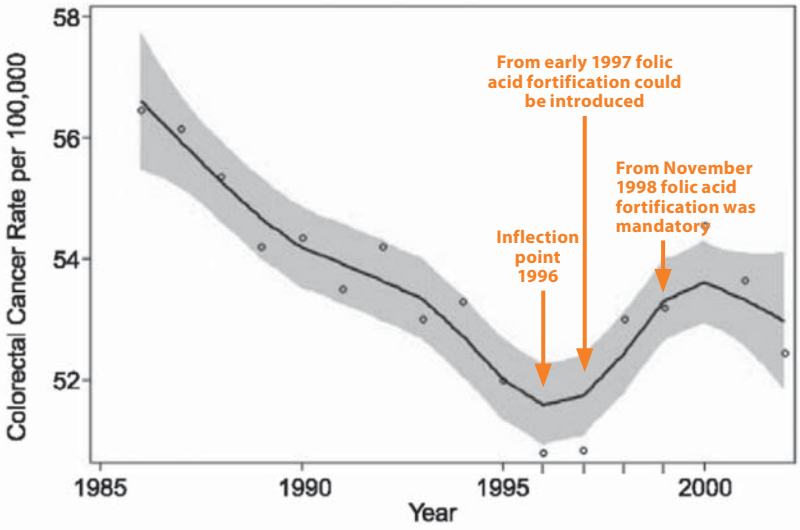
Colorectal Cancer Incidence in USA 1986-2002



** From: Lawrence et al,* Lancet, *1999, vol 354 no 9182, p. 915.*
Plot taken from: Mason et al. Cancer Epidemiol. Biomarkers Prev. *2007;16(7):1325-1329.*

Fortification cannot have caused an increase in the incidence of colorectal cancer if the increase in cancer rates occurred before the introduction of fortification.

Colorectal Cancer Incidence in Canada 1986-2002

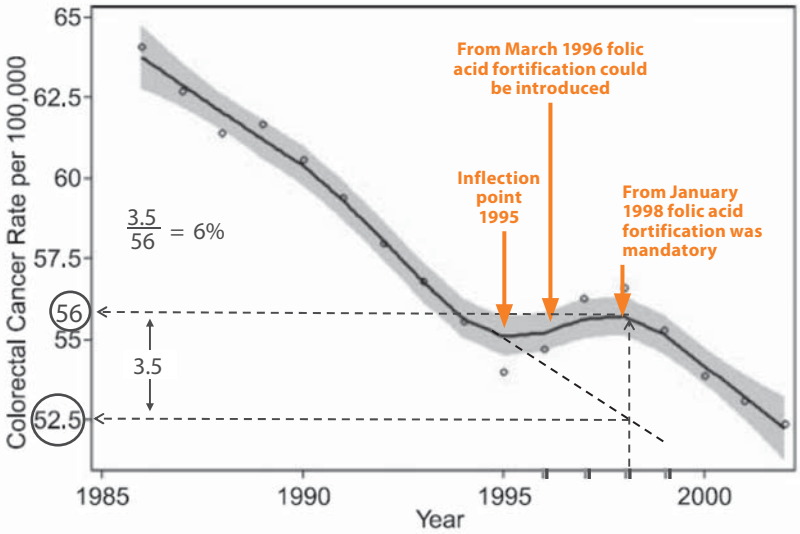


From: Ray et al. Clin. Biochem. *2003;36(5):387-391.*
Plot taken from: Mason et al. Cancer Epidemiol. Biomarkers Prev. *2007;16(7):1325-1329.*

The same temporal sequence between the introduction of fortification and the rise in colorectal cancer is seen in Canada as in the United States.

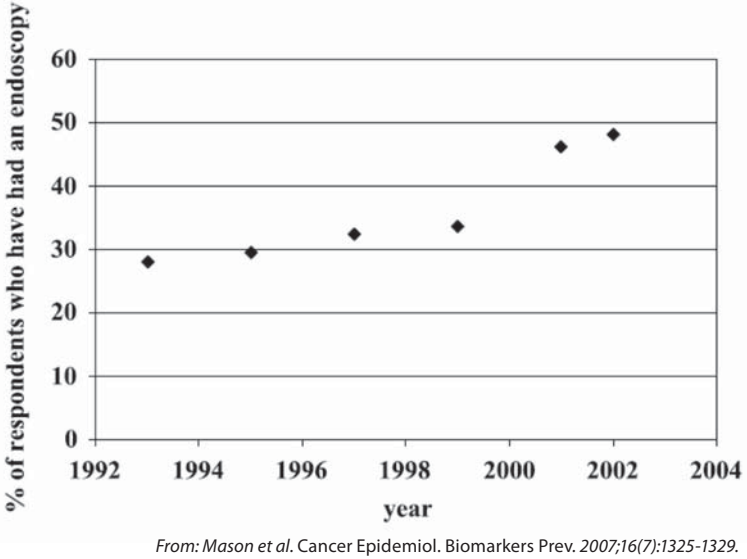
Can the temporary increase in colorectal cancer incidence be explained?

Colorectal Cancer Incidence in USA 1986-2002



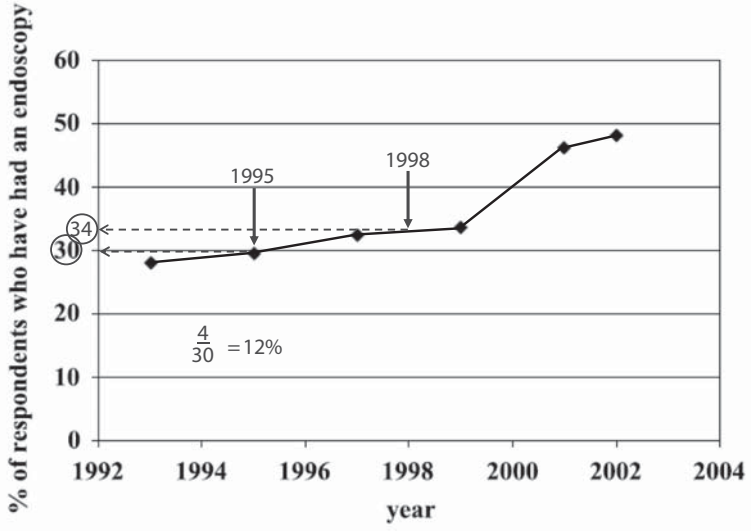
Plot taken from: Mason et al. Cancer Epidemiol. Biomarkers Prev. *2007;16(7):1325-1329.*

Endoscopy rates of the colon and/or rectum in the United States from 1993 to 2003 in persons aged 50 or more



From: Mason et al. Cancer Epidemiol. Biomarkers Prev. *2007;16(7):1325-1329.*

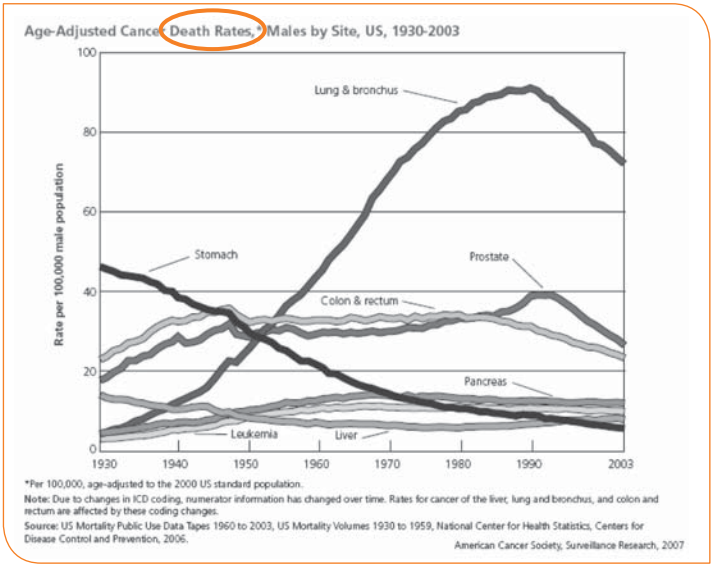
Endoscopy rates of the colon and/or rectum in the United States from 1993 to 2003 in persons aged 50 or more

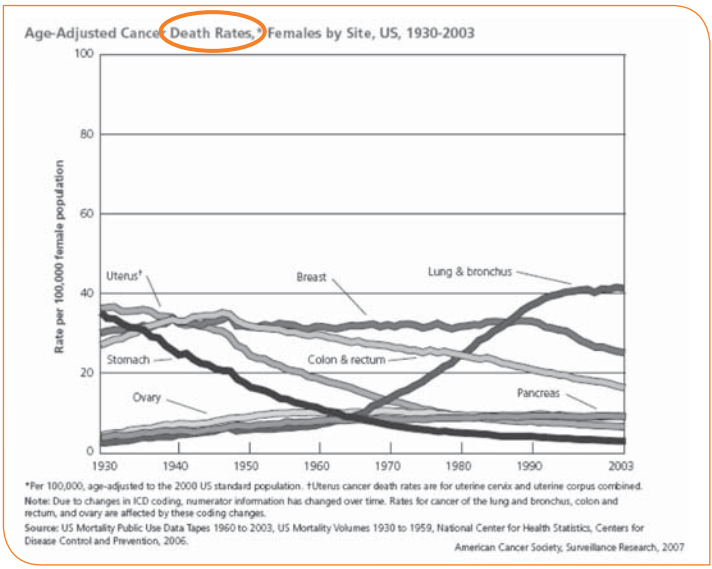


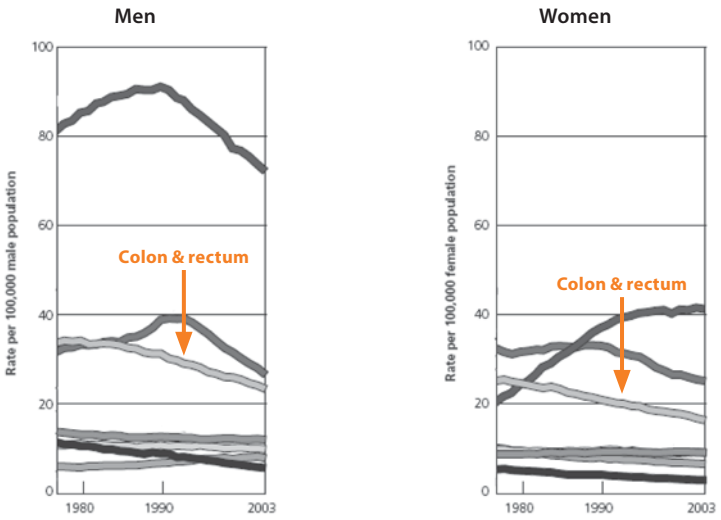
Based on: Mason et al. Cancer Epidemiol. Biomarkers Prev. *2007;16(7):1325-1329.*

In the US, between 1995 and 1998, there was a 12% increase in endoscopic colorectal screening in persons aged 50 or more and a 6% rise in colorectal cancer incidence so screening could explain the increase in incidence.

Examination of trends in mortality as well as incidence

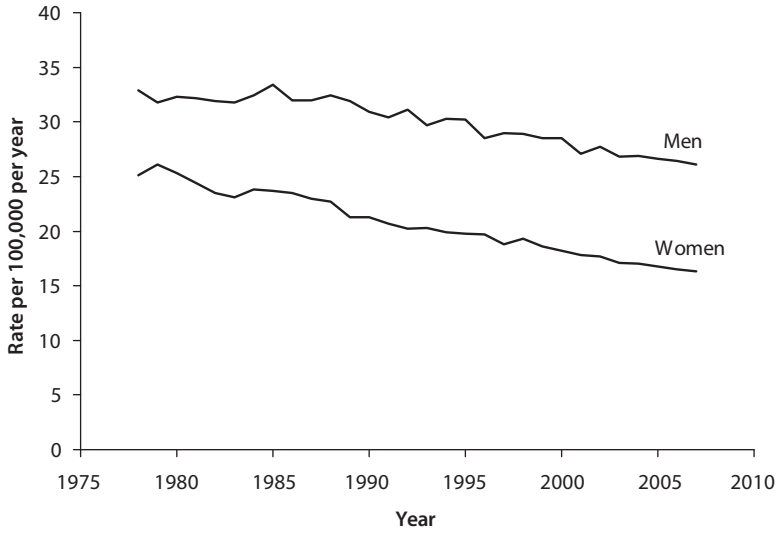






Age-Adjusted Cancer Death Rates USA, 1980-2003

Age-standardized mortality rates for colorectal cancer, Canada, 1978-2007



Canadian Cancer Statistics 2007

Since the beginning of the period covered by the introduction of fortification and the temporary increase in colorectal cancer incidence, there has been a continuous decrease in colorectal cancer mortality.

The temporary rise in colorectal cancer incidence could have two explanations:

1. Increase in exposure to a new cause
2. Earlier diagnosis from screening.

When screening for a cancer is increased there is an increased **incidence** of the cancer being screened for, because of earlier detection, without an increase in **mortality** from that cancer.

This was observed.

If an increase in incidence were due to an increased exposure to a cause of the cancer, there would be an increase in mortality from that cancer.

This was **not** observed.

We can therefore exclude folic acid fortification as a cause of the temporary increase in colorectal cancer incidence in the US and Canada.

The most likely explanation for the increase is colorectal cancer screening.

Interpretation of randomized trials of folic acid and the prevention of colorectal adenomas

Folic Acid for the Prevention of Colorectal Adenomas

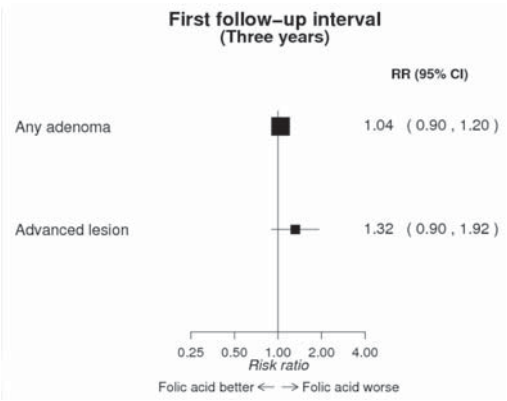
A Randomized Clinical Trial

Bernard F. Cole, PhD
John A. Baron, MD

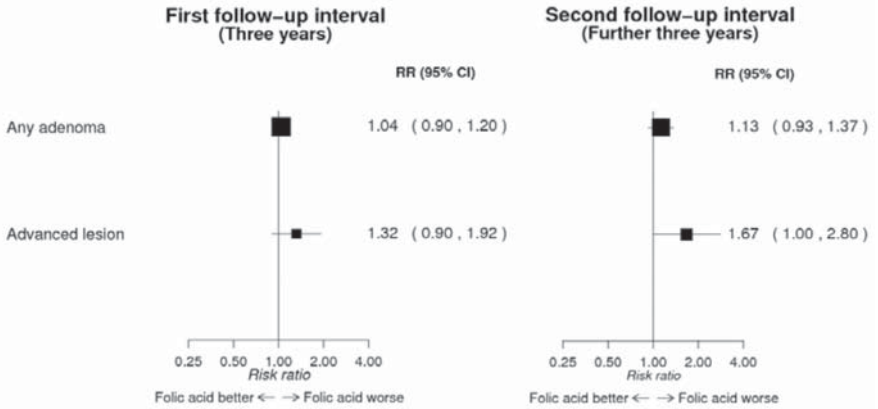
Context Laboratory and epidemiological data suggest that folic acid may have an antineoplastic effect in the large intestine.

1 mg/day folic acid vs. no folic acid

Cole et. al. JAMA *June 6 2007; 297:21; 2351-59*



Adapted from Table 3 in Cole et. al. JAMA *June 6 2007; 297:21; 2351-59*



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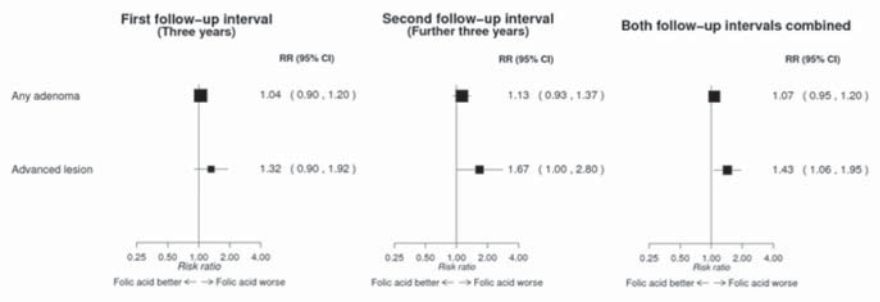
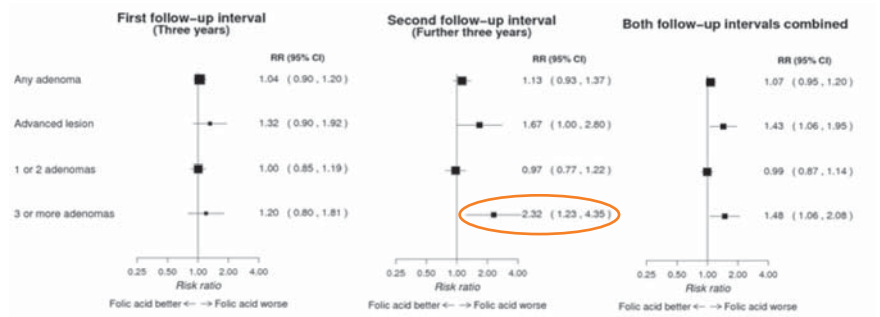


Adapted from Table 3 in Cole et. al. JAMA *June 6 2007; 297:21; 2351-59*



Adapted from Table 3 in Cole et. al. JAMA *June 6 2007; 297:21; 2351-59*

GASTROENTEROLOGY 2008;134:29–38

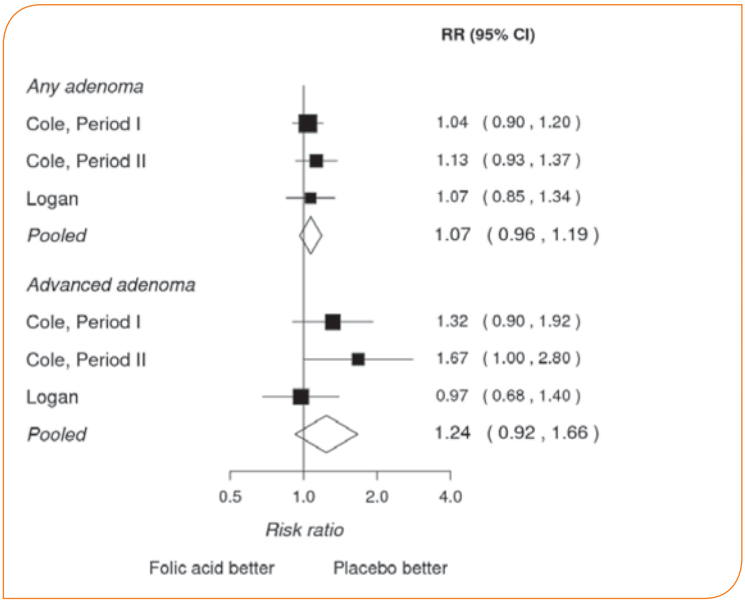
Aspirin and Folic Acid for the Prevention of Recurrent Colorectal Adenomas

RICHARD F. A. LOGAN,* MATTHEW J. GRAINGE,* VIC C. SHEPHERD,* NICHOLAS C. ARMITAGE,‡ and KENNETH R. MUIR* on behalf of the ukCAP Trial Group*

**Division of Epidemiology and Public Health, ‡Division of Surgery, University of Nottingham, University Hospital, Nottingham, United Kingdom*

0.5 mg/day folic acid vs. no folic acid

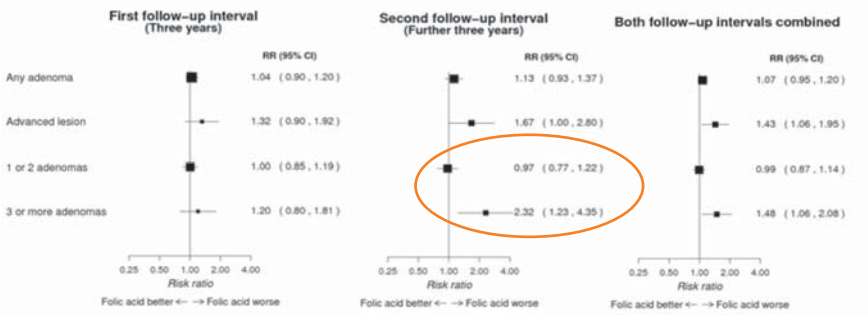
Effect of folic acid on colorectal adenomas



Interpretation of trial results in relation to colon cancer

1. Trial results related to adenomas not cancer, but adenomas are a reasonable indicator of cancer risk.
2. 1mg or 0.5mg of folic acid a day did not reduce colorectal adenoma risk, but neither did it increase it. Relative risk 1.07 (0.96, 1.19).
3. The only hint of an increased adenoma risk is in a subset analysis in one study (Cole at al) relating to the incidence of three or more adenomas. This subset analysis was based on small numbers (30 folic acid, 13 no folic acid).
4. Subset analyses are prone to the effect of chance producing formally significant results.

Folic Acid for the Prevention of Colorectal Adenomas



Adapted from Table 3 in Cole et. al. JAMA *June 6 2007; 297:21; 2351-59*

Interpretation of trial results in relation to colon cancer

5. The absence of an excess in people with 1 or 2 adenomas weighs against a causal explanation.

   If folic acid genuinely caused adenomas then one would expect not only an increase in people with 3 or more adenomas but also in people with 1 or 2.

Percentage of people with 3+ adenomas in Cole et al

	Period 1	Period 2
Folic acid	9.4	9.9
No folic acid	7.8	4.3

6. The difference in Period 2 arises from a deficiency of people with 3+ adenomas in the no folic acid group, not an excess in the folic acid group, which suggests chance as the most likely explanation.

Summary of Cole et al and Logan et al

- No overall association between folic acid and adenomas in either trial alone or in both combined.
- A suggestion of an excess in a subgroup analysis (3+ adenomas) that, in spite of being statistically significant, could have arisen by chance.
- This is the likely explanation given that the increased relative risk arises from a deficit in the no folic acid group, not an excess in the folic acid group.
- The absence of an excess of one or two adenomas supports chance as the explanation.

An example of results from subset analysis

Deaths from all causes in two randomized trials of folic acid supplementation

	Folic acid	No folic acid
Cole at al	10	19
Logan et al	1	7
Combined	11	26

Relative risk 0.4 (p=0.02)

One might conclude that folic acid reduces all-cause mortality by about 60%; this would be misleading, but no more so than concluding that folic acid causes multiple adenomas.

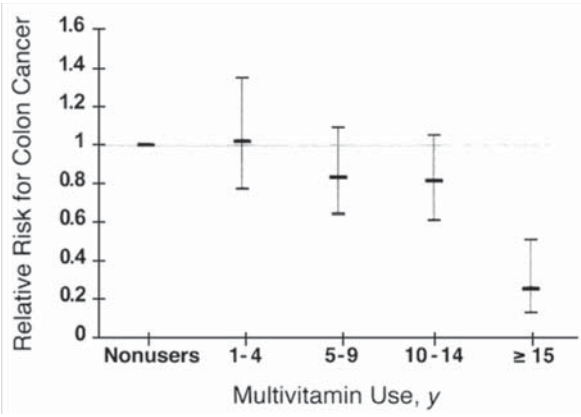
Bayston, Russell, Wald, and Hoffbrand. Lancet 2008;371:1335-1336.

The trend analyses and trial results do not provide **evidence** that folic acid fortification is a cause of colorectal cancer.

A large cohort study showed that longterm use of folic acid supplements is associated with a substantially decreased chance of colorectal cancer.

This suggests a benefit but on its own is insufficient evidence of a protective effect.

Colon cancer in women in the US Nurses' Health Study



Colon cancer relative risk according to years since the start of use of multivitamins containing folic acid

Giovannucci, E. et. al. Ann Intern Med 1998;129:517-524

Folic acid and breast cancer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CANCER DANGER OF FOLIC ACID

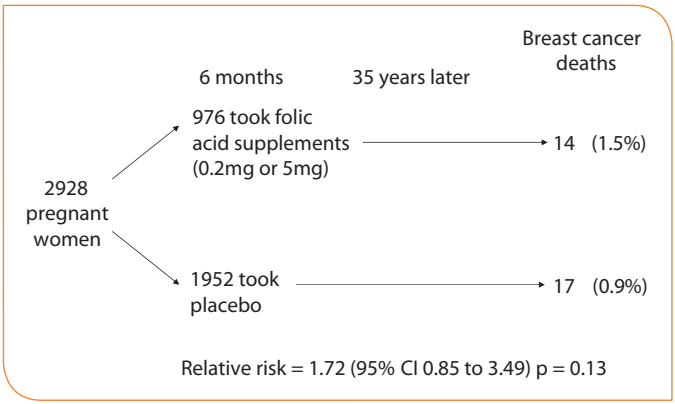
Millions warned over vitamin supplement

Daily Express, *10 December 2004*

Aberdeen folic acid supplementation trial

- Conducted 1966-7.
- 2928 pregnant women randomised to take folic acid or placebo.
- 35 years later - trial data resurrected; all deaths by cause were identified up to 2002.

Charles D, Ness AR, Campbell D, Davey Smith G, Hall MH. BMJ *2004;329: 1375-76.*



The publication of the paper, particularly in a prominent journal such as the BMJ, raised concern even though the authors stated:

> *The increase in mortality from breast cancer associated with folic acid was probably chance, it was not statistically significant and the number of deaths was small.*

The trial results are uninformative.

Even if folic acid were a cause of breast cancer, it would be extremely unlikely to be apparent given a six-month exposure followed by an interval of thirty-five years.

Even a known carcinogen would not have been detectable.

If the women had smoked cigarettes for a 6-month period in their life 30 years ago, and had not smoked before or after, we would not expect any perceptible increase in lung cancer.

Conclusions

Detailed analyses of the studies thought to suggest links between folic acid and colon adenomas and between folic acid and breast cancer do not provide evidence of a causal association.

Such concerns should not delay the decision to introduce the fortification of flour with folic acid.

Public health risk assessment

On their own, hypotheses and associations do not provide evidence.

Population risk assessment should be based on evidence.

Otherwise almost any useful public health intervention would be stalled simply on the basis of hypothesis, speculation and possible associations.

Folic acid and colorectal neoplasia: clinical trials

JOHN A.BARON
Dartmouth Medical School

Abstract:

Five randomized trials have assessed the effect of folic acid supplementation on the risk of colorectal adenomas. All these studies recruited patients with a recent colorectal adenoma history and a "clean colon," i.e. with no remaining polyps in the large bowel.

The three larger trials studied between 692 and 1021 subjects randomized to placebo or folic acid (0.5 mg in one trial, 1 mg in the two others). Overall findings from these three studies do not suggest an adenoma benefit. In two of the three larger trials, almost all the available data pertain to follow-up of about three years. One of these studies, conducted by mail in two established observational cohorts of health professionals, found indications of reduced risk from supplementation among subjects with lower baseline folate status. One of the larger trials, the Aspirin/Folate Polyp Prevention Study, had longer-term follow-up and treatment (up to 10 years post-randomization). During later follow-up, there were indications that folic acid supplementation conferred an increased risk of advanced or multiple adenomas. During both early and late follow-up, adenomas detected in the folic acid group were larger than those in the placebo group. During the early follow-up/ treatment period, dietary folate intake and baseline plasma folate levels were inversely associated with adenoma risk in the placebo group.

Two smaller trials (60 or 137 randomized subjects) studied folic acid supplements in doses of 1 or 5 mg per day for 2 or 3 years. The results from these two studies suggest that supplementation reduces adenoma risk.

Overall, these data indicate that folic acid supplementation does not reduce the risk of colorectal adenomas. There are suggestions that with prolonged treatment and/or follow-up there is an increase in risk. Differences between clinical trials and observational analyses could derive from several factors. The play of chance in secondary analyses of the clinical trials cannot be completely ruled out. Also, the trials and observational analyses have studied somewhat different exposures. The trials have used synthetic (oxidized) folic acid in relatively high doses, in clear contrast to dietary folate, which entails intake of lower doses of (methylated) natural folates in the context of other food constituents.

Presentation:

Folic acid adenoma trials

Adenoma "recurrence" studies

	# of Subjects
Pireus trial	60
Detroit trial	137

AFPPS	1021
ukCAP	945
NHS/HPS trial	692

2 smaller studies

Pireus trial

Design and findings

- 60 subjects
- Folic acid (?) 1 mg vs. placebo
- 1 & 3 year colo; 100% f/u

	Risk Ratio 1+ adenoma
Year 1	0.60 (ns)
Year 3	0.47 (ns)

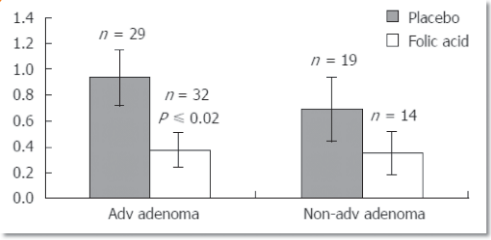
Paspatis & Karmanolis, 1994

Detroit trial

Design and findings

- 137 subjects, 5 mg folic acid vs. placebo
- 3 year colo; 69% f/u

  RR (OR?) = 0.36, p = 0.03



Jaszewski et al, 2008

3 larger studies

Aspirin/Folate Polyp Prevention Study

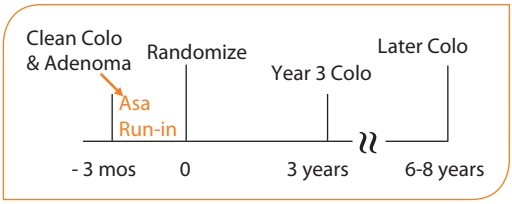
Study structure

- Recent adenoma, clean colon
- Colo Follow-up: 3 years (+ 1 cycle)
- Placebo vs 1 mg folate
  Placebo vs 81 mg Asa vs 325 mg Asa
- All adenoma endpoint
  (advanced, multiple adenomas)

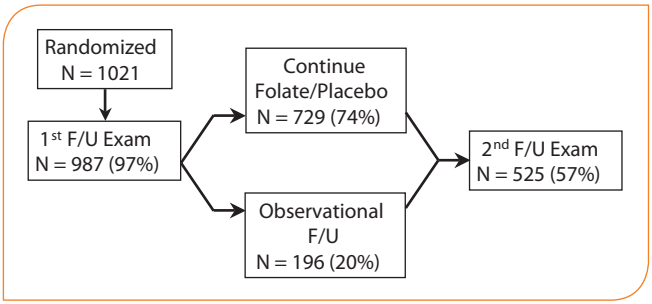
Study conduct

- 3-month asa run-in period
- NSAID/folate avoidance
- Interval questionnaire q 4 months (extensive medication Q's)
- Uniform pathology review

Basic design



Flow of subjects



Why longer-term treatment/follow-up?

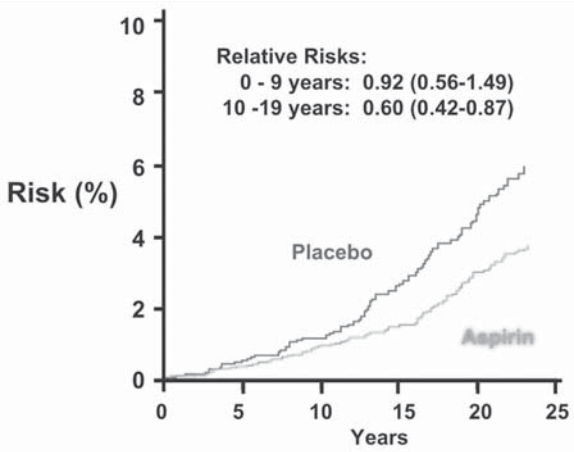
- Carcinogenesis takes time.
- Previous criticism
- Previous findings

Aspirin and colorectal cancer

Pooled analysis of 2 English trials

British Docs:
500 mg qday
(5 years)

UK-TIA:
300 mg q day or
1200 mg q day
(1-7 years)



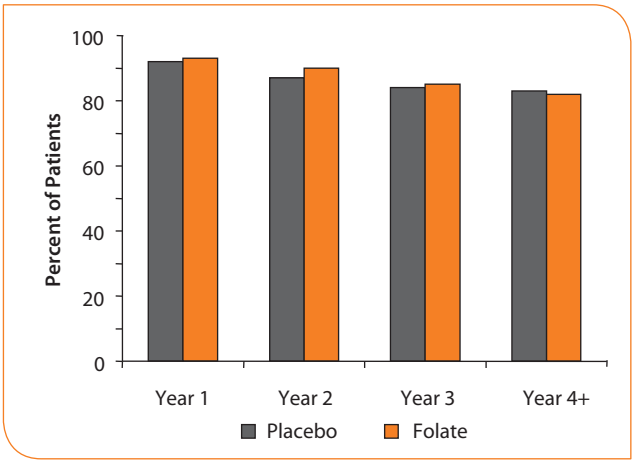
Flossmann, 2007

Characteristics of subjects

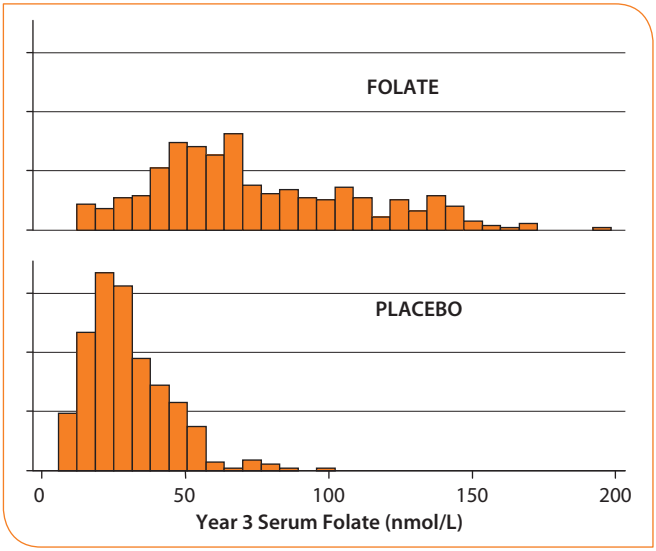
	Folate (N=516)	Placebo (N=505)
Mean age, yrs	57	57
% Male	64%	64%
% white	86%	85%
% current smoker	15%	14%
Mean ETOH drinks/day	0.6	0.6
% baseline adv lesion	29%	28%

Compliance

Study Tablets 6-7 Days/Wk



Year 3 Serum Folate



Homocysteine

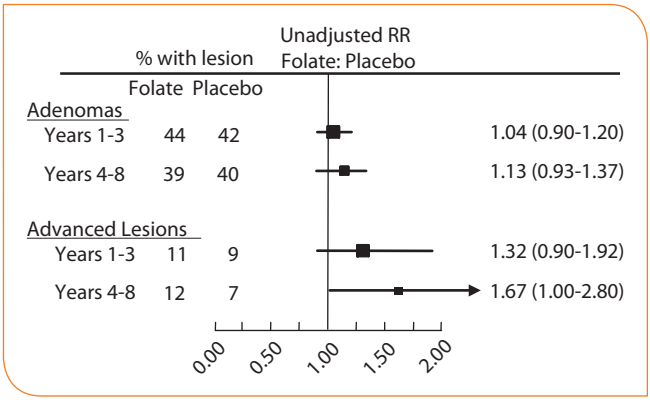
Effect of supplementation

	Total homocysteine, µmol/L	
	Baseline	**Year 3**
Placebo	9.8 (2.9)	9.2 (2.5)
Folate	9.9 (2.9)	9.0 (2.2)

Change	-0.5 (2.1)	-0.9 (2.2) p = 0.02

Adenoma/Advanced-Lesion Occurrence

Intention-to-Treat Analysis



Adenoma characteristics

Multiplicity

	FA:P Ratio (p)	RR 3+
1st F/U	1.10 (0.30)	1.20 (0.80–1.81)
2nd F/U	1.41 (0.01)	2.32 (1.23–4.35)

Medical events

	Placebo N= 505	Folate N= 516	p
Death	19 (3.8)	10 (1.9)	0.09
Hospitalization	119 (23.6)	161 (31.2)	0.01
Colorectal Ca	4 (0.8)	3 (0.6)	0.72
Other Ca	32 (6.3)	54 (10.5)	0.02
M.I.	8 (1.6)	14 (2.7)	0.28
Revasc.	16 (2.4)	16 (2.7)	0.99
Stroke	5 (1.0)	9 (1.7)	0.22

Diet, plasma levels, treatment effects

First follow-up interval (~3 years)

Treatment: 1.04 (0.90-1.20)
*Total Intake: 0.69 (0.51-0.94)
*Dietary Intake: 0.87 (0.67-1.11)
*Plasma: 0.72 (0.54-0.97)

*3rd vs. 1st tertile in placebo group

UKcap

Design and findings

- 945 Subjects; 0.5 mg folic acid vs. placebo
- Clinical colo f/u (typically 3 yrs); 91% followed
- Clinical pathology assessment

	Risk Ratio
Any adenoma	1.07 (0.85-1.34)
Advanced adenoma	0.98 (0.68-1.40)

NHS/HPFS trial

Design

- 692 subjects; 1 mg folic acid vs. placebo
- Clinical colo f/u; 70% followed
- Clinical pathology assessment

Folic acid adenoma trials

Summary

- Best studies: overall negative
- Suggestions of interactions with
  alcohol intake
  baseline folate status

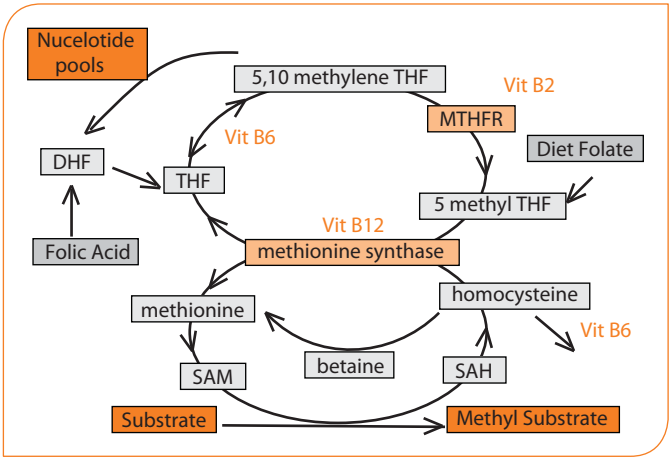
Probable conclusions:

- Benefit (if any) limited to low folate status
- Possible harm (delayed?) if high folate status

Folate findings

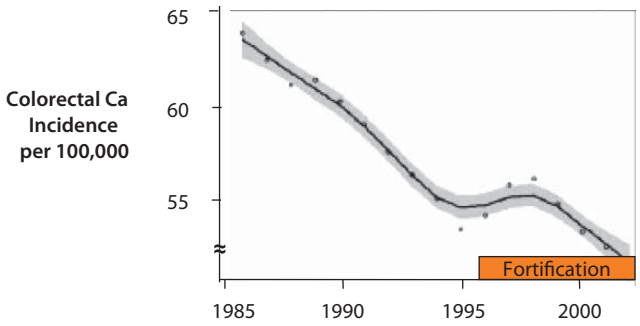
Trials vs. observational studies

- Dose differences
- (Synthetic) Folic Acid vs. (natural) folates
- Confounding, measurement error, etc.



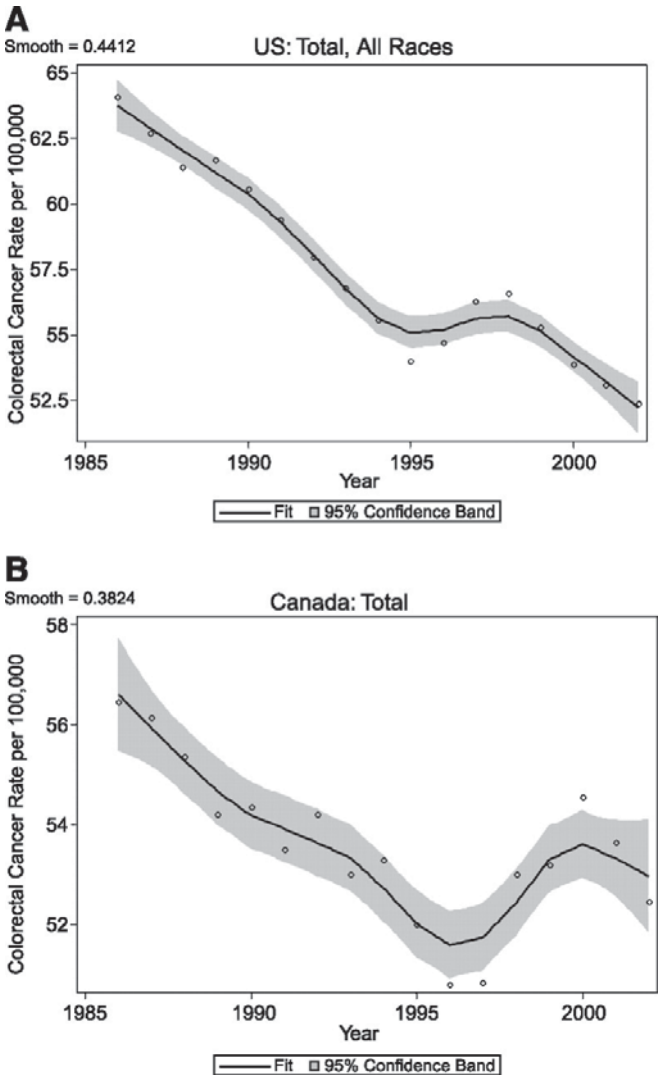
U.S. colorectal Ca incidence

1985-2002



from Mason, 2007

Colorectal cancer: age-adjusted incidence in the United States and Canada



Mason, J. B. et al. CEBP 2007;16:1325-1329

Modeling the effects of genetic polymorphisms and folate supplementation on cancer risk

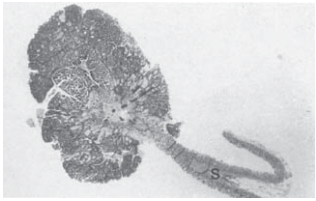
NELI ULRICH
Fred Hutchinson Cancer Research Center
University of Washington, Seattle
Shortly: Division Head, Preventive Oncology,
German Cancer Research Center, Heidelberg

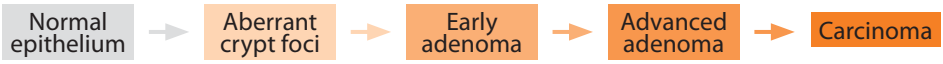
The next 20 minutes

- Genetic polymorphisms in folate metabolism
  - Phenotypic impact
  - Associations with cancer risk
  - Modeling more complex interactions
- Modeling of dual effects of folate on population cancer risk
- Folate in cancer patients
- Summary and research agenda for folate and cancer

Colon cancer

- Colon cancer third most common cancer worldwide
- Multistage process:
  - Main etiologic pathwayinvolves formation of colorectal polyps
  - Other pathways:
    - Inflammatory conditions
    - MMR deficiency (hyperplastic polyp)
    - Methylation disturbances (CIMP)





Jass J et al, 2006

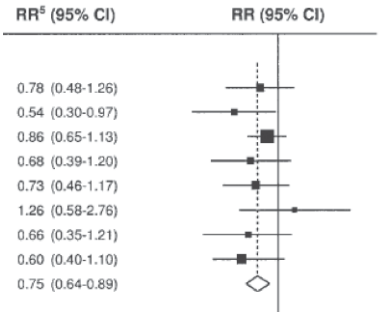
Folate and colorectal cancer risk

Consistent ↓ risk (20-40%) in both cohort and case-control studies.

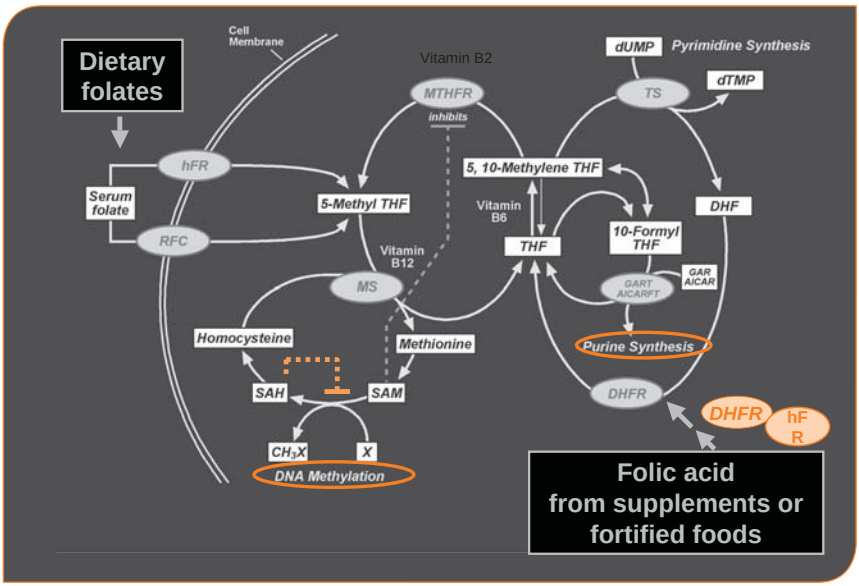
Possible interaction with alcohol intake or other one-carbon nutrients

Sanjoaquin 2005, Giovannucci 2002

Cohorts, dietary folate
RR=0.75 (0.64-0.89)



Folate-mediated one-carbon metabolism



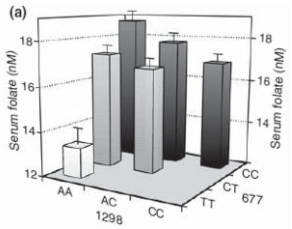
Genetic variability in folate metabolism

- Candidate polymorphisms that alter amino-acid sequences
  - Effects on protein function
- Other genetic variability incompletely explored
  - Not all genes systematically screened for polymorphisms
  - Effects on gene expression, mRNA stability, miRNA binding sites
  - Most published epidemiologic studies focus on candidate polymorphisms
- Where is folate nutrigenetics today?

MTHFR candidate polymorphisms

- Three ns SNPs in a key regulatory enzyme
  - MTHFR C677T, A1298C, R594Q

- Phenotypic impact:
  - 677 TT strong predictor
  - 1298CC ↓ serum folate ↑ Hcy on a 677 CC background
  - R594Q?



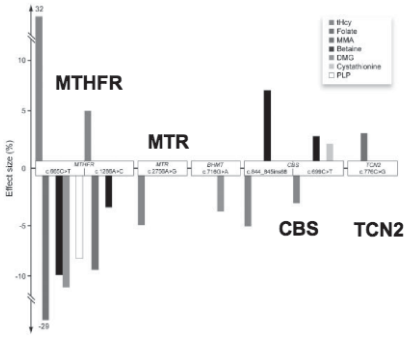
Ulvik et al. Hum Genetics 2007

DHFR *polymorphisms – central for folic acid nutrigenetics?*

- Folic acid requires reduction by *DHFR* prior to entering one-carbon metabolism
- *DHFR* polymorphism (19bp del intron 1)
  - Del/del genotype:
  - ↑ unmetabolized folic acid with ↑ folic acid intakes, compared to ins/ins (Kalmbach et al. J Nutr 2008)
- Unmetabolized folic acid:
  - Associated with reduced NK cytotoxicity? (Troen et al, J Nutr 2006)
  - Cognitive function?

Other polymorphisms

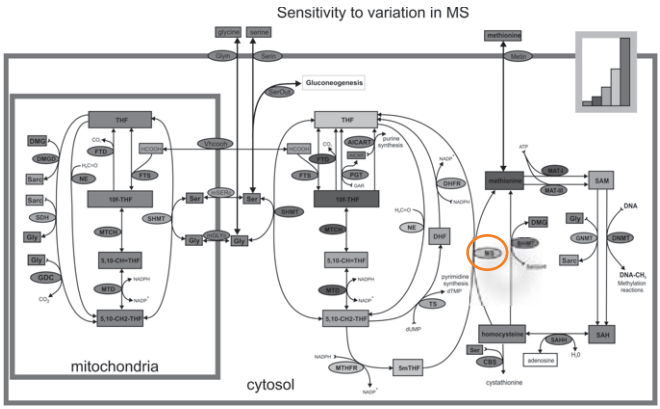
- Appear to have less impact on measurable biomarkers



Fredriksen A et al. Hum Mut 2007

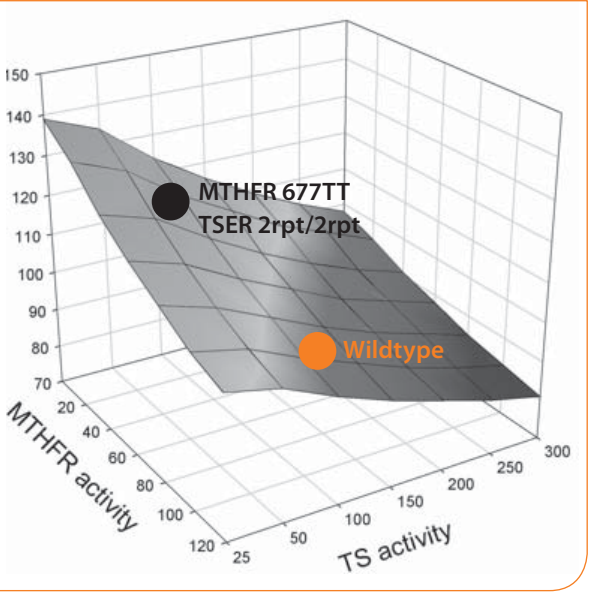
In Silico Metabolism

- Free software for modeling of folate biochemistry
- Contact Neli Ulrich (nulrich@fhcrc.org)



Slide courtesy of F. Nijhout

The combined effects of changes in MTHFR and TS activity on purine synthesis



Ulrich et al CEBP 2008

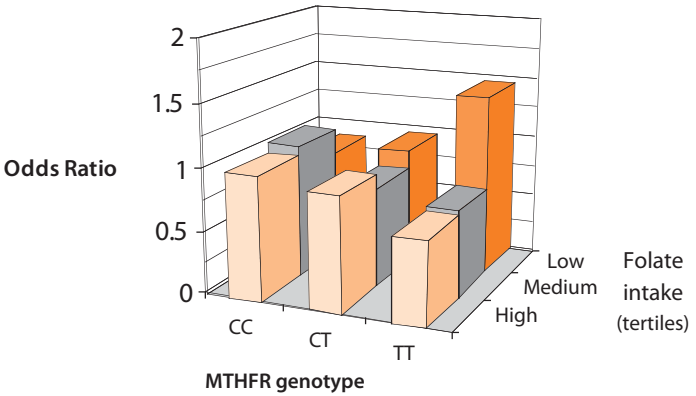
Genetic polymorphisms and cancer risk

- Meta-analyses for MTHFR SNPs with significant results:
  - Colorectal cancer:
    - 677 TT vs CC: 0.83 (0.75-0.93)
    - 1298 CC vs AA: 0.80 (0.65-0.98)
  - Adult acute lymphocytic leukemia (ALL):
    - 677 TT vs CC: 0.41 (0.24-0.72)
  - Gastric cancer:
    - 677 TT vs CC: 1.42 (1.31-1.77)
- These meta-analyses ignore gene-diet interaction

Hubner IJC 2007, Huang J Hum Gen 2007, Boccia AJE 2008, Pereira CEBP, 2006

MTHFR – *paradigm for gene-nutrient interaction*

Risk of colorectal adenomas stratified by *MTHFR* genotype and folate intake



Ulrich et al, CEBP 1999

Seattle Colon Cancer Study

- Case-control >1900 cases, >2500 controls
- Prior to fortification
- Candidate polymorphisms in ADH, SHMT, DHFR, DNMT1, MTHFD, MTHFR, MTRR, TCN2, TDG
  - Gene-diet interactions across multiple one-carbon nutrients for
  - MTHFR, *others unpublished*
  - Do associations differ by type of colon cancer?

Liu, Ulrich, Potter et al. unpublished

Folate polymorphisms and CIMP

(Colon cancer case-control study: 655 CIMP-, 261 CIMP+, 1972 controls) MINT1, MINT2, MINT31, p16, hMLH1

Polymorphism		CIMP- (0-1 gene meth)	CIMP+ (2-5 genes meth.)
MTHFR C677T	CC	1.0	1.0
	CT	0.9 (0.7-1.1)	1.0 (0.7-1.3)
	TT	0.6 (0.5-0.9)	1.0 (0.6-1.6)
MTHFR A1298C	CC	1.0	1.0
	CG	0.9 (0.7-1.1)	1.3 (1.0-1.8)
	GG	0.7 (0.5-1.0)	1.1 (0.7-1.9)

Gene-diet interaction for MTHFR A1298C

Curtin, Slattery, Ulrich et al. Carcinogenesis 2007

Summary – folate genetics and colorectal cancer

- *MTHFR*
  - 677 TT ↓ risk of neoplasia under a ↑ folate status only
  - A1298C and R594Q inadequately studied
- Possible additional gene-gene and gene-diet interactions
  - Thymidylate synthase (Ulrich 2002)
  - DHFR (Chen 2007)
  - DNMT3b (Jung 2008 and DNMT1 (Liu, unpublished)
- Associations may be limited to defined molecular subtypes (e.g., CIMP-)
- Genetic variants may affect tumor mutation spectrum and survival (Ulrich J Nutr 2006; Curtin IJC 2007)
- Complexity of the pathway → how can we integrate the biology in the data analysis?

Supplements, functional foods, fortification: Can there be too much folate?

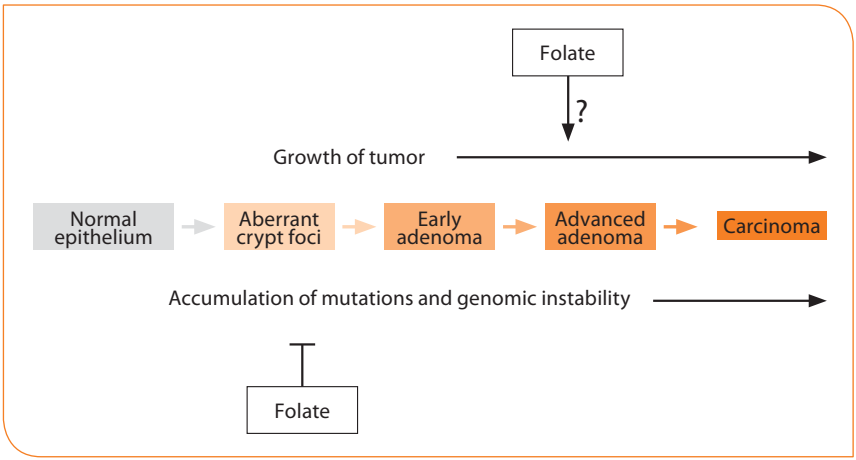
Folic acid intakes in the US population

- Multivitamins: ~ 400 µg/d
  - Used by 30-40% of the adult population
  - Women >60yrs:
    - 17% take 4+ dietary supplements
    - >50% have serum plasma folate >40 nmol/L
- Breakfast cereals: ~200-400 µg per 2/3 cup
- Health bars/drinks: often 400+ µg/serving
- Folic acid fortification: ~ 100-200 µg/day

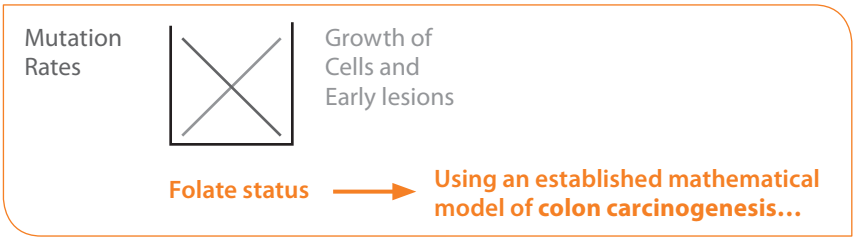
→ IOM recommended "upper limit" of 1000 µg/d folic acid can be easily surpassed in groups of the population

Rock AJCN 2007; Radimer AJE 2004; Pfeiffer AJCN 2005

A dual role of folate in tumor progression



What are the net effects of folate supplementation over lifetime?

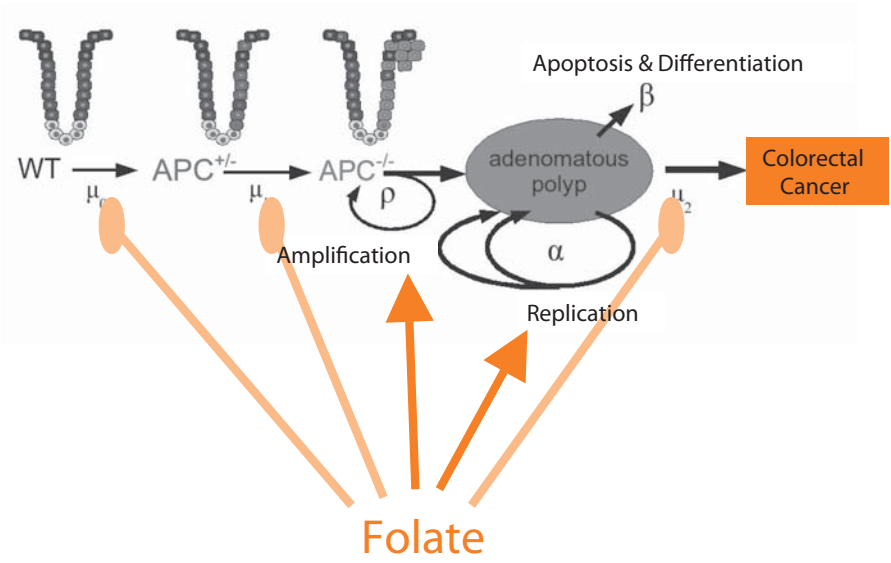


… we can calculate the expected rate of colorectal cancer in a population

… taking into account the two opposing effects of folate on carcinogenesis mutation vs replication/growth

… and calculate the **"net effect"** on **population cancer rates**

Luebeck et al. PNAS 2005

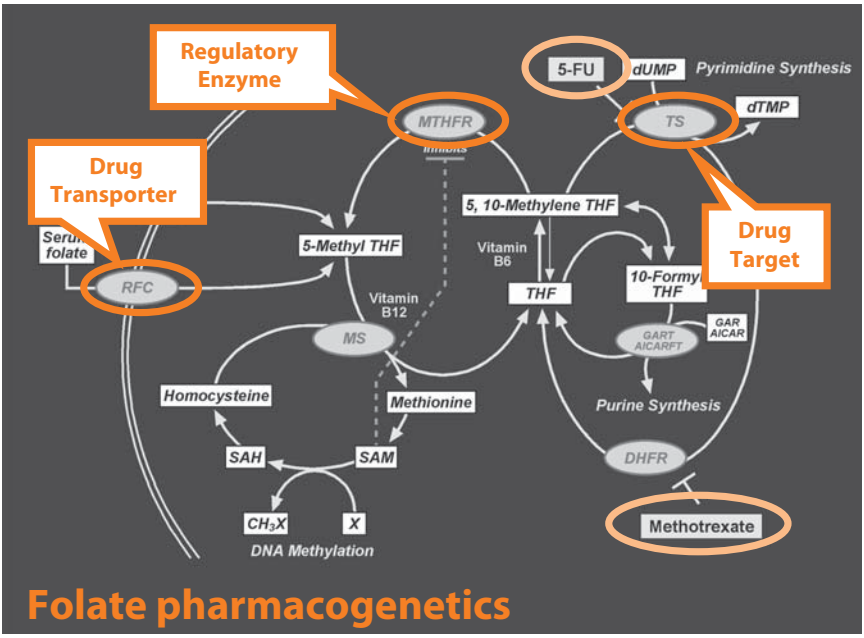


Luebeck et al. PNAS 2005; Luebeck Can Epi Biom Prev 2008

Folic acid supplementation starting at age

Proliferation Increase (%)	Mutation Reduction (%)	2	20	40
		Expected number of colorectal cancer cases per 100,000		
0	0	3319	3319	3319
		Change in colorectal cancer cases		
0	10	-687	-298	-193
0	20	-1274	-583	-397
10	0	+1286	+1223	+732
10	10	+353	+826	+511
10	20	-455	+449	+275
20	0	+2631	+2565	+1571
20	10	+1451	+2051	+1320
20	20	+419	+1570	+1053

Luebeck, Ulrich et al, CEBP 2008

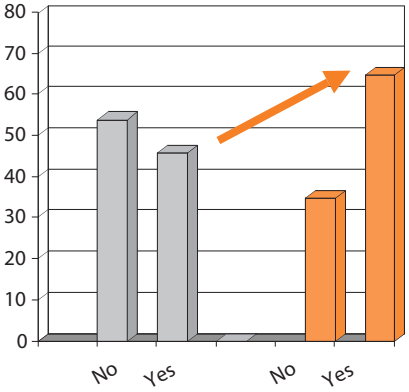


Ulrich, Nat Rev Cancer 2003

Cancer patients: Vitamin supplement use is high

- Review of 32 studies addressing vitamin and mineral supplement use among US adult cancer patients:
  - 64-81% any vitamin or mineral supplements
  - 14%-32% of survivors initiate supplement use after diagnosis
  - Up to 68% of physicians are unaware of supplement use among their patients

Increase in use of folic-acid containing supplements with diagnosis, Colon CCFR patients, n=971



Velicer & Ulrich, J Clin Onc 2008; Holmes et al. unpublished

Use of folic-acid containing supplements prior to diagnosis may be associated with greater risk of death from colon cancer

- Colon CFR Seattle: 745 cases, 172 deaths (3-year survival)

* Unpublished data*

Chia, Ulrich, unpublished

Folate in cancer patients

- Do tumors differ depending on folic acid supplementation?
  - Differences in folate receptors, or gene expression?
- Do treatment effects differ with folic acid supplementation?
- Does growth of micrometastases and risk of recurrence differ with folic acid supplementation?

Summary - Genetics (1)

- **Genetic variability** in folate metabolism
  - Still inadequately characterized
  - Can modify a person's biochemical profile (e.g., Hcy)
  - Can affect cancer risk, particularly GI and leukemia
  - Cancer risk may differ depending on molecular cancer subtype
- Our ability to investigate jointly multiple factors in a biologic pathway is very limited
  - Incomplete answers for nutrigenomics
  - Only the effects of MTHFR C677T are well understood
  - Personalized nutrition recommendations?

Summary - Epidemiology (2)

- Epidemiologic associations between folate and cancer risk may differ by
  - Supplement use
  - Fortification status
- Many epidemiologic studies have failed to use dietary folate equivalents (DFE) as a measure of total folate
- Other nutrients relevant to one-carbon metabolism play an increasingly important role
  - Particularly vitamin B6

Summary – Public Health (3)

- Evidence for a dual role of folate in carcinogenesis
  - Mathematical modeling generally predicts
    ↑ population cancer rates with fortification
    → if folate ↑ cell proliferation
    - Incomplete *in vitro* and *in vivo* data on dual effects
    - Human studies unethical
- Concern over folic acid among cancer patients
  - If growth promoting → then potential for ↑ recurrence rates
  - Potential effects on efficacy/toxicity of chemotherapy (5-FU, MTX)

Summary – Research Needs (4)

- Animal studies of folate administration on pre-cancerous-resected lesions (e.g., with imaging)
- Patient cohorts to investigate effects of folic-acid fortification and supplementation on recurrence/metastatic tumor potential
- *In vitro* and *in vivo* studies on proliferation effects
- Effects of unmetabolized folic acid on health outcomes
- Pathway-based data analysis
- **Ulrich CM, Cancer Epi Biom Prev 2008;17:2226-30**
  "Folate and Cancer Prevention – Where to Next?"

FHCRC:

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John Baron (Dartmouth)

Colleagues from the Colon CFR and the Aspirin/Folate Prevention Trial

Grant support by NIH

B-vitamins and risk of vascular disease, cancer and all-cause mortality: meta-analysis of the homocysteine-lowering trials

ROBERT CLARKE
Clinical Trial Service Unit, University of Oxford,
Oxford, United Kingdom

Abstract:

Elevated plasma total homocysteine is a risk factor for cardiovascular disease (CVD), but the randomized trials of dietary supplementation with B-vitamins to lower homocysteine have not yet provided clear evidence for any beneficial effects for risk of vascular and non-vascular outcomes. Recently, it has been suggested that folic acid supplementation might increase the risk of cancer.

The B-Vitamin Treatment Trialists' Collaboration was set up to combine data from all randomized trials assessing the effects of lowering homocysteine levels with B-vitamins on risk of vascular and non-vascular outcomes. Among the 12 randomized homocysteine-lowering trials for prevention of CVD, data were available from 8 trials involving 37,491 participants by the end of 2008 for the 1st cycle of this collaboration. Data were available on 9179 major vascular events, 3083 cancer events and 5125 deaths in the trials with prior vascular disease. Additional data were collected on cancer outcomes in 2652 individuals from 3 smaller trials of folic acid in patients with colorectal adenoma. The primary analysis assessed the effects on major vascular events, stroke, major coronary events, any cancer and cancer at specific sites. Additional analyses assessed the effects on vascular and cancer outcomes in sub-groups defined by age, sex, population level of fortification and pre-treatment levels of folate and homocysteine and duration of treatment.

Among the participants with a prior history of vascular disease, supplementation with B-vitamins lowered plasma homocysteine levels by about 24% for an average duration of about 5 years. However in this population with prior vascular disease, B-vitamins had no beneficial effects on risk of major vascular events (Hazard ratio: 1.01; 95%CI: 0.97-1.05) or on coronary heart disease, stroke or revascularisation events. Moreover, B-vitamins had no harmful effects on risk of cancer, (Hazard ratio: 1.05; 95%CI: 0.98-1.13) overall, or at any site or in any of the pre-specified sub-groups by age, sex, level of folic acid fortification and pre-treatment levels of folate or homocysteine, or by duration of treatment. B-vitamins had no beneficial or adverse effect on risk of all-cause mortality. Among participants with a prior history of colorectal adenoma, dietary supplementation with B-vitamins had no significant adverse effect on any cancer.

Dietary supplementation with B-vitamins did not reduce the risk of heart disease, stroke or revascularisation events, nor did it have any significant adverse effect on cancer or cause-specific mortality.

Folate/folic acid and breast/prostate risk

ANDERS EKBOM
Institutionen för Medicin
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Abstract:

Folate/folic acid and breast/prostate risk

Breast and prostate cancers are the most common cancer forms in females and males in the westernized world. There has been an increase in the incidence for both cancers during the last decades in contrast to the mortality which has been stable or even has showed a slight decrease. The trends in incidence and mortality can at least partly be explained by screening and a higher diagnostic intensity compared to earlier time periods. This means that, especially for breast cancer, the age specific incidence rates have changed substantially, which can affect the interpretation of the results assessing the impact of folate on both cancer forms.

There are some known risk factors for breast cancer such as age at menarche and menopause, parity, age at first birth, BMI, height, HRT and alcohol. These factors can either act during the initiation of the malignant process or during the progression of a tumor already in place. As there are reasons to believe that folic acid might be protective during the initiation process but act as a risk factor during the progression, there is a problem to disentangle the role of folic acid during a malignant transformation. Making the assumption that alcohol acts as an initiator it would therefore mean that folic acid supplementation could be of interest as a chemo preventive measure for breast cancer in contrast to the progression, which seems to occur during pregnancy.

There are no intervention studies of folic acid and breast or prostate cancer, but there are results from some observational studies, which are contradictory. During the 20th century, there was a consensus that there is an inverse association between folic acid and breast cancer which, to some extent has been contradicted by results from the 21'st century. However, there is a consistent finding of an interaction between alcohol consumption and exposure for folic acid, i.e. folic seems to be protective.

There are very few known risk factors for prostate cancer besides genetics and ethnicity. As for breast cancer, there are reasons to believe, that there are factors acting during the initiation and/or during the progression but the process still remains an enigma. Similar to breast cancer there were reports during the 20th century and early 21st century of an inverse association between folic acid and the risk of prostate cancer, something which some extent has been contradicted by observational studies published later during the 21st century.

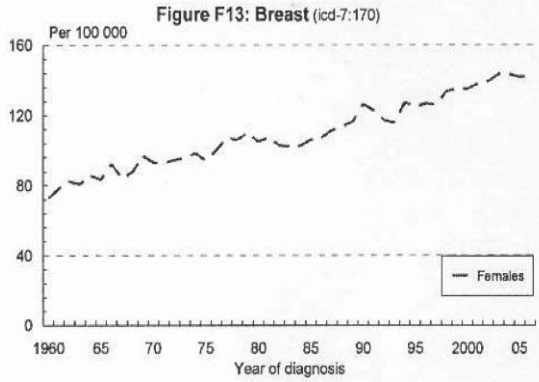
There are, therefore, too little data to form any firm conclusions, but folate supplementation can not be recommended as a chemo preventive measure against breast or prostate cancer at present, although women who consume moderate to high amounts of alcohol possibly could benefit from such measures.

Presentation:

Breast and prostate cancers

The two most common cancer forms in females and males

Breast cancer



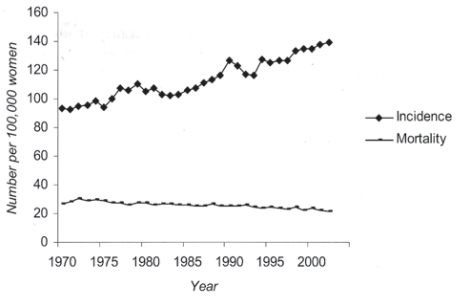


Fig. 1. Age-standardized breast cancer incidence and mortality per 100,000 women in Sweden 1970-2001. Adapted from the National Board of Health and Welfare.

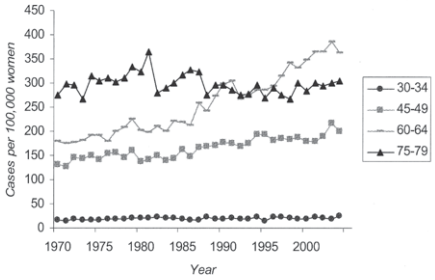


Fig. 2. Age-standardized breast cancer incidence per 100,000 women in Sweden 1970-2004 in selected age-groups. Adapted from the National Board of Health and Welfare.

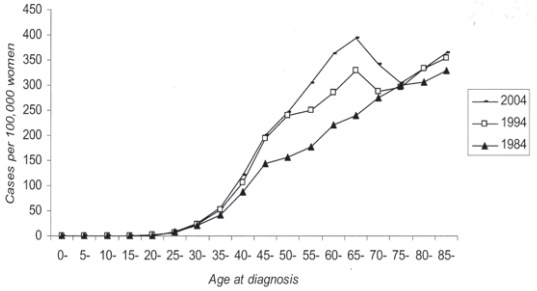


Fig 3. Age-specific incidence rates of breast cancer in Sweden in 1984, 1994, and 2004. Adapted from the National Board of Health and Welfare

Risk factors

- Genetics
- Menarche
- Age at first birth
- Parity
- Menopause
- BMI
- Height
- Radiation
- HRT
- Alcohol

Initiation
Progression

Age at first birth

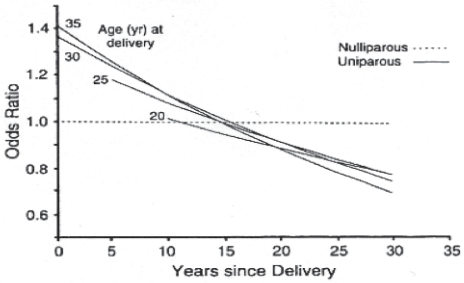


Figure 1. Odds Ratios for the Risk of Breast Cancer in Uniparous Women of Various Ages at Delivery, According to the Number of Years since Delivery.

A group of nulliparous women were used as the reference group. Ratios were derived from a conditional logistic-regression model. Age at delivery and years since delivery were included in the model as continuous variables.

Alcohol

20th Century

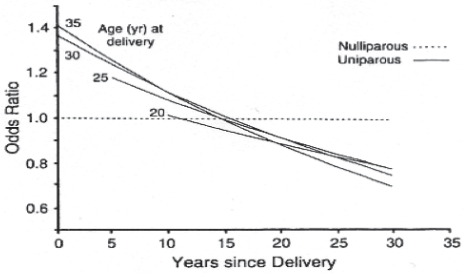
Inverse association between folate and breast cancer

21st Century

Interaction between alcohol and folate i.e folate protective among those exposed to alcohol

21st Century

Conflicting results!



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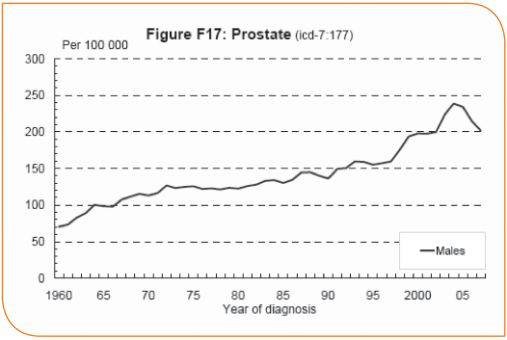
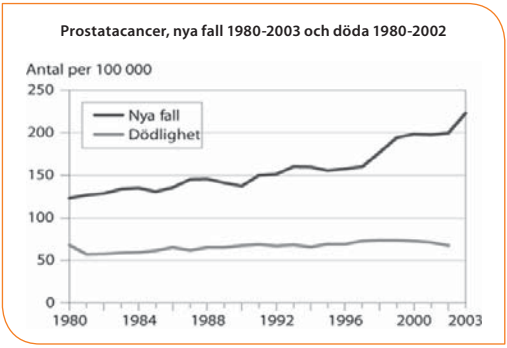
A group of nulliparous women were used as the reference group. Ratios were derived from a conditional logistic-regression model. Age at delivery and years since delivery were included in the model as continuous variables.

Prostate cancer





Risk factors

- Genetics
- Ethnicity

20th Century

Inverse association between folate and prostate cancer

Initiation
Progression

21st Century

Conflicting results!

Conclusion

Folate supplementation cannot be recommended as a chemo-preventive measure against either breast or prostate cancer

Relation of dietary sources of folic acid to blood folate concentrations for the assessment of cancer risk NHANES 2001–2004

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Division of Birth Defects and Developmental Disabilities
Centers for Disease Control and Prevention, Atlanta

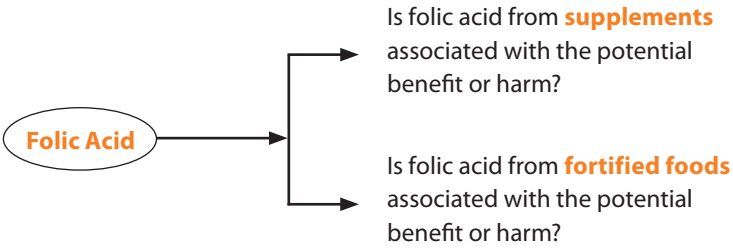
Disclaimer: The findings and conclusions in this presentation have not been formally disseminated by the Centers for Disease Control and Prevention and should not be construed to represent any agency determination or policy.

Abstract:

The three main sources of synthetic folic acid intake in the United States — supplements containing folic acid and folic acid added to food under two U.S. Food and Drug Administration regulations: (1) mandatory fortification of enriched cereal-grain products and (2) optional fortification of ready-to-eat products such as breakfast cereals. Dietary data from the National Health and Nutrition Examination Survey (NHANES) contains information on individual dietary intake, which allows quantification of daily intake of these different sources of folic acid. A report on non-pregnant participants 19 years of age or older from NHANES 2001–2004 related daily intake of these sources to serum folate concentrations. This report shows that high serum folate concentrations were influenced primarily by the use of supplements containing folic acid. Folic acid fortification alone at the current U.S. level does not produce high serum folate concentrations. The source and quantity of folic acid should be considered when interpreting findings associated with potential beneficial and adverse effects of folic acid.

Presentation:

An understanding of the association between folic acid and cancer may be complicated by dosage: consider the sources



Background

- Sources of folic acid associated with colorectal cancer
  - Supplements
    - CRC clinical trials
  - Mandatory fortification
    - CRC incidence - Mason

Fortification of food with folic acid in the United States

- Before 1996 only a few products were voluntarily fortified with folic acid
- Since 1996, the number of fortified products increased
- After 1998, mandatory fortification operational

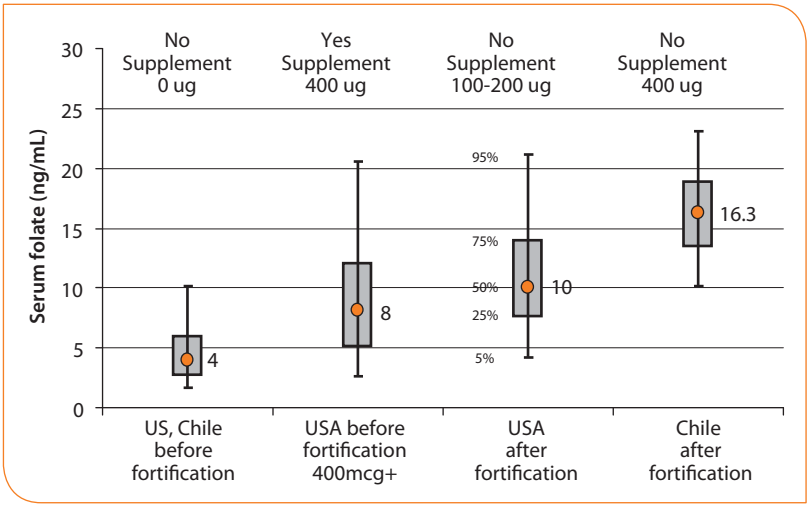
National Health and Nutrition Examination Survey (NHANES)

- National representative sample of U.S.
  - Continuous 2 year cycles since 1999
- Interview
  - Self-reported dietary assessments using 24 hour recall
    - Consumption of foods
    - Consumption of every supplement
- Laboratory measurements
  - Serum and RBC folate

Content

- Relative contribution of different sources of folic acid added to food and of supplements from NHANES
  - Relation to blood folate concentrations
  - Relation to daily intake of folic acid

Median serum folate among non-pregnant reproductive age women, before and after fortification , US and Chile



Sources of folates in diet (NHANES)

- Three sources of daily total folate intake from individual self-reports
  - Naturally occurring food folate
    - e.g., dark green leafy vegetables
  - Folic acid added to food (fortified foods)
    - e.g., pastas, grains, ready to eat cereals, special foods, and protein supplements
    - Dietary date in NHANES from 2001 onwards
  - Supplements containing folic acid
    - e.g., multivitamins, folic acid only supplements

Folic acid added to food

- Two different standards of identity
  - Enriched cereal grain products (ECGP)
    - In 1996, FDA mandated fortification of ECGP at 140 µg of folic acid per 100 grams of flour by January 1, 1998.
  - Ready-to-Eat products (RTEs)
    - In 1996, FDA allowed that RTE foods could be fortified with up to 400 µg of folic acid per serving

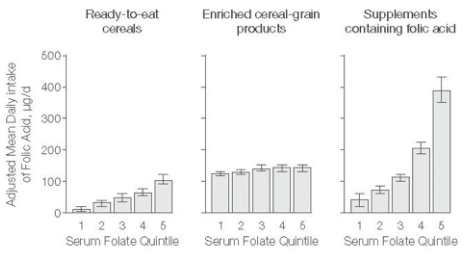
Sources of daily folic acid

Folic Acid From Supplements	**~400 µg / supplement**
Folic Acid From Fortified Breakfast Cereals	**0 - 400 µg / serving**
Folic Acid From Fortified Staples	**100-150 µg / day**

NHANES 2001 - 2004

- Relative contribution of different sources of dietary folic acid to plasma folate concentrations
- Geometric means of daily folic acid intake from ECGP, RTEs, and supplements

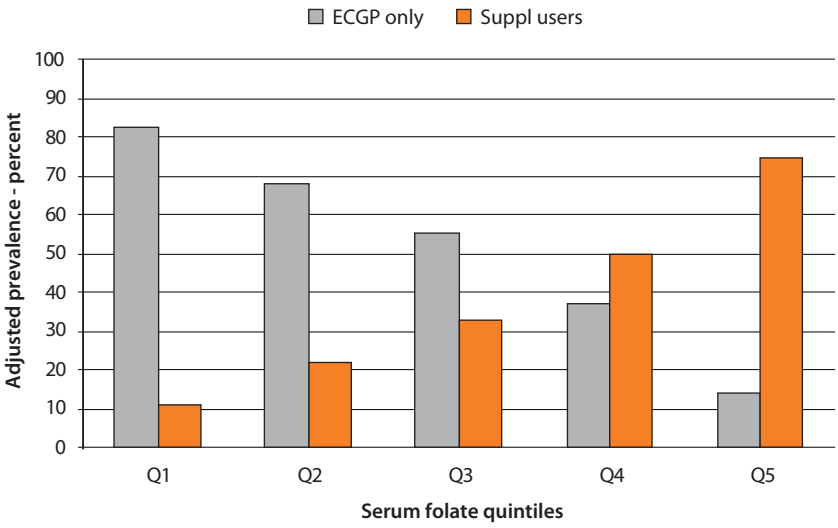
Figure. Estimated Daily Intake of Folic Acid by Serum Folate Quintile



Data were obtained from the National Health and Nutrition Examination Survey (NHANES) 2001-2004 for 8655 nonpregnant adults aged 19 years and older. For ready-to-eat cereals, $P < .001$; for enriched cereal-grain products, $P = .01$; and for supplements, $P < .001$ (Satterthwaite adjusted F statistic). All analyses were adjusted for age, sex, and race/ethnicity. Error bars indicate 95% confidence intervals. Refer to Table for quintile ranges.

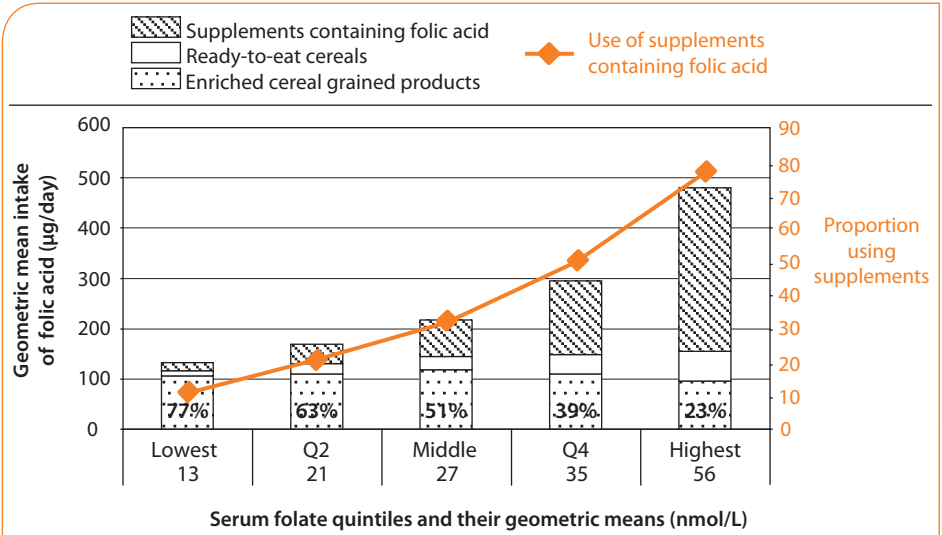
Yeung, LY et al. JAMA *2008;300:2486-7*

Adjusted prevalence of users of ECGP and supplements by serum folate quintile among 8655 non-pregnant adults ≥ 19 years, NHANES 2001-2004



Yeung, LY et al. JAMA 2008;300:2486-7

Geometric mean intake- folic acid, from enriched cereal-grain products, ready-to-eat cereals & supplements, and proportion of participants who used supplements containing folic acid – by serum folate quintiles NHANES 2001-2004, ≥19 years, n=8,655



Yeung, LY et al. JAMA *2008;300:2486-7*

Summary

- Association with high serum folate concentration
  - Strongest with average daily use of supplements.
  - Weak association with daily intake of folic acid from RTE breakfast cereals.
  - No association with daily intake folic acid from enriched cereal grain products.

Conclusions

- Total folic acid intake
  - Some participants have very large intakes of folic acid from supplements
- Blood folate levels
  - Supplements are main contributor to higher daily intakes and to higher blood folate concentrations
- Fortification of ECGP in the absence of consumption of supplements is unlikely to produce elevated serum folate concentrations

What is the source and dosage of folic acid?

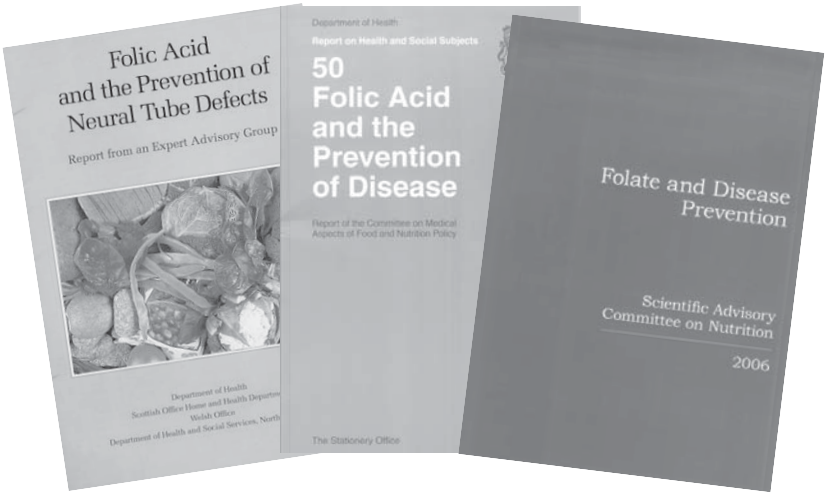
- Supplements – **400 µg / day**
  - Associated with high blood folate concentrations
  - Less use outside of the United States
- Ready to eat breakfast cereals – **400 µg / serving**
  - High consumption may lead to high blood folate concentrations
  - Low use in the developing world
- Enriched cereal grain products – **100 – 150 µg / day**
  - Mandatory fortification
  - Likely only source of folic acid in the developing world

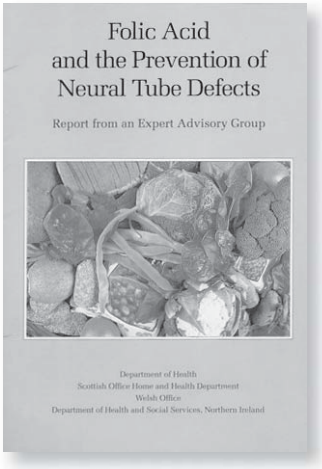
Acknowledgments

- Centers for Disease Control and Prevention,
  - Joe Mulinare, NCBDDD
  - Quanhe Yang, NCBDDD
  - Lorraine Yeung, NCBDDD
  - Mary Cogswell, NCBDDD

Folic acid and cancer

ALAN A JACKSON
Scientific Advisory Committee on Nutrition, UK





- Randomised controlled trial
- Preconceptual dietary supplementation with folic acid
- significantly reduced likelihood of recurrence of NTD
- affected pregnancy by about 70%

Wald N, Sneddon J, Densem J, Frost C, Stone R. Prevention of neural tube defects: results of the MRC Vitamin Study. Lancet *1991; **338**: 132-137.*

Folic acid preconceptually: neural tube defect

- commonest developmental abnormality
- failure of brain and spinal cord to develop normally
- first 4 weeks of pregnancy
- 700 to 900 affected conceptions/year
  - therapeutic termination
  - incompatible with independent life
  - lifelong disability

Current advice

- Folate status of population poor
- Women who are trying to conceive or who are likely to become pregnant are advised to:
  - take a daily supplement of 400µg of folic acid.
  - eat foods **voluntarily fortified with folic acid** and folate rich foods.
- **Younger mothers and those from the most socio-economically deprived areas were the least likely to report taking any action.**

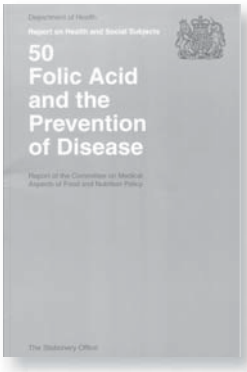
1998

USA and Canada: mandatory fortification of flour

Reduced NTD by up to 50%

No demonstrable adverse effects in population

No formal systems in place to identify possible adverse effects



On scientific, medical and public health grounds, the Committee concluded that universal folic acid fortification of flour at **240 µg/100g** in food products as consumed would have a significant effect in **preventing NTD-affected conceptions** and births **without resulting in unacceptably high intakes** in any group of the population.

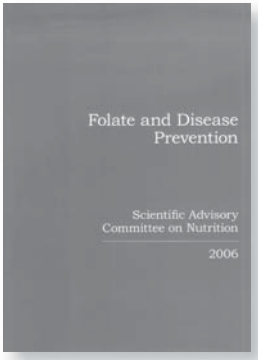
The use of folic acid supplements in pregnancy in the UK

- 55% of mothers who planned their pregnancy reported taking supplements or modifying their diet
- Younger mothers and those from the most socio-economically deprived areas were the least likely to report taking any action
- Around 50% of pregnancies are unplanned in England

Percentage below reference nutrient intake (200µg/d)

Women	
19-24 years	35%
25-34 years	35%
35-49 years	26%

Elderly		
Free living	25% men	48% women
Institutionalised	41% men	53% women



- Review evidence since previous report
- Make recommendations
- Modelling exercise
  - fortification of flour
  - balance risks and benefits

Mandatory fortification with folic acid, concerns:

- interaction with vitamin B-12, neurological damage
- cardiovascular disease,
- cancer

Modelling: effect of fortification with folic acid.

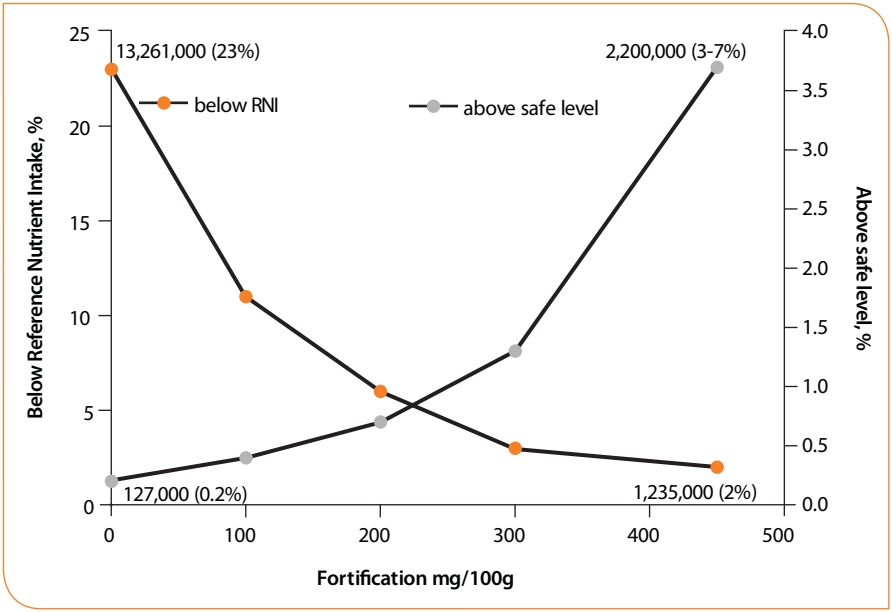
- Reference Nutrient Intake: 200 µg/d
- Supplement for pregnancy: 400 µg/d
- Guidance/Safe Upper Level: 1 mg/d (B-12 interaction)

- Currently:
  - women at risk of pregnancy less than 200 µg/d
  - people consuming foods fortified with folic acid or supplements containing folic acid > 1mg/d

Effects on the UK population of fortification of flour with folic acid: includes folate and folic acid from all sources

Fortification level of folic acid µg/100g flour (level in food after processing)	Average increase in folic acid intake (µg/day)	Estimated numbers (%) with intakes below RNI	Estimated numbers (%) exceeding the UL of folic acid/day	Estimated number aged 65y+ with low vitamin B_{12} status exceeding 1mg/d folic acid	Estimated NTD pregnancies prevented per year (% reduction in NTD risk)
0	0	13,261,000 (23%)	127,000 (0.2%)	900	0
100 (75)	51	6,471,000 (11%)	225,000 (0.4%)	1,700	42-93 (6-10%)
200 (150)	102	3,424,000 (6%)	404,000 (0.7%)	2,000	82-180(12-20%)
300 (225)	152	1,888,000 (3%)	773,000 (1.3%)	2,500	114-261(16-29%)
450 (338)	228	1,235,000 (2%)	2,200,000 3.7%)	6,300	163-369 (23-41%)

Effect of Folic Acid Fortification of Flour

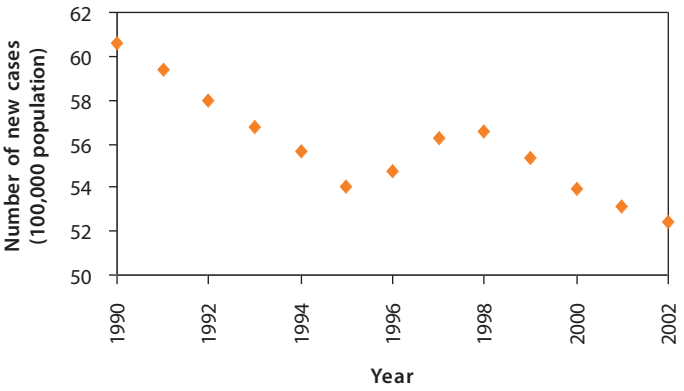


Problem: need for risk benefit analysis

	Benefit	Harm
Folate status	✓	
Neural tube defects	✓	
Cancer	?	?
Heart disease	?	?
Clinically manifest vitamin B_{12} deficiency		✓

Cancer – evidence of risk

Figure 1: Trends in colorectal cancer incidence in the USA



National Cancer Institute, USA, 2005

Do nothing:

voluntary increase in intake not effective,

voluntary fortification increases intake of those least likely to need greater intake, does not reach those most likely to need greater intake.

continue with part of population not getting enough and other part of population getting more than desirable (may increase with time if voluntary fortification increases)

Mandatory fortification;

- reduces those at risk of inadequate intake,
- increases numbers possibly at risk of excess, those consuming more than the safe upper level

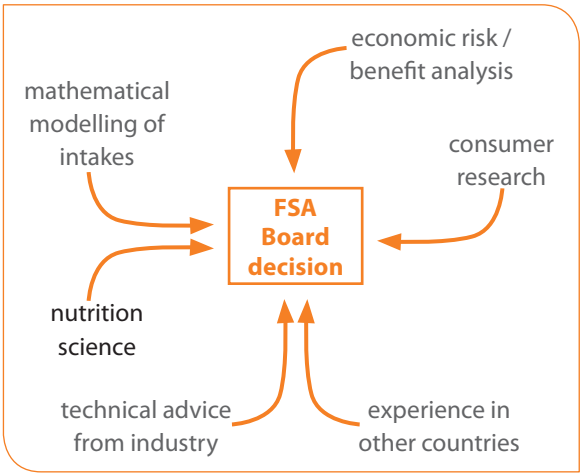
Mandatory fortification with limit on voluntary fortification and use of supplements.

- reduces those at risk of inadequate intake,
- limits the numbers possibly at risk of excess those consuming more than the safe upper level.

SACN recommendations

- All women who could become pregnant should take 400µg/day folic acid prior to conception and until the twelfth week of pregnancy. (5mg/d for women with a previous NTD-affected pregnancy.)
- Mandatory fortification should only be introduced in the UK if it is accompanied by:
  - action to reduce folic acid intakes from voluntarily fortified foods
  - measures for monitoring emerging evidence on effects of long-term exposure to intakes above the GL/UL per day including postulated adverse effects.
- Clear guidance is needed on the use of folic acid containing supplements by the general population.

Evidence



Conclusions

Complexity and nature of the evidence

Any evidence:

- related to cut point: intake > 1mg/d
- dose response, any effect < 1mg/d

At risk:

- food/fortification/supplementation,
- total intake folic acid/folates

Voluntary fortification:

- widen gap,
- how to advise/manage those with intakes > 1mg/d

Shift pattern of distribution of intakes.

Interventions with folic acid: WHO perspective for evidence-based guidelines

JUAN PABLO PENA-ROSAS
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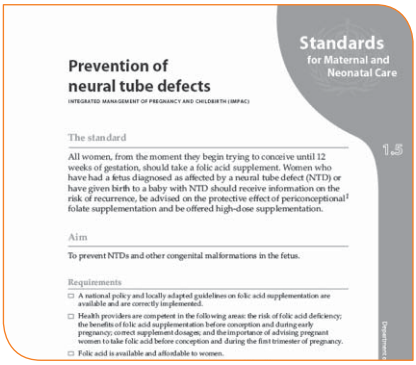
World Health Organization
Geneva, Switzerland

Consequences of folate deficiency

- Neural tube defects
  Spina bifida:
  - Paralysis
  - Loss of bowel & bladder control
  - Learning disabilities
  - Anencephaly:
  - Still births or death after delivery
- Anemia

Folic acid supplementation



Standards
for Maternal and
Neonatal Care

Prevention of neural tube defects

INTEGRATED MANAGEMENT OF PREGNANCY AND CHILDBIRTH (IMPAC)

1.5

The standard

All women, from the moment they begin trying to conceive until 12 weeks of gestation, should take a folic acid supplement. Women who have had a fetus diagnosed as affected by a neural tube defect (NTD) or have given birth to a baby with NTD should receive information on the risk of recurrence, be advised on the protective effect of periconceptional[1] folate supplementation and be offered high-dose supplementation.

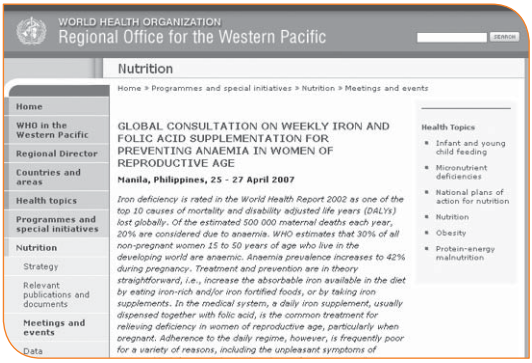
Aim

To prevent NTDs and other congenital malformations in the fetus.

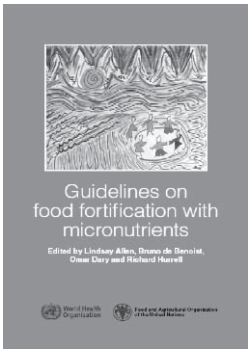
Requirements

- ☐ A national policy and locally adapted guidelines on folic acid supplementation are available and are correctly implemented.
- ☐ Health providers are competent in the following areas: the risk of folic acid deficiency; the benefits of folic acid supplementation before conception and during early pregnancy; correct supplement dosages; and the importance of advising pregnant women to take folic acid before conception and during the first trimester of pregnancy.
- ☐ Folic acid is available and affordable to women.

Weekly iron-folic acid supplementation (WIFS) in women of reproductive age: its role in promoting optimal maternal and child health



Folic acid fortification



WHO/FAO Guidelines on Food Fortification with Micronutrients, 2006

FFI and Partners

Second Technical Workshop on Wheat Flour Fortification: Practical Recommendations for National Application

March 30 to April 3, 2008

Wheat flour fortification: practical recommendations for national application

www.who.int/nutrition

WHO, FAO, UNICEF, GAIN, MI, & FFI. Recommendations on wheat and maize flour fortification. Meeting Report: Interim Consensus Statement. *Geneva, World Health Organization, 2009 (http://www.who.int/nutrition/publications/micronutrients/wheat_maize_fort.pdf, accessed [date])*


World Health Organization

Recommendations on Wheat and Maize Flour Fortification
Meeting Report: Interim Consensus Statement

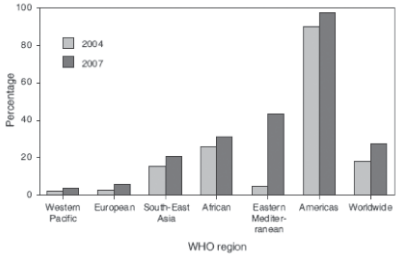
Average levels of nutrients to consider adding to fortified wheat flour based on extraction, fortificant compound, and estimated per capita flour availability

Nutrient	Flour Extraction Rate	Compound	Level of nutrient to be added in parts per million (ppm) by estimated average per capita wheat flour availability (g/day)[1]			
			<75[2] g/day	75-149 g/day	150-300 g/day	>300 g/day
Iron	Low	NaFeEDTA	40	40	20	15
		Ferrous Sulfate	60	60	30	20
		Ferrous Fumarate	60	60	30	20
		Electrolytic Iron	NR[3]	NR[3]	60	40
	High	NaFeEDTA	40	40	20	15
Folic Acid	Low or High	Folic Acid	5.0	2.6	1.3	1.0
Vitamin B_{12}	Low or High	Cyanocobalamin	0.04	0.02	0.01	0.008
Vitamin A	Low or High	Vitamin A Palmitate	5.9	3	1.5	1
Zinc[4]	Low	Zinc Oxide	95	55	40	30

1. *These estimated levels consider only wheat flour as main fortification vehicle in a public health program. If other mass-fortification programs with other food vehicles are implemented effectively, these suggested fortification levels may need to be adjusted downwards as needed.*
2. *Estimated per capita consumption of <75 g/day does not allow for addition of sufficient level of fortificant to cover micronutrients needs for women of childbearing age. Fortification of additional food vehicles and other interventions should be considered.*
3. *NR = Not Recommended because very high levels of electrolytic iron needed could negatively affect sensory properties of fortified flour.*
4. *These amounts of zinc fortification assume 5 mg zinc intake and no additional phytate intake from other dietary sources.*

Mandatory fortification 2007
Iron and folic acid: 50 countries
Folic acid: 2 countries

FIGURE. Percentage of wheat flour processed in roller mills that was fortified — worldwide and by World Health Organization (WHO) region, 2004 and 2007



Source: CDC. Trends in Wheat-Flour Fortification with Folic Acid and Iron -Worldwide, 2004 and 2007. MMWR 2008: 57(01);8-10

Fortification software programme

- Key component of a fortification programme is the calculation of the level of micronutrient fortification in a food
  - Must be both effective and safe
- Programme being designed to aid public health managers in calculating the optimal level of additional micronutrients in a user-friendly way, using locally available data
- Input programme
  - Upload food and nutrient intake data for population group(s) of interest
- Output programme
  - Present descriptive data on the population and the percent of population with inadequate intakes and excessive intakes

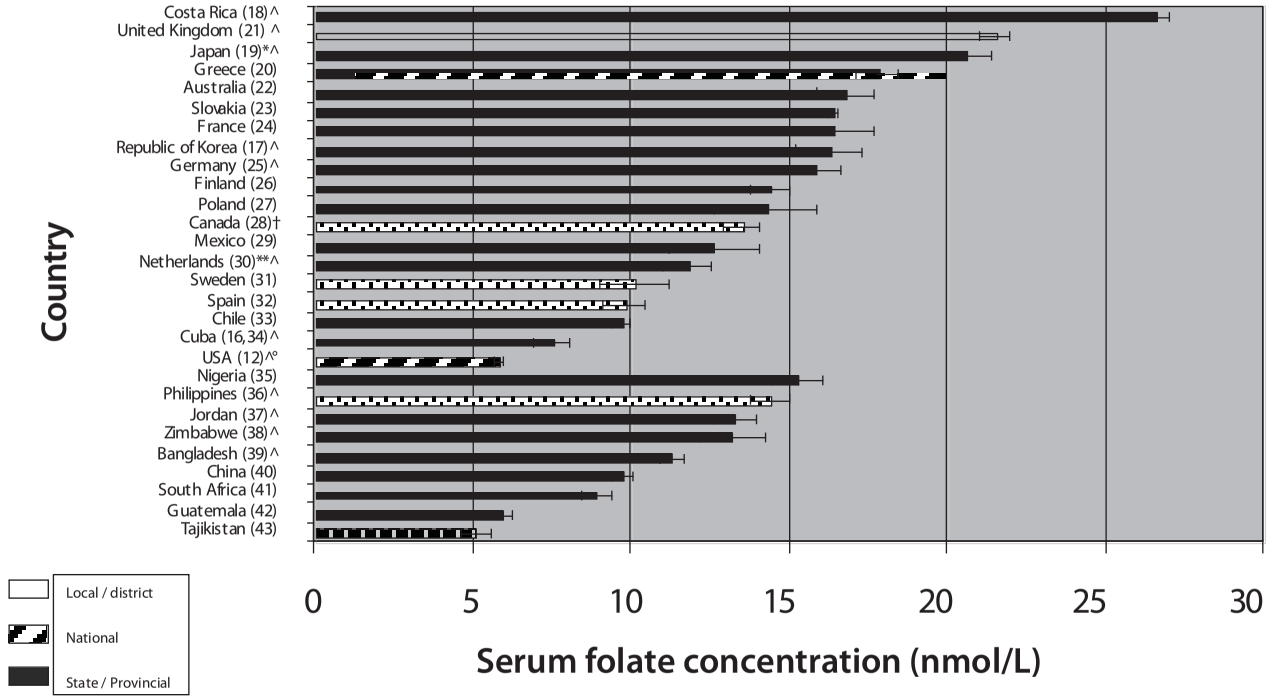
- Fortification programme
  - Evaluates the effect of fortifying one or more foods with one or more nutrients, as specified by the user.
  - Recalculates percent of population with inadequate intakes and excessive intakes
  - May be able to suggest optimal levels of nutrients for fortification based on desired population prevalence of inadequate and excessive intakes

Folate and vitamin B_{12} deficiencies WHO technical consultation Geneva, Switzerland October 18-21, 2005

published as supplement *Food and Nutrition Bulletin* June 2008

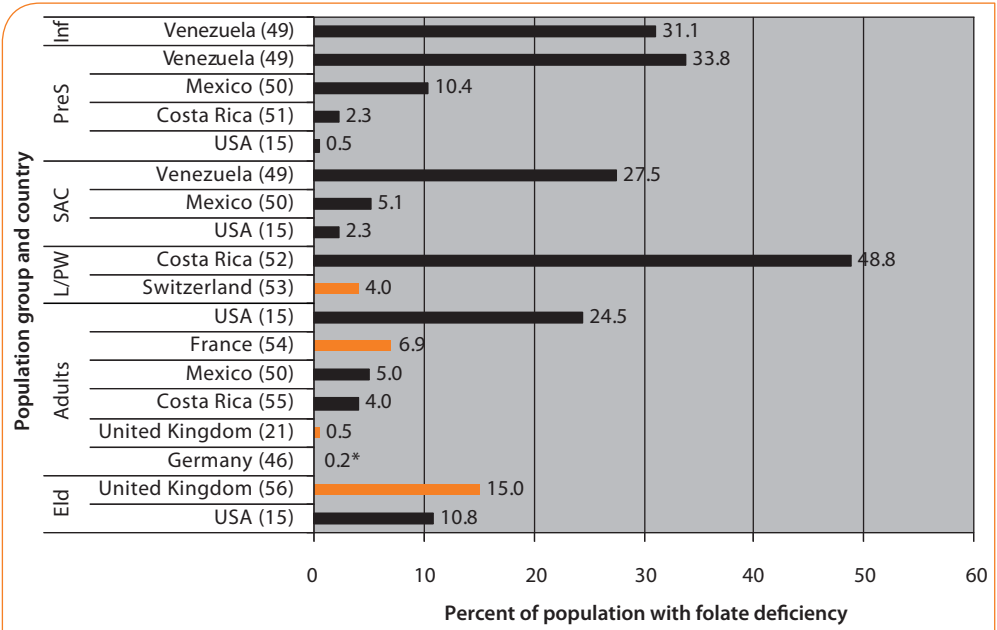
- The public health significance of folate and vitamin B_{12} deficiency
- Indicators to be used for assessing and monitoring vitamin status
- Strategies to control folate and vitamin B_{12} deficiency

Mean folate concentrations in adults, 2005.



Source: Mclean E et al. Review of the magnitude of folate and vitamin B_{12} deficiencies worldwide. Food and Nutrition Bulletin, 2008.

Prevalence of folate deficiency in countries with nationally representative data, 2005.



Source: Mclean E et al. Review of the magnitude of folate and vitamin B_{12} deficiencies worldwide. Food and Nutrition Bulletin, 2008.

Use of evidence in WHO recommendations

Oxman, Lavis & Fretheim. Lancet. 2007;369(9576):1883-9.

WHO guidelines are insufficiently transparent and not evidence based

- Lack of use of systematic reviews
- Lack of transparency about judgements
- Too much dependence on expert opinion
- Lack of emphasis on adapting global guidelines to end users' needs
- Tension between time taken and when advice needed
- Lack of resources

Solution 2007 - Effective as of 2008

- A review committee – Guidelines Review Committee
- Minimum standards for:
  - Reporting
  - Processes
  - Use of evidence
- Revised *WHO Handbook for Guideline Development*
- Different processes for documents to fit different purposes
  - Rapid advice guidelines
  - Standard guidelines
  - Full guidelines

WHO Standards for guidelines Principles

- Initial definition of scope and target audience
- Development of 'questions'
- Systematic and comprehensive evidence retrieval and synthesis
- Development of recommendations based on interpretation of evidence
- Management of conflicts of interest
- Standards for reporting
- Plan for implementation and update

Minimum standards for reporting *in WHO guidelines*

- Who was involved and their declaration of interests
- How the guideline was developed, including
  - How the evidence was identified
  - how the recommendations were made
- Use by date (review by date)

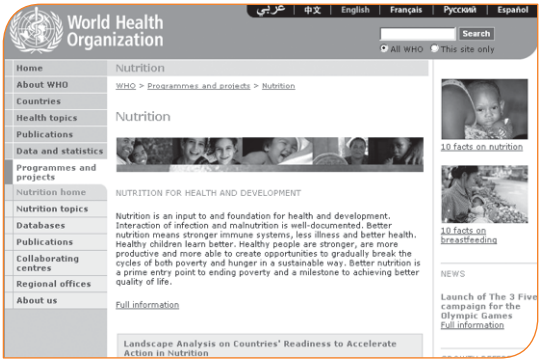
Standards for evidence Principles

- For recommendations:
  - Synthesis of all available evidence
  - Evidence summaries for group meetings using standard template
  - Formal assessment of quality of evidence
  - Consideration of resource use and costs
  - Linked evidence to recommendations, explaining reasons for judgements
- System for assessing evidence for interventions: GRADE

Nutrition Guidelines

- Questions needing review include:
  - Effects and safety of folic acid supplementation during periconception for preventing other birth defects
  - Effects and safety of (6S)-5-methyltetrahydrofolate in supplementation programs for WRA
  - Supplementation of folic acid in WRA in malaria endemic areas
  - Benefits and safety of fortification of staple foods with folic acid
  - Risks: Maternal folate status coupled with low vitamin B_{12} status and higher adiposity and insulin resistance in babies

http://www.who.int/nutrition/en/

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