FOLIC ACID AND COLORECTAL CANCER RISK: REVIEW OF RECOMMENDATION FOR MANDATORY FOLIC ACID FORTIFICATION

September 2009

Introduction

1. This report has been prepared in response to a request by the Chief Medical Officer (CMO) for detailed consideration by the Scientific Advisory Committee on Nutrition (SACN) of evidence suggesting potential adverse effects of folic acid on colorectal cancer risk. It updates previous advice provided by the Committee in their report, *Folate and Disease Prevention* (SACN, 2006).

Background

2. Folate is a generic term for a naturally occurring family of B-group vitamins. It is found naturally in a variety of foods. Folic acid is a synthetic form of folate which is widely used in supplements and for food fortification.

3. In December 2006, SACN published their report, *Folate and Disease Prevention* (SACN, 2006), which recommended that mandatory fortification of flour with folic acid would improve the folate status of women most at risk of pregnancies affected by neural tube defects (NTD). However, the Committee recognised that mandatory fortification combined with the current practice of voluntary fortification of foods with folic acid and general supplement use would increase the numbers in the population with intake levels of folic acid above the Guidance/Tolerable Upper Intake Level (GL/UL)[1]. SACN therefore recommended that mandatory fortification should only be introduced in the UK if it is accompanied by controls on voluntary fortification, guidance on supplement use, measures for careful monitoring of emerging evidence on the effects of long-term exposure to folic acid intakes above the GL/UL per day, and a review of the evidence on benefits and risks after five years. The previous recommendations of the SACN report are attached as *Annex 1*.

[1] In the UK, the Expert Group on Vitamins and Minerals (EVM) set aGL of 1 mg/day of folic acid for adults (EVM, 2003); GLs were not set for children. The GL is based on limited data and is an approximate indication of intakes that would not be expected to cause adverse effects. The evidence for adverse effects of folic acid were considered insufficient to establish a safe upper level (SUL); the SUL represents the amount of a nutrient that can consumed over a lifetime without significant risk to health and is based on adequate available evidence. In the USA and Europe, a UL of 1 mg/d of folic acid was set for adults (FNB, 1998; SCF, 2000); the UL represents the highest level of daily nutrient that is likely to pose no risk to health. ULs were set for children in the USA and Europe based on body weight. ULs for children, Europe: 4-6y, 300 µg/d; 7-10y, 400 µg/d; 11-14y, 600 µg/d; 15-17y, 800 µg/d. ULs for children, USA: 1-3y, 300 µg/d; 4-8y, 400 µg/d; 9-13y, 600 µg/d; 14-18y, 800 µg/d.

4. In June 2007, the Food Standards Agency recommended mandatory fortification of bread or flour with folic acid, alongside controls on voluntary fortification and guidance on supplement use, to UK Health Ministers. The recommendation was based on SACN's advice (see above paragraph). The purpose of the combined approach was to reduce the number of NTD-affected pregnancies without increasing the number of people with intakes of folic acid above the GL/UL per day compared to the current situation.

5. In October 2007, SACN was asked by the CMO to consider in depth two papers published in June 2007, Cole *et al* (2007) and Mason *et al* (2007), suggesting that folic acid may increase the risk of colorectal cancer. Preliminary data from both studies had previously been considered by SACN and the Committee had concluded that the relationship between folic acid and increased or reduced cancer risk was unclear. As there were insufficient data for a full assessment of folic acid intake levels in relation to cancer risk, the report recommended that, as a precaution, there should not be a substantial increase in average population intakes of folic acid or in the numbers consuming intakes above the GL/UL.

6. SACN found no evidence to suggest that high intakes of natural folates found in foods are associated with increased cancer risk.

Process

7. It was agreed to co-opt members of the Committee on Carcinogenicity (COC) and external experts in cancer epidemiology for further consideration of the evidence on folic acid and colorectal cancer risk.

8. The SACN Working Group (WG) on Folic Acid and Colorectal Cancer Risk was chaired by Professor Alan Jackson (Chair, SACN) and comprised members of SACN (the late Professor Sheila Bingham, Professor Tim Key, Dr Paul Haggarty), COC (Professor David Phillips, Professor Alan Boobis) and an external cancer epidemiologist (Professor Elio Riboli).

9. The WG met three times between January 2008 and March 2009 (21 January 2008, 7 January 2009, and 2 March 2009). The first meeting considered the studies by Cole *et al* (2007) and Mason *et al* (2007). The second and third meetings considered the results of a meta-analysis of a number of trials which had investigated the association between B vitamins (including folic acid) and cardiovascular disease (CVD) and had also collected data on cancer outcomes. A report[2] prepared by Dr Robert Clarke, co-ordinator of the B-Vitamin Trialists' Collaboration, with further details and results of these trials is attached as *Annex 2*. Some of the results reported in the summaries of the WG meetings are slightly different from those in *Annex 2* as they were based on earlier preliminary analyses.

10. The study by Cole *et al* (2007) was also considered by the COC in July 2007 and results from the B-Vitamin Trialists' Collaboration were considered in April 2009.

[2] Published in 2013: Vollset SE, Clarke R, Lewington S et al. Effects of folic acid on overall and site-specific cancer incidence during the randomised trials: meta-anlayses of data on 50,000 individuals. *Lancet.* 2013; 381: doi: 10.1016/S0140-6736(12)62001-7. The published results may differ slightly from those considered by SACN, which were based on preliminary analyses. http://www.ncbi.nlm.nih.gov/pubmed/23352552

Further consideration of the evidence on folic acid and colorectal cancer risk

11. The discussions at the meetings of the WG, the COC, and SACN are summarised below.

First meeting of the WG: 21 January 2008

12. The papers by Cole *et al* (2007) and Mason *et al* (2007) were considered. Another randomised controlled trial from the UK, which had investigated the effect of aspirin and folic acid on colorectal adenomas (Logan *et al*, 2008), was also considered.

13. The study by Cole *et al* (2007) was a double-blind randomised controlled trial in the USA which investigated the potential of folic acid supplementation (1 mg/day[3]) with or without aspirin for prevention of new colorectal adenomas in persons with a recent history of colorectal adenomas (Aspirin/Folate Polyp Prevention Study). Adenoma occurrence was determined by 2 colonoscopic examinations (at 3 years and 6-8 years).

14. Participants were recruited in 1994-1998 (voluntary folic acid fortification was introduced in 1996 and became mandatory in 1998). Since folic acid fortification was mandatory by the time of the second phase, the folic acid intakes of the folic acid supplemented group would have been higher than the 1 mg/day provided in the trial and much higher than the estimated increase in population average intakes of folic acid if mandatory fortification is introduced in the UK.

15. Results of the study showed 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 group in the first follow-up interval after 3 years (unadjusted risk ratio, 1.04; 95% CI, 0.90-1.20; p=0.58) or the second follow-up interval after 6-8 years (unadjusted risk ratio, 1.13; 95% CI, 0.93-1.37; p=0.23).

16. In the second interval (after 6-8 years) there was a significantly greater incidence of advanced lesions in the folic acid group compared to the placebo group (unadjusted risk ratio, 1.67; 95% CI, 1.00-2.80; p=0.05), but after adjustment[4] the difference was no longer significant (risk ratio, 1.57; 95% CI, 0.92-2.67). However there were significantly more people in the folic acid group with three or more adenomas in the second interval (unadjusted risk ratio, 2.32; 95% CI, 1.23-4.35; p=0.02); the difference remained significant after adjustment (risk ratio, 2.20; 95% CI, 1.15-4.21).

17. The design of the study by Cole *et al* (2007) was considered to be robust. It was agreed that the study raised concerns as it suggested that folic acid at doses in excess of 1 mg/day may increase the risk of developing multiple/advanced adenomas and consequently increase colorectal cancer risk in people with existing premalignant colorectal adenomas, a prior history of colorectal adenomas, or older people who are at increased risk of developing colorectal adenomas.

18. In the study by Logan *et al* (2007), which also investigated the potential of folic acid supplementation for the prevention of colorectal adenoma recurrence in people with a recent history of colorectal adenomas (n=853), participants in the folic acid group had received 0.5 mg/day of folic acid for 3 years. The relative risk (RR) of adenoma incidence for the folic acid group compared to the placebo group was not significant (RR, 1.07; 95% CI, 0.85-1.34; p=0.58).

[3] Equivalent to the GL/UL for adults.

[4] Age, sex, study centre, length of follow-up, lifetime number of adenomas at baseline, aspirin treatment assignment, smoking status [never, former, current], large [≥1 cm] baseline adenoma [yes/no], baseline advanced adenoma [yes/no]).

19. The paper by Mason *et al* (2007) is an ecological study which highlights a temporal association between folic acid fortification of enriched cereal grains and an increase in colorectal cancer incidence in the USA and Canada. In both countries the incidence of colorectal cancer decreased in the years after 1985 for about 10 years but then increased for 2 or 3 years between 1996-1998 (USA) and 1998-2000 (Canada) before recommencing a downward trend. The authors reported that the increase in colorectal cancer incidence represented a *"highly statistically significant deviation from the previous downward trend, resulting in about 4-6 additional cases per 100,000 individuals"*. The authors interpreted this pattern as an *"excess incidence"* which remained superimposed upon an overall downward trend in rates, and stated that the "*excess incidence"* had not returned to the earlier baseline by 2002. The paper hypothesises that the significant deviation from the downward trend in colorectal cancer incidence around this period could not be due to chance and that folic acid fortification, rather than improved screening for colorectal cancer, may have been responsible.

20. The WG considered whether the upturn in colorectal cancer incidence could be explained by an improvement in screening rates. With improved screening an immediate increase in colorectal cancer incidence would be expected in the short-term because the cancer is detected at an earlier stage, followed by a return to background levels once all previously unidentified cases had been accounted for; however, if screening rates increased gradually over a number of years, this delay could offset the return to previous incidence rates. In 1995 the US Preventive Services Task Force endorsed screening with fecal occult blood testing (FOBT) and sigmoidoscopy for people at average risk of colorectal cancer and this step change in policy might have led to the observed increase in colorectal cancer incidence. However the available data are insufficient to confirm whether the changes in colorectal cancer screening rates can explain the changes in colorectal cancer incidence in the USA or Canada.

21. There were differences of opinion in the interpretation of the study by Mason *et al* (2007). One view was that the increase in colorectal cancer incidence was transient, that it occurred at different times for men and women in the US and Canada, and that the changes in plasma and red cell folate concentrations pre and post folic acid fortification (Pfeiffer *et al*, 2007) were not clearly consistent with the trend data. Although a causal effect of folic acid fortification on colorectal cancer incidence could be consistent with the similarities and differences between the USA and Canada in changes in colorectal cancer incidence, the effect on tumour progression would have to have been almost immediate; current knowledge does not adequately explain a causal link between folic acid and colorectal cancer on this timescale (colorectal tumours usually develop from adenomas over a period of 10 or more years). It was noted that the absolute incidence in the USA is currently lower than it was before the introduction of folic acid fortification.

22. The other view was that, after adjustment for the prefortification trend, the increase in colorectal cancer incidence was statistically significant, was not transient but persisted while exposure to folic acid was elevated, and occurred at the same time for men and women.

23. The WG agreed that there was no certain explanation for the increase in colorectal cancer incidence observed in the USA and Canada at around the same time as the introduction of folic acid fortification and that increased rates of colorectal cancer screening, higher intakes of folic acid at the time of fortification, or other factors, could have been responsible.

24. After consideration of the evidence by Cole *et al* (2007) and Mason *et al* (2007), the WG agreed, on balance, with SACN's previous recommendation that mandatory fortification should only be introduced with controls on voluntary fortification and guidance on supplement use. However, it was agreed that the recommendation to restrict voluntary fortification should be strengthened if mandatory fortification is introduced.

SACN meeting: 7 February 2008

25. At the meeting of the full SACN committee on 7 February 2008, Members were informed that most of the data from the trials on B-vitamins and CVD would be available at the end of 2008. It was therefore agreed to delay making a decision regarding mandatory folic acid fortification until the results from these trials could be considered.

Second meeting of WG: 7 January 2009

26. Dr Robert Clarke, co-ordinator of the B-Vitamin Trialists' Collaboration, presented *preliminary* results from the completed B-vitamin trials. The Collaboration was set up to combine data from all randomised trials assessing the effects of B-vitamins on risk of vascular outcomes in participants with a prior history of vascular disease. The meta-analysis had more than 99% power to detect a 10% difference in major vascular events predicted by observational studies. The primary analysis assessed effects on major vascular events, stroke, major coronary events, any cancer and cancer at specific sites. Additional analyses assessed effects on vascular and cancer outcomes in sub-groups defined by age, sex, population level of fortification, pre-treatment levels of folate and homocysteine, and duration of treatment.

27. Preliminary data were available for 7 trials which had completed (n=35,429); data from another trial (n=2056) which had finished were still to be included. Results from 3 remaining trials (n=15,000) were not expected to be available until 2011.

28. The meta-analysis of individual participant data from the 7 trials which had completed found no effect of B-vitamins on major vascular events (hazard ratio, 1.01; 95% CI, 0.97-1.06), stroke (hazard ratio, 0.96; 95% CI, 0.87-1.06), or major coronary events (hazard ratio, 1.03; 95% CI, 0.96-1.09).

29. Cancer data were available from 6 trials (n=33,547). The meta-analysis of individual participant data from these trials found no evidence of a statistically significant increase on overall risk of cancer with B-vitamin supplementation after an average of 5 years; the hazard ratio for any cancer event for all trials was 1.06 (95% CI, 0.98-1.14). Neither was there any statistically significant effect of B-vitamins on cancer incidence by duration of trial, dose, or type of cancer (hazard ratio for colorectal cancer incidence was 1.11; 95% CI, 0.83-1.47).

30. There was an extensive discussion on whether the meta-analysis was sufficiently powered to detect an association between folic acid and colorectal cancer risk and it was agreed to reconvene once additional data (n=2056) from the other recently completed trial (see paragraph 27) had been included in the meta-analysis.

Third meeting of WG: 2 March 2009

31. Power calculations provided by Dr Robert Clarke indicated that the data from the completed B-vitamin trials had 72% power to detect a 10% higher risk of all cancer, 13% power to detect a 10% higher risk of colorectal cancer and 18% power to detect a 10% higher risk of prostate cancer. In 2011, the completed trials should collectively have an 84% power to detect a 10% higher risk of all cancers, a 16% power to detect a 10% higher risk of colorectal cancer and a 22% power to detect a 10% higher risk of prostate cancer.

32. The updated meta-analysis showed that inclusion of additional data (n=2056) from the other completed trial had little effect on the previous findings. The hazard ratio of any cancer event for the 7 trials was 1.05 (95% CI, 0.98-1.13) compared to the previous hazard ratio of 1.06 (95% CI, 0.98-1.14) of 6 trials; the hazard ratio for colorectal cancer incidence was 1.12 (95% CI, 0.85-1.49). There was also no trend in cancer risk by duration of treatment, by prior plasma homocysteine concentration, percentage reduction in plasma homocysteine, age, sex, or dose of folic acid.

33. The WG also considered a paper on colon cancer in Chile (Hirsch *et al*, 2009) which compared rates of hospital discharges due to colon cancer before (1992-1996) and after (2001-2004) mandatory fortification of flour with folic acid in 2000. However the study was considered to have a number of methodological limitations including lack of information and lack of clarity in the way the information was presented. Members agreed that it was not possible to draw any conclusions from this study.

34. The majority view of the WG was that, on balance, they supported SACN's original recommendation and that the new evidence they had considered did not provide a substantial basis for changing the recommendation. It was agreed that in the event of fortification, concerns about cancer risk should be addressed by careful monitoring of emerging evidence on any adverse effects of folic acid fortification.

35. The view of one member of the WG was that it would be unsafe to proceed with mandatory folic acid fortification because of the uncertainties in relation to colorectal cancer risk.

COC meeting: 2 April 2009

36. The Chair of the COC reminded Members that in July 2006, the COC had been asked by SACN for advice on the possible association between folic acid and cancer risk. The Committee had recommended a precautionary approach in considering mandatory fortification of flour with folic acid, i.e., increasing low intakes whilst ensuring that high intakes did not increase. The study by Cole *et al* (2007) had been discussed by the COC in July 2007. After consideration of this study, the Committee had concluded that, *'on balance, it was content with the proposals regarding mandatory fortification recommended by the FSA Board which includes monitoring of the folic acid intakes and status of the UK population and postulated risks, including cancer incidence, and a review of the data on the benefits and possible risks 5 years after introduction of mandatory fortification'.*

37. At the COC meeting in April 2009, Dr Robert Clarke presented the results from the meta-analysis of 7 B-vitamin trials which showed no statistically significant effect of folic acid on risk of cancer or colorectal cancer. The COC agreed that there was still considerable uncertainty since the meta-analysis was underpowered to detect an association between folic acid and cancer risk. There was no realistic way of reducing the uncertainty since inclusion of the results from the 3 outstanding trials would not provide the necessary increase in power; therefore, there would be no additional benefit in postponing the advice about folic acid and cancer risk until 2011 when the results from these trials are expected to be available. Members observed that many of the trials involved exposure to folic acid doses considerably higher than the fortification levels considered by SACN and that these high levels were additional to different background exposures depending on the fortification policy of the country in which the study was carried out.

38. The Committee also considered a paper by Figueiredo *et al* (2009) which reported secondary findings regarding prostate cancer incidence in the Aspirin/Folate Polyp Prevention Study by Cole *et al* (2007). Men in the folic acid supplemented group were reported to be at greater risk of developing prostate cancer compared to those in the placebo group (age-adjusted hazard ratio, 2.63; 95% CI, 1.23-5.65; p=0.01). The COC noted that the analysis was based on a small number of cases which could lead to spurious results and that co-dosing with aspirin may not have been adequately considered as a confounder. This publication was not considered to alter the weight of evidence.

39. In conclusion, the COC agreed with the decision of the WG to support SACN's previous recommendation for mandatory folic acid fortification. Members also agreed that it would be prudent to monitor emerging evidence of any adverse effects of folic acid fortification.

SACN Meeting: 10 June 2009

40. The Chair informed Members that, after consideration of further evidence on folic acid and cancer risk, the majority of the WG had supported SACN's previous recommendation for mandatory folic acid fortification and had agreed that the best way to address concerns about folic acid and cancer risk was to ensure that careful monitoring procedures were in place to identify risk at the earliest opportunity.

41. Members were asked if they agreed with the conclusions of the WG and whether they still supported SACN's original recommendation for the introduction of mandatory folic acid fortification.

42. Members agreed that the uncertainties regarding folic acid and cancer risk remained. SACN's original recommendation had taken this uncertainty into account by trying to limit exposure to high intakes of folic acid.

43. Concerns were raised regarding use of folic acid supplements by people who may be at increased risk of developing colorectal adenomas and those with existing premalignant adenomas. It was agreed that the recommendation needed to express this concern.

44. The majority of Members were in support of the previous recommendation to introduce mandatory fortification alongside controls on voluntary fortification. One Member was in support of the previous recommendation with the condition that it was amended to include specific guidance on supplement use for women planning a pregnancy and for people with existing colorectal adenomas or at increased risk of developing colorectal adenomas. One Member did not support mandatory fortification because of the possibility of an association between folic acid and increased colorectal cancer risk (the same person who did not support mandatory folic acid fortification on the WG).

45. A number of amendments to the wording of the original recommendation, to take account of the concerns raised by Members, were agreed.

Revised recommendations on mandatory fortification of flour with folic acid

46. As previously recommended by the Committee on Medical Aspects of Food and Nutrition Policy (Department of Health, 2000), all women who could become pregnant should take 400 µg/day folic acid as a medicinal or food supplement prior to conception and until the twelfth week of pregnancy. Women with a history of a previous NTD-affected pregnancy are advised to take 5 mg/day of folic acid prior to conception and until the twelfth week of pregnancy.

47. Individual long-term intakes of folic acid from fortified foods and supplements above the GL/UL per day for folic acid (1 mg/day for adults; lower amounts for children[5]) should be avoided. A proportion of the UK population[6] is currently exceeding the GL/UL per day due to consumption of foods fortified with folic acid on a voluntary basis and supplement use. The current risk posed by voluntary fortification of food with folic acid and supplement use in contributing to intakes above the GL/UL per day for folic acid needs to be addressed.

48. Mandatory fortification of flour with folic acid would improve the folate status of women most at risk of pregnancies affected by NTDs. It would also improve the folate status of other population groups in the UK. However, mandatory fortification, combined with the current practice of voluntary fortification of foods with folic acid and inappropriate supplement use, would increase the numbers in the population consuming levels of folic acid above the GL/UL per day. Therefore, mandatory fortification should only be introduced in the UK if it is accompanied by:
    - Action to restrict voluntary fortification of foods with folic acid;
    - measures for careful monitoring of emerging evidence on any adverse effects of long-term exposure to intakes of folic acid above the GL/UL per day; and
    - guidance on supplement use for particular population groups.

49. Mandatory fortification of flour[7] alongside restrictions on voluntary fortification will confer a more even distribution of folic acid intakes across the population compared to current voluntary fortification and supplement use. It will not lead to a substantial increase in the average population intake of folic acid but will reduce the risk of intakes exceeding the GL/UL and increase intakes of those currently consuming the lowest total folate intakes (from foods containing naturally occurring folates and foods fortified with folic acid).

50. The introduction of mandatory fortification will require: acquisition of new baseline data on folic acid intakes and blood folate concentration to ensure that mandatory fortification does not lead to an increase in folic acid intakes above the GL/UL and to permit monitoring of trends in future surveillance programmes; adoption of a sufficiently robust common standard analytical method for measurement of folate status at baseline and in all future surveillance studies; and establishment of suitable reference ranges to predict folate adequacy and deficiency.

51. If mandatory fortification were introduced, all women who could become pregnant and those with a history of a previous NTD-affected pregnancy should continue to supplement their diet with 400 µg and 5 mg per day of folic acid respectively prior to conception and until the twelfth week of pregnancy.

[5] GLs were not set for children in the UK. ULs were set for children in the USA and Europe based on body weight. ULs for children, Europe: 4-6y, 300 µg/d; 7-10y, 400 µg/d; 11-14y, 600 µg/d; 15-17y, 800 µg/d. ULs for children, USA: 1-3y, 300 µg/d; 4-8y, 400 µg/d; 9-13y, 600 µg/d; 14-18y, 800 µg/d.

[6] Approximately 106,000 people.

[7] Careful consideration would need to be given to the issue of overage.

52. There are no specific recommendations on folic acid supplementation for other population groups (i.e., children, women above child bearing age, and men) except on medical advice. For people who choose to take supplements, as a precaution, it would be advisable for those aged over 50 years not to consume supplements containing folic acid above the recommended nutrient intake (RNI)[8] for folate of 200 µg/day since the risk of developing colorectal adenomas/colorectal cancer increases after this age (Winawer *et al*, 1997; American Cancer Society, 2008). For people with a previous history of colorectal adenomas, folic acid supplementation should also not exceed 200 µg/day without medical guidance. This recommendation is relevant to current consumption patterns and those which would prevail if mandatory fortification were introduced.

53. Evidence on the benefits and hypothesised risks of folic acid should be reviewed after an appropriate period of time which should be no later than five years.

54. There are a number of uncertainties regarding the GL/UL per day set for folic acid which is based on limited data and relates to concerns regarding vitamin B_{12} deficiency. Further research is required on safe upper levels of folic acid intake in relation to other postulated risks, such as cancer.

55. More reliable diagnostic indices to identify vitamin B_{12} deficiency should be developed. The development of a clinical strategy to manage issues related to vitamin B_{12} is necessary irrespective of a decision on future mandatory fortification of flour with folic acid.

56. The prevalence of poor vitamin B_2 (riboflavin) status in the UK population needs to be addressed.

Acknowledgements:
Thanks to Professor David Phillips, Professor Alan Boobis, and Professor Elio Riboli for their time and assistance in consideration of the evidence on folic acid and colorectal cancer risk. Special thanks to the B-Vitamin Treatment Trialists' Collaboration, particularly Dr Robert Clarke, for sharing pre-publication results of the B-vitamin trials with the WG, COC, and SACN.

[8] The RNI represents the amount of a nutrient that that is sufficient to meet the requirements of 97.5% of the population.

References

American Cancer Society. *Cancer Facts & Figures 2008.* Atlanta: American Cancer Society; 2008.

Cole BF *et al*. Folic acid for the prevention of colorectal adenomas. *JAMA*. 2007; 297:2351-2359**.**

Department of Health. *Folic acid and the prevention of disease.* Report on health and social subjects 50. London: TSO, 2000.

Expert Group on Vitamins and Minerals. *Safe Upper Levels for Vitamins and Minerals.* London: Food Standards Agency, 2003.

Figueiredo JC, Grau MV, Haile RW, *et al*. Folic acid and risk of prostate cancer: results from a randomized clinical trial. *J Natl Cancer Inst.* 2009; 101(6):432-5.

Food and Nutrition Board, Institute of Medicine. *Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline.* Washington, DC: National Academy Press, 1998.

Hirsch S, Sanchez H, Albala C *et al*. Colon cancer in Chile before and after the start of the flour fortification program with folic acid. *Eur J Gastroenterol Hepatol.* 2009; 21(4):436-9.

Logan RFA *et al*. Aspirin and folic acid for the prevention of recurrent colorectal adenomas. *Gastroenterology*. 2008; 134(1):29-38.

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

Pfeiffer CM, Johnson CL, Jain RB. Trends in blood folate and vitamin B-12 concentrations in the United States, 1988-2004. *Am J Clin Nutr.* 2007; 86:718-27.

Scientific Advisory Committee on Nutrition. *Folate and Disease Prevention.* London: TSO, 2006.

Scientific Committee on Food. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Folate, 2000. Online at http://ec.europa.eu/food/fs/sc/scf/out80e_en.pdf

Winawer SJ, Fletcher RH, Miller L, *et al*. Colorectal cancer screening: clinical guidelines and rationale. *Gastroenterology.* 1997; 112:1060 and 1998; 114:625.

ANNEX 1

SACN's previous recommendations on mandatory folic acid fortification
Folate and Disease Prevention **(SACN, 2006)**

Previous recommendations in SACN report: *Folate and Disease Prevention* (2006)

1. As previously recommended by COMA (DH, 2000), all women who could become pregnant should take 400 µg/day folic acid as a medicinal or food supplement prior to conception and until the twelfth week of pregnancy. Women with a history of a previous NTD-affected pregnancy are advised to take 5 mg/day of folic acid prior to conception and until the twelfth week of pregnancy.

2. Individual long-term intakes of folic acid from fortified foods and supplements above the GL/UL per day for folic acid should be avoided. A proportion of the UK population is currently exceeding the GL/UL per day due to consumption of foods fortified with folic acid on a voluntary basis and supplement use. The current risk posed by voluntary fortification of food with folic acid and supplement use in contributing to intakes above the GL/UL per day for folic acid needs to be addressed.

3. Mandatory fortification of flour with folic acid would improve the folate status of women most at risk of NTD-affected pregnancies. It would also improve the folate status of other population groups in the UK. However, mandatory fortification, combined with the current practice of voluntary fortification of foods with folic acid and inappropriate supplement use, would substantially increase the numbers in the population consuming levels of folic acid above the GL/UL per day.

4. Mandatory fortification should only be introduced in the UK if it is accompanied by:
   - Action to reduce folic acid intakes from voluntarily fortified foods to ensure that the numbers of people with intakes above the GL/UL per day do not exceed current levels and there is no substantial increase in mean intakes or in the folate status of the UK population;
   - Measures for careful monitoring of emerging evidence on the effects of long-term exposure to intakes of folic acid above the GL/UL per day and the postulated adverse effects, including neurological damage, CVD, and cancer.

5. The introduction of mandatory fortification will require:
   - The establishment of a new baseline for folic acid intakes and blood folate concentration prior to fortification to ensure that mandatory fortification does not lead to substantial increases in folic acid intake or folate status and so that trends can be monitored in future surveillance programmes;
   - The adoption of a sufficiently robust common standard analytical method for measurement of folate status at baseline and in all future surveillance studies;
   - The establishment of suitable reference ranges to predict folate adequacy and deficiency.

6. If mandatory fortification is introduced, the evidence on benefits and postulated adverse effects should be reviewed after a period of five years.

7. Mandatory fortification of flour with folic acid, accompanied by action to reduce folic acid intake from voluntarily fortified foods, would lead to a redistribution of folic acid intakes of the population. This could provide the most secure method of balancing the benefits and possible risks to the UK population as, relative to current practice, it would reduce exposure to intakes of folic acid above the GL/UL and increase the intake of low consumers. Careful consideration would need to be given to the issue of overage.

8. Clear guidance is needed on the use of folic acid containing supplements by the general population.

9. If mandatory fortification is introduced in the UK, all women who could become pregnant and those with a history of a previous NTD-affected pregnancy should continue to supplement their diet with 400 µg/day and 5 mg/day of folic acid respectively prior to conception and until the twelfth week of pregnancy.

10. There are a number of uncertainties regarding the GL/UL per day set for folic acid which is based on limited data and is related to concerns regarding vitamin B12 deficiency. Further research is required on safe upper levels of folic acid intake in relation to other postulated risks, such as cancer.

11. More reliable diagnostic indices to identify vitamin B_{12} deficiency should be developed. The development of a clinical strategy to manage issues related to vitamin B_{12} is necessary irrespective of a decision on future mandatory fortification of flour with folic acid.

12. The prevalence of poor vitamin B2 (riboflavin) status in the UK population needs to be addressed.

ANNEX 2

Pre-publication paper which includes data considered by SACN

Effects of homocyteine-lowering on vascular disease, cancer and cause-specific mortality in 37, 485 individuals with a prior history of vascular renal disease

Information relevant to folic acid and cancer risk can be found in:

Paragraphs 12, 13, 17, 18

Figures

4 Effects of folic acid on cancer events, in different categories
5 Effects of folic acid on site specific cancers
7 Effects of folic acid on cancer events by trials
e5 Effects of folic acid on cancer incidence in additional categories

Effects of homocysteine-lowering on vascular disease, cancer and cause-specific mortality in 37,485 individuals with a prior history of vascular or renal disease

Robert Clarke FRCP[1], Jim Halsey BSc[1], Sarah Lewington DPhil[1], Jane Armitage FRCP[1], Eva Lonn MD[2], JoAnn E Manson MD[3], Kaare H Bonaa MD[4], David Spence MD[5], Ottar Nygard MD[6], Rex Jamison MD[7], J Michael Gaziano MD[8], Peter Guarino PhD[9], Fraz Mir MD[10], Derrick Bennett PhD[1] Richard Peto FRS[1] and Rory Collins FRCP[1] on behalf of the B-Vitamin Treatment Trialists' Collaboration

[1]Clinical Trial Service Unit and Epidemiological Studies Unit, University of Oxford, Oxford, UK; [2]Population Health Research Institute and Department of Medicine, Division of Cardiology, McMaster University, Hamilton, Ontario, Canada; [3]Department of Medicine, Harvard Medical School, Brigham and Women's Hospital, Boston, USA; [4]Department of Cardiology, University of Trosmso, Tromso, Norway; [5]Department of Neurology, Robarts Institute, London, Ontario, Canada;
[6]Department of Cardiology, University of Bergen, Bergen, Norway;[7] Department of Medicine, Stanford University School of Medicine, Palo Alto, California, USA;
[8]Massachussetts Veterans Research, Roxbury, Boston, USA; [9]Veterans Affairs Connecticut Healthcare System, Westhaven, Connecticut, USA;[10]Department of Clinical Pharmacology, Addenbrookes' Hospital, Cambridge, UK.

Correspondence to:

Dr Robert Clarke, Clinical Trial Service Unit and Epidemiological Studies Unit
Richard Doll Building, University of Oxford, Old Road Campus, Roosevelt Drive
Oxford, OX3 7LF, United Kingdom.
Telephone: 01865-743743
Fax: 01865-743985
E-mail: robert.clarke@ctsu.ox.ac.uk

Abstract: 299 Text 3604

Confidential Report for the Scientific Advisory Committee on Nutrition

22 September 2009

Abstract

Context Elevated plasma homocysteine concentrations have been associated with risk of cardiovascular disease, but the relevance of folic acid supplementation to lower homocysteine, or of vitamin B6, for prevention of vascular disease is uncertain.

Objectives To test the effects of supplementation with folic acid and other B-vitamins on risk of major vascular and non-vascular events.

Data sources An individual participant data meta-analysis of serious events (major vascular events, cancer and mortality) involving 37,485 individuals in 8 trials of people with prior vascular or renal disease.

Study selection Trials of B-vitamin therapy for prevention of vascular disease involving more than 1000 participants treated for at least one year.

Data extraction Data were collected on 9326 major vascular events (3990 major coronary events, 1528 strokes and 5068 revascularisations), 3008 cancer events and 5125 deaths.

Data synthesis The comparisons were intention-to-treat analyses of first events during the scheduled treatment period by allocation to B-vitamin supplements versus control. On average, folic acid allocation yielded a 24% reduction in homocysteine levels. During a median follow-up of 5 years, folic acid allocation had no significant effects on vascular outcomes, overall, or in any of the pre-specified sub-groups, with rate ratios (RR) and 95% confidence intervals (CI) of 1.01 (0.97-.05) for major vascular events, 1.03 (0.97-1.10) for major coronary events, and 0.96 (0.87-1.06) for stroke. Importantly, folic acid had no significant adverse effects on cancer (RR 1.05; 0.98-1.13) overall, or in any pre-specified sub-groups, or on total mortality (RR 1.02; 0.97-1.08). Vitamin B6 allocation also had no effect on plasma homocysteine levels or on any of the serious events studied.

Conclusions Dietary supplementation with folic acid or vitamin B6 had no significant effects on risk of cardiovascular events, cancer or mortality. These results do not support the routine use of B-vitamins for prevention of cardiovascular disease.

Introduction

1. Elevated plasma homocysteine has been identified as a potentially modifiable risk factor for coronary heart disease (CHD), stroke and other vascular diseases.[1-4] Observations on vascular disease in untreated children with homocystinuria, a rare autosomal recessive condition with plasma homocysteine levels greater than 100 µmol/L, prompted the hypothesis that moderate elevations of homocysteine may be relevant to vascular disease in the general population.[5] Many observational studies reported that cases with CHD or stroke had 3 to 5 µmol/L higher homocysteine concentrations than age and sex-matched controls.[1-3] In 1995, the first meta-analysis of such studies reported that a 5 µmol/L higher baseline homocysteine level was associated with a 70% higher risk of CHD.[2] Subsequently, prospective cohort studies of homocysteine and vascular disease reported more modest associations of homocysteine with incident vascular disease.[3,4] In 2002, a collaborative meta-analysis involving individual data reported that after adjustment for known vascular risk factors in prospective studies, a 25% lower usual homocysteine was associated with an 11% (95% CI: 4%-17%) lower risk of CHD and a 19% (5%-31%) lower risk of stroke.[4]

2. A meta-analysis of folic acid trials assessing the effects on homocysteine concentrations demonstrated that in populations without folic acid fortification, folic acid lowered homocysteine by 23%, vitamin B12 provided a 7% additional reduction, and the combination lowered it by 30%.[6] The effects of folic acid are attenuated in populations with mandatory folic acid fortification, where combination therapy typically lowers homocysteine by 20%.[6] Supplementation with vitamin B6 does not affect plasma homocysteine concentrations, but observational studies have reported inverse associations of dietary intake of vitamin B6 with CHD[7] and of circulating vitamin B6 concentrations with CHD[8], independent of plasma homocysteine concentrations. Moreover, supplementation of individuals with homocystinuria with B-vitamins (particularly vitamin B6) is highly effective for the prevention of vascular complications.[9]

3. Several large trials of B-vitamin supplements to lower homocysteine concentrations in patients with prior vascular or renal disease were designed to test the "homocysteine hypothesis" of vascular disease[10-20] but the results to date have not been encouraging. Many of these trials were designed to detect a 30% reduction in CHD risk, based on quantitative reviews of the observational studies published by the late 1990s,[2,3] and these trials individually lacked statistical power to detect the effects of homocysteine-lowering on vascular risk.[21] Consequently, a collaborative prospective meta-analysis involving individual participant data from all large homocysteine-lowering trials for the prevention of cardiovascular disease was established in 2004.[21,22] The present report describes the effects of folic acid on serious events (major vascular events, cancer and mortality) in 37,485 participants from 8 homocysteine-lowering trials that were completed before 2009 for the first cycle of this collaboration.[10-17] The aims of this report were: (i) to examine the effects of lowering homocysteine with folic acid on vascular or non-vascular outcomes, overall and in pre-specified sub-groups; and (ii) to assess the effects of vitamin B6, alone or in combination with folic acid on vascular and non-vascular outcomes.

Methods

Trial eligibility

4. Randomized trials were eligible if (i) they involved a randomized comparison of B-vitamin supplements containing folic acid versus placebo (irrespective of whether any other treatment was administered factorially) in trials designed for the prevention of vascular disease; (ii) the relevant treatment arms differed only with respect to the homocysteine-lowering intervention (i.e. they were unconfounded); and (iii) the trial involved 1000 or more participants for a scheduled treatment duration of at least one year. Unpublished trials were sought through electronic searches and discussions with other experts in the field, but none were found. Individual participant data were provided from all trials completed before 2009 for the 1st cycle of this collaboration. Data are not yet available from 3 trials involving 15,000 participants (8000 from VITATOPS[18], 4000 from FAVORIT[19] and 3000 from SU.FOL.OM3[20]) that are not expected to report their results before 2010. An additional trial of 15,000 patients with hypertension living in China, that started enrolment in 2008, will assess the effects on stroke risk of folic acid or placebo when added to blood pressure lowering therapy.[23] The present report describes the results for the 1st cycle of this collaboration, involving data on 37,485 individuals (72%) of 52,000 included in any large trial for prevention of vascular disease in Western populations.

Baseline and follow-up data collected

5. Data were sought about each randomised participant for certain characteristics prior to randomisation, details of the randomly allocated treatments, and the type and date of any of the prospectively agreed outcomes occurring during the scheduled treatment period. As well as providing these data on each participant, investigators were also asked to provide summary data on the number of patients allocated to each treatment group, and plasma concentrations of total homocysteine, folate and vitamin B12 before and after starting treatment, and the numbers who developed each of the prospectively defined outcomes. The individual patient data were checked for consistency with any published reports (and directly with the trialists) to help ensure that the individual study results were incorporated correctly into the meta-analysis and, hence, that the results are reliable.

Pre-specified analyses

6. The comparisons were intention-to-treat analyses of first events during the scheduled treatment period in all participants allocated to folic acid-based B-vitamins or control (irrespective of any other treatment allocated factorially) and separately among participants allocated vitamin B6. The main outcomes were major vascular events (and its components: major coronary events, strokes, coronary and non-coronary artery revascularisations), cancers, total and cause-specific mortality. Major coronary events were defined as the first occurrence of non-fatal myocardial infarction or coronary death (including death due to heart failure and sudden or unexpected deaths that are assumed to be coronary in origin). Stroke was defined as the first occurrence of either ischemic or hemorrhagic or unclassified strokes (but not including transient cerebral ischemic attacks). Coronary revascularisation events included coronary artery bypass grafting or coronary angioplasty (with or without stent insertion) and non-coronary revascularisation included carotid endarterectomy or carotid artery angioplasty, repair of aortic aneurysm, peripheral arterial surgery, or

angioplasty. A major vascular event was defined as the first occurrence of any major coronary event, stroke, or coronary or non-coronary revascularisation. A cancer event was defined as the first occurrence after randomisation of any malignancy, excluding non-melanoma skin cancers.

Statistical analysis

7. The primary comparisons were of the effects of B-vitamins to lower homocysteine levels on risk of major vascular events, stroke and major coronary events, cancer and mortality during the scheduled treatment period. The log-rank observed minus expected (o-e) statistics and their variances (v) from each trial were summed to produce, respectively, a grand total observed minus expected (G) and its variance (V).[24] The one step estimate of the log of the event rate ratio is G/V with variance 1/V. The effects on vascular outcomes were assessed in pre-defined sub-groups: trial; sex; age, approximate thirds of pre-treatment plasma or serum concentrations of folate (<10, 10-18 and 18 nmol/L or greater) and homocysteine (<11, 11-14, and 15 μmol/L or greater); mandatory folic acid fortification; years since randomisation. Additional sub-groups included baseline smoking, alcohol drinking, presence of diabetes, statin use, aspirin use, body mass index (<25, 25-29, 30 kg/m^2 or greater) and creatinine (<80, 80-94, 95 μmol/L or greater). In addition, the rate ratios for major vascular events in each trial were compared with the percentage homocysteine reduction achieved in the trial. The X^2 test statistic (χ^2_{n-1}) for heterogeneity between n trials is $S-(G^2/V)$, where S is the sum over all the trials of $(o-e)^2/v$. Heterogeneity of the rate ratios among multiple sub-groups defined by baseline characteristics was investigated by a global heterogeneity test, which helps to avoid misinterpreting false positive results arising from multiple comparisons.[25] For analyses of rate ratios [RR], 99% confidence intervals [CI] were used for the individual trials or sub-groups and 95% confidence intervals [CI] were used for the summary estimates. All analyses were carried out using SAS (Version 9.1).

Results

Characteristics of the participating trials

8. Selected characteristics of the 8 individual trials are shown in **Table 1** (and eTable 1). Six trials recruited individuals with prior coronary heart disease,[10, 12, 13, 15-17] one with prior stroke[11] and one with end-stage renal disease[14]. Two-thirds of the participants were men, and the mean (SD) age at entry was 65 (10) years. The results of four trials[10,12,16,17] that recruited people from non-fortified populations (20,785 individuals, 5878 major vascular events) were compared with four trials[11,13,14,15] that were carried out in fortified populations (16,700 individuals, 3448 major vascular events). Two trials[11,13] included participants from both non-fortified and fortified populations and the results were analyzed by fortification status of the individual participants. The median duration of treatment varied from 1.8 to 7 years (median: 5 years). All trials included a comparison of B-vitamins containing folic acid (hereafter, referred to as "folic acid") with placebo, except the VISP trial[11] that compared the effects of 2.5 mg with 0.02 mg of folic acid. The daily doses of folic acid used in most trials ranged from 0.8 mg to 5 mg, but the HOST trial used 40 mg of folic acid[14]. All trials, except the CHAOS-2 trial[10] also included vitamin B12 (dose range 0.4 to 1 mg) with folic acid. Four trials[11, 13-15] assessed the effects of combinations of folic acid (dose range: 2.5 to 40 mg), vitamin B12 (dose range: 0.4

to 1 mg), and vitamin B6 (dose range: 5 to 100 mg) with placebo. Two trials (6839 individuals, 2642 major vascular events) [12, 17] assessed the effects of vitamin B6 (40 mg) vs placebo, independently of the folic acid comparison. The results of the effects of vitamin B6, with or without added folic acid, on serious events were considered separately. About 18% were current smokers, 20% had diabetes and 30% were obese (defined as body mass index of 30 kg/m^2 or greater) (eTable 2).

Effect of folic acid on plasma homocysteine concentrations

9. **Table 2** shows the median plasma homocysteine and folate concentrations prior to treatment and the first available post-treatment value after randomisation among those allocated to folic acid and placebo. The median pre-treatment plasma folate concentrations were higher in fortified compared with non-fortified populations (22.3 vs 11.8 nmol/L, respectively). Consequently, allocation to folic acid treatment was associated with a greater proportional reduction in homocysteine concentrations in non-fortified compared with that in fortified populations (25.8% vs 20.3%). Overall, the meta-analysis assessed the effects of folic acid on risk of vascular and non-vascular events associated with an average 24% reduction in homocysteine levels (about 3 μmol/L) maintained for a median duration of 5 years.

Effect of folic acid on major vascular events

10. Among the 37,485 participants in 8 trials, there were 9326 incident major vascular events during the scheduled treatment period (**Table 3** and eTable 2). Allocation to folic acid treatment had no overall effect on major vascular events, with 4670 (24.9%) among 18,723 allocated to folic acid and 4656 (24.8%) among 18,762 allocated to control (RR 1.01; 95% CI 0.97-1.05; **Figure 1**). Nor was there any effect of supplementation with folic acid on any component of major vascular events. Major coronary events were reported in 2019 (11.4%) in the folic acid treated group and 1971 (11.1%) in the control group (RR 1.03; 0.97-1.10; Figure 1). Despite consistent evidence for stronger associations in observational studies of blood homocysteine levels with risk of stroke compared with CHD[4], folic acid had no significant effects on the overall risk of stroke: (747 [4.2%] vs 781 [4.4%] stroke events; RR 0.96; 0.87-1.06; Figure 1). Nor was there any effect of supplementation with folic acid on ischemic stroke (RR 0.96; 0.81 -1.14) or hemorrhagic stroke (RR 1.08; 0.66 -1.77) or unclassified stroke (RR 0.94; 0.75-1.18) or on fatal or non-fatal strokes (eFigure 1). Moreover, treatment with folic acid had no beneficial effects on coronary or non-coronary artery revascularisation events (Figure 1).

11. The overall lack of effect of B-vitamins on major vascular events was mirrored in each of the pre-specified sub-groups (**Figure 2** and eFigure 2). Despite substantial differences in folate status before treatment and dose of folic acid assessed and other folic acid used in the different trials, there was no significant heterogeneity between the effects of folic acid on major vascular events in the individual trials (test for heterogeneity, χ_7^2=8.09; p=0.3). Nor was there was any significant heterogeneity between any of the pre-specified subgroups (global test for heterogeneity, χ_5^2=3.25 p=0.66). Even among the one third with the highest homocysteine levels (mean: 21 μmol/L) that experienced the greatest reduction in homocysteine concentrations (about 27%), folic acid allocation was not associated with any reduction in risk of major vascular events. There was no trend of increasing benefit or hazard with increasing follow-up on treatment for up to 7 years (χ_1^2=0.49) (Figure 2).

Supplementation with folic acid had no effects on major vascular events in additional categories defined by smoking, alcohol intake, history of diabetes, use of statins or aspirin, body mass index, or creatinine (global test for heterogeneity, χ_7^2=7.59; p=0.37: eFigure 2). Consistent with the absence of any heterogeneity of effect of folic acid on major vascular events, there was no heterogeneity in the effects of folic acid on major coronary events (eFigure 3) or on stroke (eFigure 4) in different subgroups. **Figure 3** shows that even taking into account the large differences in homocysteine response to folic acid achieved in the trials, there was no suggestion that even in those trials where a larger homocysteine reduction was achieved was there any beneficial effects of folic acid on risk of any vascular disease event.

Effect of folic acid on cancer

12. Data were available on 3008 incident cancers among the 35,603 individuals included in the 7 vascular disease trials (one trial[10] did not collect data on cancer). Cancer events occurred in 1539 (8.7%) of 17,783 allocated to folic acid and in 1469 (8.2%) of 17,820 allocated to control treatment (RR 1.05; 95%CI: 0.98-1.13; **Figure 4**). There was no significant heterogeneity in the effects on cancer between the individual trials (test for heterogeneity, χ_6^2=4.62; p=0.6) despite the daily doses of folic acid used ranging from 0.8 mg to 40 mg, nor was there any heterogeneity in the effects of folic acid on cancer in any of the pre-specified sub-groups (global test for heterogeneity, χ_5^2=5.9; p=0.32). Importantly, even among those patients who took folic acid treatment up to 7 years, there was no suggestion of hazard beginning to emerge with longer duration of treatment (χ_1^2 for trend=0.01; p= 0.9). In addition, there was no heterogeneity in the effects of folic acid on any cancer events in additional categories defined by smoking, alcohol consumption, and body mass index, use of aspirin or statins or creatinine (global test for heterogeneity, χ_7^2=4.33; p=0.74: eFigure 5). There was no significant effect of folic acid on cancers at any site, including colorectal cancer, prostate cancer, lung cancer or cancers at any other site (**Figure 5**).

Effects of folic acid on mortality

13. Data were available on 5125 deaths among the 37,485 participants. Allocation to folic acid was not associated with any significant differences in overall mortality. There were 2578 deaths (13.8%) among 18,723 allocated folic acid vs 2547 deaths (13.6%) among 18,762 allocated to control (RR 1.02; 95%CI: 0.97-1.08; **Figure 6**). Nor was there any significant effect of folic acid on specific causes of death. Consistent with the overall null results on risk of major vascular events, folic acid had no significant effects on mortality from CHD (RR 1.02; 0.90-1.16), stroke (RR 0.92; 0.67-1.25) or from any other vascular cause (RR 0.99; 0.83 -1.17). Importantly, allocation to folic acid had no effect on cancer mortality (RR 1.00; 0.85-1.18).

Effect of vitamin B6 on all outcomes

14. Dietary supplementation with vitamin B6 alone had no effect on homocysteine concentrations in the two trials[12,17] that directly assessed the effects of vitamin B6 with control (eTable 3). Allocation to vitamin B6 alone had no effect on major vascular events (RR 1.00; 95%CI: 0.87-1.16; eFigure 6), nor did allocation to vitamin B6 with folic acid (RR 0.99; 0.92-1.06) or allocation to folic acid acid alone (RR 1.04; 0.96 -1.12). Nor did allocation to vitamin B6 alone have any significant effect on

cancer (RR 0.96; 0.61-1.52; eFigure 7), or on total mortality (RR 1.01; 0.72-1.42; eFigure 8).

Discussion

15. The present meta-analysis involving 37,485 individuals assessed the effects of folic acid on 9326 major vascular events (3990 coronary events, 1528 stroke events and 5068 revascularisations), and had more than 99% power to detect a 10% difference in major vascular events predicted by the observational studies.[4,21] Despite a 24% average reduction in homocysteine concentrations (26% in non-fortified populations and 20% in fortified populations) maintained for a median duration of 5 years, this meta-analysis demonstrated that lowering homocysteine levels had no effect on risk of cardiovascular disease events. The dose of folic acid used in the individual trials exceeded the minimum daily doses associated with a maximum reduction in homocysteine concentrations and most trials included vitamin B12. With data on individual participants, this meta-analysis was able to assess the effects of treatment on standardized outcomes, and in a wide range of pre-specified sub-groups. Contrary to suggestions from observational studies indicating stronger associations of homocysteine with stroke than CHD[4], and in women than men[4], lowering homocysteine had no beneficial effects on either stroke or CHD, either in women or in men. Significantly, folic acid had no effects on major vascular events in individuals with either low plasma folate concentrations or high homocysteine concentrations. While treatment continued for up to 7 years was not associated with any beneficial effects, the possibility that a longer duration might be associated with some effects on vascular risk cannot be excluded. However, the available evidence refutes the "homocysteine hypothesis" of vascular disease that was suggested by the observational studies[1-4], and also that of any relevance of vitamin B6 that may be independent of homocysteine[7,8]. The reasons for the discrepant effects of the observational studies of vascular disease and the randomised trials are not fully understood, but as homocysteine is highly correlated with creatinine, it may reflect some other correlate of renal impairment that is unaltered by B-vitamins.

16. These results highlight the importance of individual patient data meta-analysis of all the available evidence from large-scale trials when assessing moderate differences in risk. For example, the present meta-analysis had more than six times the number of incident stroke events than the HOPE-2 trial that reported a significant protective effect of B-vitamins on risk of stroke.[26] Moreover, the findings from this meta-analysis also refute the findings of a previous meta-analysis of B-vitamin trials that reported that folic acid was effective for the prevention of stroke.[27] The results of the present meta-analysis differ from an earlier report on secular trends in stroke mortality in the United States and the United Kingdom, which attributed the greater reduction in stroke mortality between 1990 and 2002 in the United States compared with that in the United Kingdom to the introduction of fortification.[28] The present meta-analysis demonstrated unequivocally that folic acid supplementation does not influence the risk of stroke in Western populations.

17. While observational studies have reported that folate status was inversely related to risk of colorectal cancer[29] and breast cancer[30], concerns have been expressed that increasing folic acid intake either from supplements, or from mandatory folic acid fortification, might transform adenomas into cancers or small cancers into larger

ones.[31] While the Aspirin and Folate Polyp Prevention Trial in the United States of 1021 individuals with prior colorectal adenoma reported an excess risk of advanced or multiple adenomas[32], and of prostate cancer[33] associated with folic acid treatment for 7 years, a trial of 0.5 mg of folic acid daily in 945 patients with colorectal adenoma treated for 3 years in the United Kingdom reported no excess of cancer (9 vs 8 events).[34] An analysis of trends in colorectal cancer incidence in the United States from 1986 to 2002 indicated a transient reversal in the downward trends coinciding with the introduction of folic acid fortification in 1996 that the authors suggested might be causal also raised concerns about folic acid and cancer.[35]

18. The present cancer analysis, involving 35,603 individuals at high-risk of vascular disease demonstrated no statistically significant adverse effect of folic acid on cancer incidence (RR 1.05; 95%CI: 0.98-1.13), overall, or in any of the pre-specified sub-groups. Moreover, there was no trend of an emerging hazard for duration of treatment of up to 7 years. Moreover, the meta-analysis demonstrated no heterogeneity in the effects on cancer by dose of folic acid, ranging from 0.8 mg to 40 mg daily. For example, the HOST trial, that evaluated the effects of 40 mg of folic acid that resulted in a 100-fold increase in plasma folate concentration, had no adverse effect on cancer (RR 0.94; 95%CI: 0.61-1.47).[14] Further details of the effects of B-vitamins on site-specific cancers will be provided in a separate report.

19. One third of adults living in the United States,[36] and one fifth of adults in the United Kingdom,[37] report taking daily multivitamin supplements containing B-vitamins. The present meta-analysis refuted the “homocysteine hypothesis” of vascular disease and demonstrated that routine use of folic acid or vitamin B6 has no significant effects on vascular or non-vascular events and suggests that dietary supplementation with B-vitamins cannot be recommended for the prevention of vascular disease in the general population. The daily doses of folic acid used in these trials (0.8 mg to 40 mg) were substantially greater than the dose of folic acid used for mandatory fortification (140 µg/100 gm cereal grain products) in the United States, but the results do not demonstrate any significant hazards for any serious events (vascular or cancer or mortality events) to warrant concern about existing public health strategies on folic acid fortification that have proven efficacy for the prevention of neural tube defects.[38,39]

Contributions

All members of the writing committee contributed to the collection or analysis of the data, or both, to the interpretation of the results, and the preparation of the report.

B-vitamin Treatment Trialists' Collaboration

Writing Committee for this report R Clarke, J Halsey, S Lewington, J Armitage, E Lonn, JE Manson, KH Bonaa, D Spence, O Nygard, F Mir, D Bennett, R Peto, R Collins for the B-Vitamin Treatment Trialists' Collaboration.
.

Collaborators in trials assessing effects on vascular events

CHAOS-2: F Mir, M Brown; **FAVORIT**: A Bostom, I Rosenberg; **HOPE-2**: E Lonn, S Yusuf; **NORVIT**: KH Bonaa, I Njolstad; **SEARCH**: J Armitage, L Bowman, R Collins; **Secretariat** R Clarke, S Lewington, J Halsey, D Bennett, J Armitage, P Sherliker, R Collins, **SU.FOL.OM3**: P Galan, S Hercberg; **HOST:** R Jamison, JM Gaziano, P Guarino; **VITATOPS**: G Hankey, JW Eikelboom; **VISP**: J Toole, MR Malinow, LE Chambless, JD Spence, LC Pettigrew, VJ Howard, EG Sides, CH Wang, M Stampfer; **WAFACS**: JE Manson, R Glynn, F Grodstein; **WENBIT**: O Nygard and M Ebbing, DWT Nilsen, PM Ueland, H Refsum, SE Vollset.

Collaborators in additional trials assessing effects on cancer

AFPPS: J Baron; **ukCAP** J Logan, KR Muir; **Harvard**: E Giovannuci.

Conflicts of interest

The Clinical Trial Service Unit and Epidemiological Studies Unit (CTSU), where the BVTT Secretariat is located has a policy of staff not accepting fees or honoraria, or paid consultancies. The CTSU receives funding from the British Heart Foundation (BHF) and the UK Medical Research Council (MRC) and support for the SEARCH trial was received from Merck. Partial support for this project was provided by a grant from the UK Food Standards Agency.

Acknowledgements

We thank the trial participants and the investigators. Sources of funding for the individual trials are described in their separate publications. The CTSU is supported by a core grant from the UK MRC, BHF and by Cancer Research UK. R Collins is supported by the BHF Personal Chair.

List of Tables

Table 1	**Design and eligibility criteria of participating trials**
Table 2	**Vitamin status before and after treatment in the included trials, by presence of fortification**
Table 3	**Distribution of serious events and event rates by trial**
eTable 1	**Distribution of major vascular events (MVE), cancer and total mortality in individual trials, by allocated treatment**
eTable 2	**Distribution of cardiovascular risk factors in individual trials**
eTable 3	**Vitamin status before and after treatment in trials with independent allocation to vitamin B6 or placebo**

List of Figures:

Figure 1	**Effects of folic acid on major vascular events**
Figure 2	**Effects of folic acid on major vascular events in different categories**
Figure 3	**Effects of folic acid on major vascular events, by percentage reduction in homocysteine**
Figure 4	**Effects of folic acid on cancer events in different categories**
Figure 5	**Effects of folic acid on site-specific cancers**
Figure 6	**Effects of folic acid on cause-specific mortality**
Figure 7	**Effects of folic acid on cancer events by trial**
eFigure 1	**Effects of folic acid on stroke types**
eFigure 2	**Effects of folic acid on major vascular events, in additional categories**
eFigure 3	**Effects of folic acid on major coronary events, in different categories**
eFigure 4	**Effects of folic acid on stroke, in different categories**
eFigure 5	**Effects of folic acid on cancer, in additional categories**
eFigure 6	**Effects of vitamin B6 on major vascular events**
eFigure 7	**Effects of vitamin B6 on cancer events**
eFigure 8	**Effects of vitamin-B6 on total mortality**

References

1. Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B et al. Hyperhomocysteinemia: an independent risk factor for vascular disease. N Engl J Med 1991;324(17):1149-55.

2. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA 1995;274(13):1049-57.

3. Danesh J, Lewington S. Plasma homocysteine and coronary heart disease: systematic review of published epidemiological studies. J Cardiovasc Risk 1998;5(4):229-32.

4. Homocysteine Studies Collaboration. Homocysteine and Risk of Ischemic Heart Disease and Stroke: a meta-analysis. JAMA 2002;288:2015-22.

5. McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol 1969;56(1):111-28.

6. Homocysteine Lowering Trialists' Collaboration. Dose-dependent effects of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials. Am J Clin Nutr 82(4), 806-812. 2005.

7. Rimm EB, Willett WC, Hu FB et al. Folate and vitamin B6 from diet and supplements in relation to risk of coronary heart disease among women. JAMA 1998; 279: 359-364.

8. Robinson K, Arheart K, Refsum H, et al.; European COMAC Group. Low circulating folate and vitamin B6 concentrations: risk factors for stroke, peripheral vascular disease, and coronary artery disease. Circulation 1998; 97:437-443.

9. Yap S, Naughten ER, Wilcken B, Wilcken DE, Boers GH. Vascular complications of severe hyperhomocysteinemia in patients with homocystinuria due to cystathionine beta-synthase deficiency: effects of homocysteine-lowering therapy. Semin Thromb Hemost 2000;26(3):335-40.

10. Baker F, Picton D, Blackwood S, Hunt J, Erskine M, Dyas M et al. Blinded comparison of folic acid and placebo in patients with ischaemic heart disease: an outcome trial. Circulation 2002;106:3642 (abst).

11. Toole JF, Malinow MR, Chambless LE, Spence JD, Pettigrew LC, Howard VJ et al. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. JAMA 2004;291(5):565-75.

12. Bonaa KH, Njolstad I, Ueland PM, Schirmer H, Tverdal A, Steigen T, Wang H, Nordrehaug JE, Arnesen E, Rasmussen K, and the NORVIT Trial Investigators. Homocysteine Lowering and Cardiovascular Events after Acute Myocardial Infarction. N Engl J Med 354(15), 1578-1588. 2006.

13. Lonn E, Yusuf S, Arnold MJ, Sheridan P, Pogue J, Micks M Sheridan P, Pogue J et al. Homocysteine lowering with folic acid and B-vitamins in vascular disease. N Engl J Med 2006;354:1567-77.

14. Jamison RL, Hartigan P, Kaufman JS, Goldfarb DS, Warren SR, Guarino PD et al. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: a randomized controlled trial. JAMA 2007;298(10):1163-70.

15. Albert CM, Cook NR, Gaziano JM, Zaharris E, MacFadyen J, Danielson E et al. Effect of folic acid and B vitamins on risk of cardiovascular events and total mortality among women at high risk for cardiovascular disease: a randomized trial. JAMA 2008;299(17):2027-36.

16. Bowman L, Armitage J, Bulbulia R, Parish S, Collins R. Study of the effectiveness of additional reductions in cholesterol and homocysteine (SEARCH): characteristics of a randomized trial among 12064 myocardial infarction survivors. Am Heart J 2007;154(5):815-23.

17. Ebbing M, Bleie O, Ueland PM, Nordrehaug JE, Nilsen DW, Vollset SE et al. Mortality and cardiovascular events in patients treated with homocysteine-lowering B vitamins after coronary angiography: a randomized controlled trial. JAMA 2008;300(7):795-804.

18. Hankey GJ, Algra A, Chen C, Wong MC, Cheung R, Wong L et al. VITATOPS, the VITAmins TO prevent stroke trial: rationale and design of a randomised trial of B-vitamin therapy in patients with recent transient ischaemic attack or stroke. Int J Stroke 2007;2(2):144-50.

18. Bostom AG, Carpenter MA, Hunsicker L, Jacques PF, Kusek JW, Levey AS et al. Baseline characteristics of participants in the Folic Acid for Vascular Outcome Reduction in Transplantation (FAVORIT) Trial. Am J Kidney Dis 2009;53(1):121-8.

20. Galan P, Briancon S, Blacher J, Czernichow S, Hercberg S. The SU.FOL.OM3 Study: a secondary prevention trial testing the impact of supplementation with folate and B-vitamins and/or Omega-3 PUFA on fatal and non fatal cardiovascular events, design, methods and participants characteristics. Trials 2008;9:35.

21. B-Vitamin Treatment Trialists' Collaboration. Homocysteine-lowering trials for prevention of cardiovascular events: a review of the design and power of the large randomized trials. Am Heart J 2006;151(2):282-7.26.

22. B-Vitamin Treatment Trialists' Collaboration. Homocysteine-lowering trials for prevention of vascular disease: protocol for collaborative meta-analysis. Clin Chem Lab Med 2007;45: 1571-76.

23. China Stroke Primary Prevention Trial. ClinicalTrials.gov.

24. Early Breast Cancer Trialists' Collaborative Group. Treatment of early breast cancer. Vol 1 Worldwide evidence 1985-1990. Oxford: Oxford University Press, 1990.

25. Cochran WG. Some methods for strengthening the common Chi-squared tests. Biometrics 1954; 10:417-51..

26. Saposnik G, Ray JG, Sheridan P, McQueen M, Lonn E and the HOPE-2 Investigators. Homocysteine-lowering therapy and stroke risk, severity, and disability: additional findings from the HOPE-2 trial. Stroke 2009; 40: 1365-1372.

27. Wang X, Qin X, Demirtas H, Li J, Mao G, Huo Y et al. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. Lancet 2007; 369(9576):1876-82. 32-5.

28. Yang Q, Botto LD, Erickson JD, Berry RJ, Sambell C, Johansen H et al. Improvement in stroke mortality in Canada and the United States, 1990 to 2002. Circulation 2006;113:1335-43.

29. Giovannucci E. Epidemiologic studies of folate and colorectal neoplasia: a review. J Nutr 2002;132(8 Suppl):2350S-5S.

30. Larsson SC, Giovannucci E, Wolk A. Folate and risk of breast cancer: a meta-analysis. J Natl Cancer Inst 2007;99(1):64-76.26.

31. Kim YI. Will mandatory folic acid fortification prevent or promote cancer? Am J Clin Nutr 2004;80(5):1123-8.

32. Cole BF, Baron JA, Sandler RS, Haile RW, Ahnen DJ, Bresalier RS et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. JAMA 2007; 297(21):2351-9.

33. Figueiredo JC, Grau MV, Haile RW, Sandler RS, Summers RW, Bresalier RS et al. Folic acid and risk of prostate cancer: results from a randomized clinical trial. J Natl Cancer Inst 2009; 101(6):432-5.

34. Logan RFA, Grainge MJ, Shepherd VC, Armitage NC, Muir KR on behalf of the ukCAP Trial Group. Aspirin and folic acid for the prevention of recurrent colorectal adenomas. Gastenterology 2008; 134:29-38.

35. Mason JB, Dickstein A, Jacques PF, Haggarty P, Selhub J, Dallal G et al. A temporal

association between folic acid fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis. Cancer Epidemiol Biomarkers Prev 2007;16(7):1325-9.

36. Radimer K, Bindewald B, Hughes, Ervin B, Swanson C, Picciano MF. Dietary supplement use by US adults: data from the National Nutrition Survey 1999-2000. Am J Epidemiol 2004; 160:339-349.

37. Henderson L, Gregory J, Swan G. The National Diet and Nutrition Survey: adults aged 19 to 64 years, types and quantities of food consumed. London, HMSO 2003

38. Honein MA, Paulozzi LJ, Erickson JD, Wong LY. Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. JAMA 2001;285:2981-2986.

39. Ray JG. Folic acid fortification in Canada. Nutr Rev 2004;62:S35-39.

Table 1: Design and eligibility criteria of participating trials

Trial	No randomised	Prior disease	Country	Mandatory fortification	Duration of treatment (years)	Daily dose of B-vitamins (mg)		
						Folic acid	B12	B6
CHAOS-2	1882	CHD	UK	-	2.0	5.0	-	-
HOST	2056	Renal	USA	+	5.0	40.0	0.5	100
WENBIT†	3090	CHD	Norway	-	3.1	0.8	0.4	40
VISP	3680	Stroke	*USA/UK	+/-	1.8	2.5	0.4	25
NORVIT	3749	CHD	Norway	-	3.0	0.8	0.4	40
WAFACS†	5442	CHD	USA	+	7.0	2.5	1.0	5.0
HOPE-2	5522	CHD	**Canada/Europe	+/-	4.6	2.5	1.0	5.0
SEARCH	12064	CHD	UK	-	6.7	2.0	1.0	-
ALL	**37485**							

* 3634 from US; 46 from UK
** 3982 from US; 1540 from Europe
† Factorial comparison of vitamin B6 versus placebo

Table 2: Vitamin status before and after treatment in the included trials, by presence of fortification

Trial	Number of participants	Median plasma Folate (nmol/L)				Median plasma Homocysteine (μmol/L)				Percent homocysteine reduction (%)
		Treated		Control		Treated		Control		
		Before	After	Before	After	Before	After	Before	After	
Fortified										
VISP	3634	22.9	62.9	23.2	22.6	12.3	9.8	12.3	11.7	17.2
HOST	2056	15.7	2019.0	15.5	16.5	22.5	16.5	22.2	21.6	24.7
HOPE-2	3982	28.8	45.4	28.9	23.2	11.0	9.0	11.0	12.0	23.6
WAFACS	5442	19.9	88.1	20.1	35.0	12.1	9.8	12.5	11.8	18.4
Sub-total	15114	22.4	69.2	22.3	22.3	13.2	11.0	13.2	13.5	20.3
Non-fortified										
VISP	46	17.6	65.9	13.2	12.6	13.5	8.7	14.0	14.4	34.7
HOPE-2	1540	14.5	45.4	13.3	14.0	13.0	9.0	12.7	13.1	28.9
CHAOS-2	1882	14.5	45.3	14.3	16.3	9.3	8.2	9.9	9.2	10.8
WENBIT	3090	9.7	61.6	10.1	8.5	10.0	7.7	10.1	10.2	26.4
NORVIT	3749	8.1	62.4	8.0	7.3	12.1	8.9	12.1	12.4	27.6
SEARCH	12064	14.0	50.1	13.8	15.1	12.6	8.8	12.5	12.5	27.0
Sub-total	22371	11.9	50.1	11.8	15.1	12.0	8.4	12.0	11.4	25.8
All trials	37485	13.9	50.1	13.6	15.1	12.3	9.3	12.3	12.2	23.6

Table 3: Distribution of serious events and event rates by trial

Trial	Number randomised	Major vascular events		Cancer		Total mortality	
		No. of events	Rate* (n/1000/yr)	No. of events	Rate* (n/1000/yr)	No. of events	Rate* (n/1000/yr)
CHAOS-2	1882	206	70	-	-	144	48
HOST	2056	471	89	137	24	884	143
WENBIT	3090	642	77	144	17	131	15
VISP	3680	613	101	187	33	216	32
NORVIT	3749	2000	321	149	15	365	30
WAFACS	5442	778	24	413	12	506	14
HOPE-2	5522	1586	71	661	28	945	34
SEARCH	12064	3030	42	1317	18	1934	25
ALL	**37485**	**9326**	**-**	**3008**	**-**	**5125**	**28**

* Adjusted for age and sex

eTable 1: Distribution of Major Vascular Events (MVE), Cancer and total mortality in individual trials, by allocated treatment

	CHAOS-2[1]			VISP[2]			NORVIT			HOPE-2			WAFACS			HOST[2]			WENBIT			SEARCH			All trials		
	Active	Control	All	Active	Control	All	Active	Control	All	Active	Control	All	Active	Control	All	Active	Control	All	Active	Control	All	Active	Control	All	Active	Control	All
Number randomised	940	942	1882	1827	1853	3680	1872	1877	3749	2758	2764	5522	2721	2721	5442	1032	1024	2056	1540	1550	3090	6033	6031	12064	18723	18762	37485
Major coronary events																											
Non-fatal MI	.	.	.	51	47	98	236	214	450	238	241	479	56	64	120	110	131	241	88	77	165	416	400	816	1195	1174	2369
CHD death	.	.	.	34	38	72	116	123	239	180	194	374	34	42	76	47	49	96	38	23	61	402	362	764	851	831	1682
Any	.	.	.	85	85	170	352	337	689	404	410	814	89	105	194	150	175	325	135	113	248	804	746	1550	2019	1971	3990
Strokes																											
Ischemic	.	.	.	37	39	76	35	30	65	79	98	177	69	62	131	1	1	2	28	37	65	190	193	383	439	460	899
Hemorrhagic	.	.	.	5	2	7	6	6	12	8	10	18	9	6	15	2	5	7	0	2	2	26	21	47	56	52	108
unclassified	.	.	.	117	114	231	20	20	40	24	39	63	1	1	2	37	44	81	0	0	0	53	51	104	252	269	521
Any	.	.	.	159	155	314	61	56	117	111	147	258	79	69	148	40	50	90	28	39	67	269	265	534	747	781	1528
Revascularisations																											
Coronary	.	.	.	20	30	50	779	833	1612	375	346	721	253	255	508	32	38	70	238	227	465	589	591	1180	2286	2320	4606
Non-coronary	.	.	.	38	31	69	21	19	40	98	93	191	49	51	100	48	54	102	5	7	12	31	21	52	290	276	566
Any	.	.	.	57	61	118	790	842	1632	457	422	879	285	293	578	77	88	165	241	231	472	615	609	1224	2522	2546	5068
Any MVE	111	95	206	311	302	613	987	1013	2000	790	796	1586	392	386	778	214	257	471	328	314	642	1537	1493	3030	4670	4656	9326
Any cancer	.	.	.	92	95	187	82	67	149	341	320	661	200	213	413	65	72	137	81	63	144	678	639	1317	1539	1469	3008
Total mortality	70	74	144	99	117	216	185	180	365	470	475	945	250	256	506	448	436	884	73	58	131	983	951	1934	2578	2547	5125

[1] Only provided information on all-cause and cardio-vascular deaths

[2] Cancers were not adjudicated

eTable 2: Distribution of cardiovascular risk factors in individual trials

Trial	Number randomised	Male %	Age years Mean(SD)	Current smoker %	Current drinker %	Diabetes mellitus %	Obesity %	Baseline statin use %	Baseline aspirin use %
CHAOS-2	1882	80	63 (10)	24		12	20		
HOST	2056	98	66 (12)	19	18	54	28	59	
WENBIT	3090	79	62 (10)	24		11	18	88	90
VISP	3680	62	66 (11)	16	57	29	31	40	60
NORVIT	3749	73	63 (12)	46	44	9	14	75	
WAFACS	5442	0	63 (9)	11	45	21	50	34	
HOPE-2	5522	71	69 (7)	11	41	40	34	60	74
SEARCH	12064	82	64 (9)	12	61	10	28	72	91
Overall total	**37485**	**66**	**65 (10)**	**18**	**43**	**20**	**30**	**59**	**53**

eTable 3: Vitamin status before and after treatment in trials with independent allocation to vitamin B6 or placebo

Trial	Number of participants	Median plasma Folate (nmol/L)				Median plasma Homocysteine (μmol/L)			
		B6 only		Placebo		B6 only		Placebo	
		Before	After	Before	After	Before	After	Before	After
WENBIT	1550	10.1	7.5	10.1	9.7	10.0	10.1	10.2	10.4
NORVIT	1877	7.9	6.5	8.0	8.2	12.0	12.2	12.2	12.5
All trials	3427	8.7	6.9	8.8	8.9	11.0	11.3	11.2	11.5

Figure 1: Effects of folic acid on MAJOR VASCULAR EVENTS

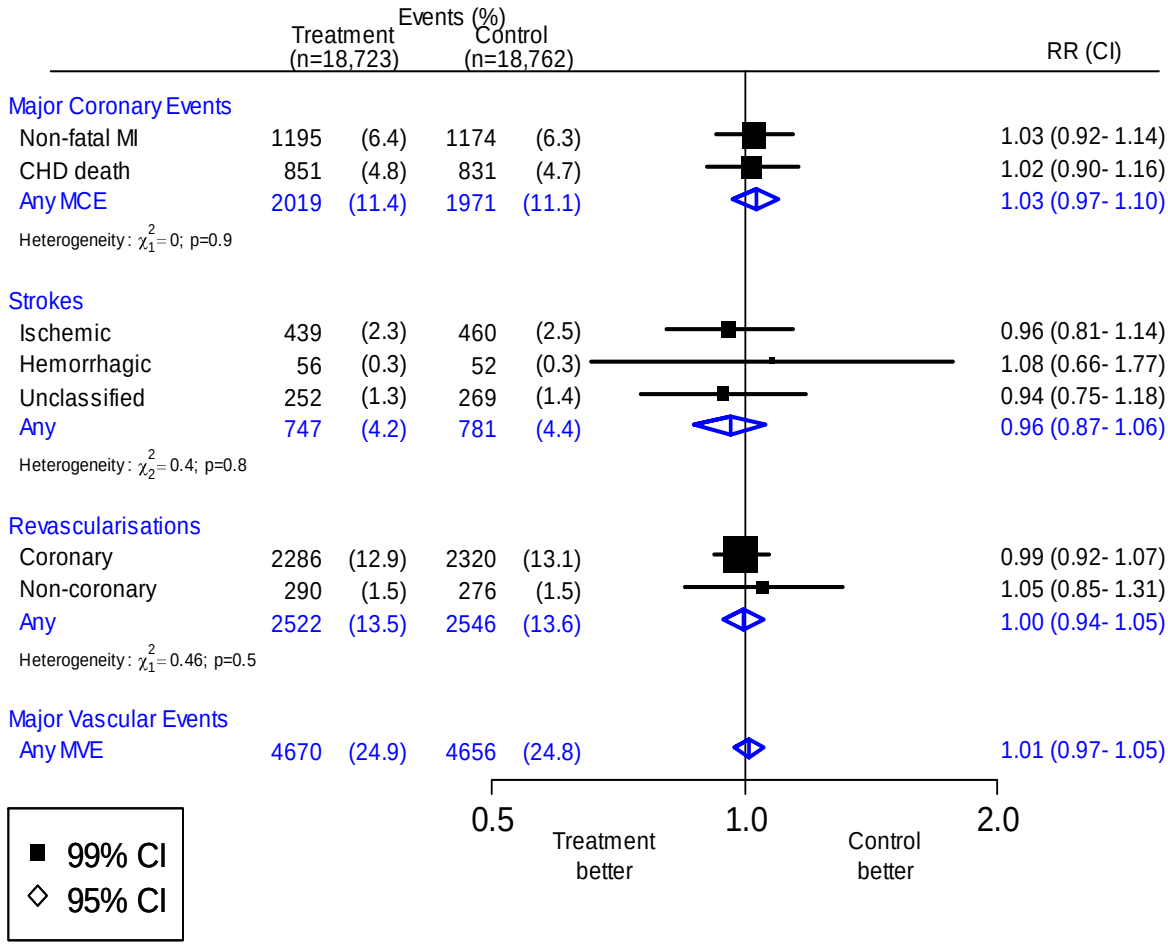


Figure 2: Effects of folic acid on MAJOR VASCULAR EVENTS, in different categories

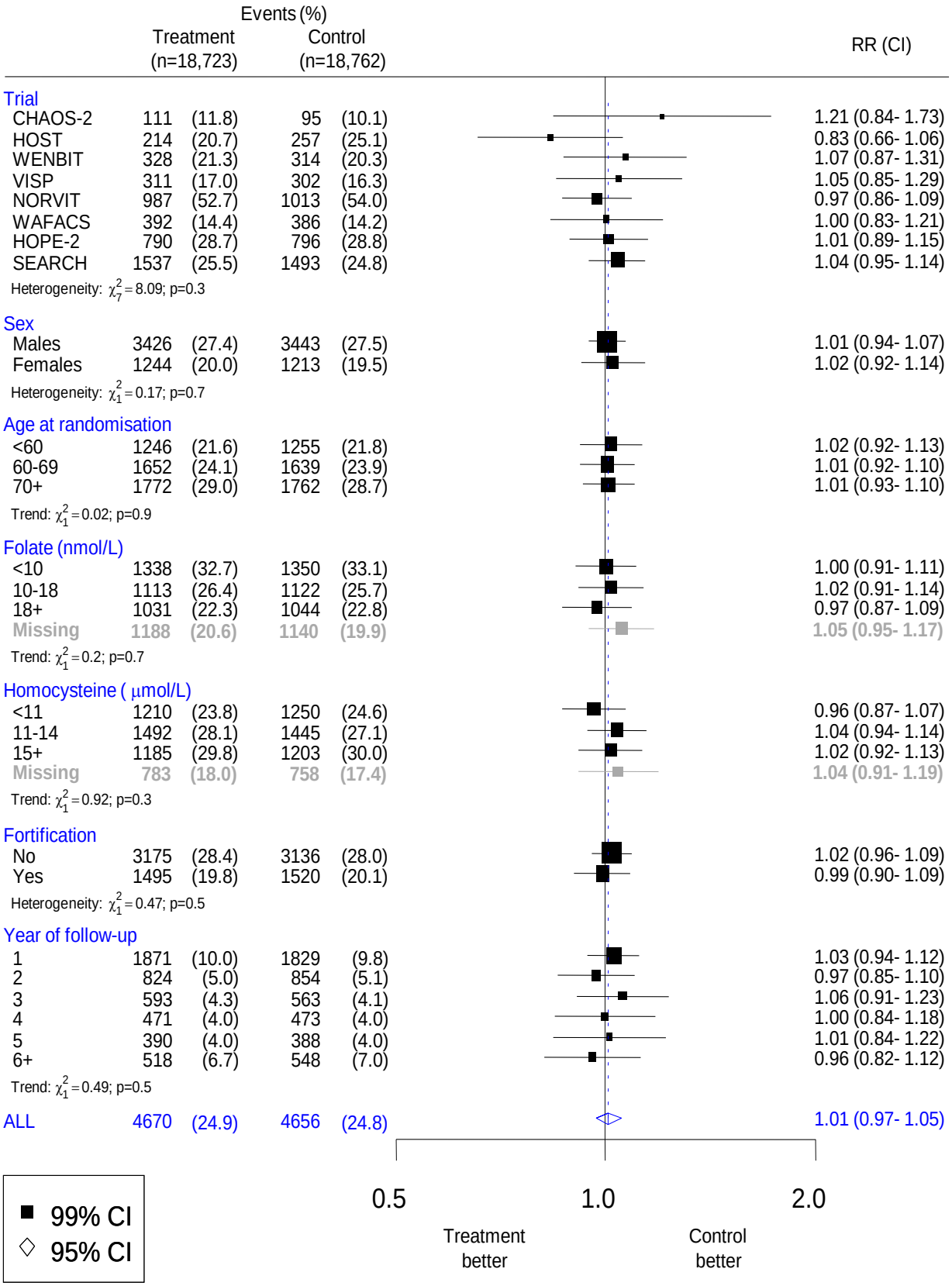


Figure 3: Effects of folic acid on MAJOR VASCULAR EVENTS, by percentage reduction in homocysteine

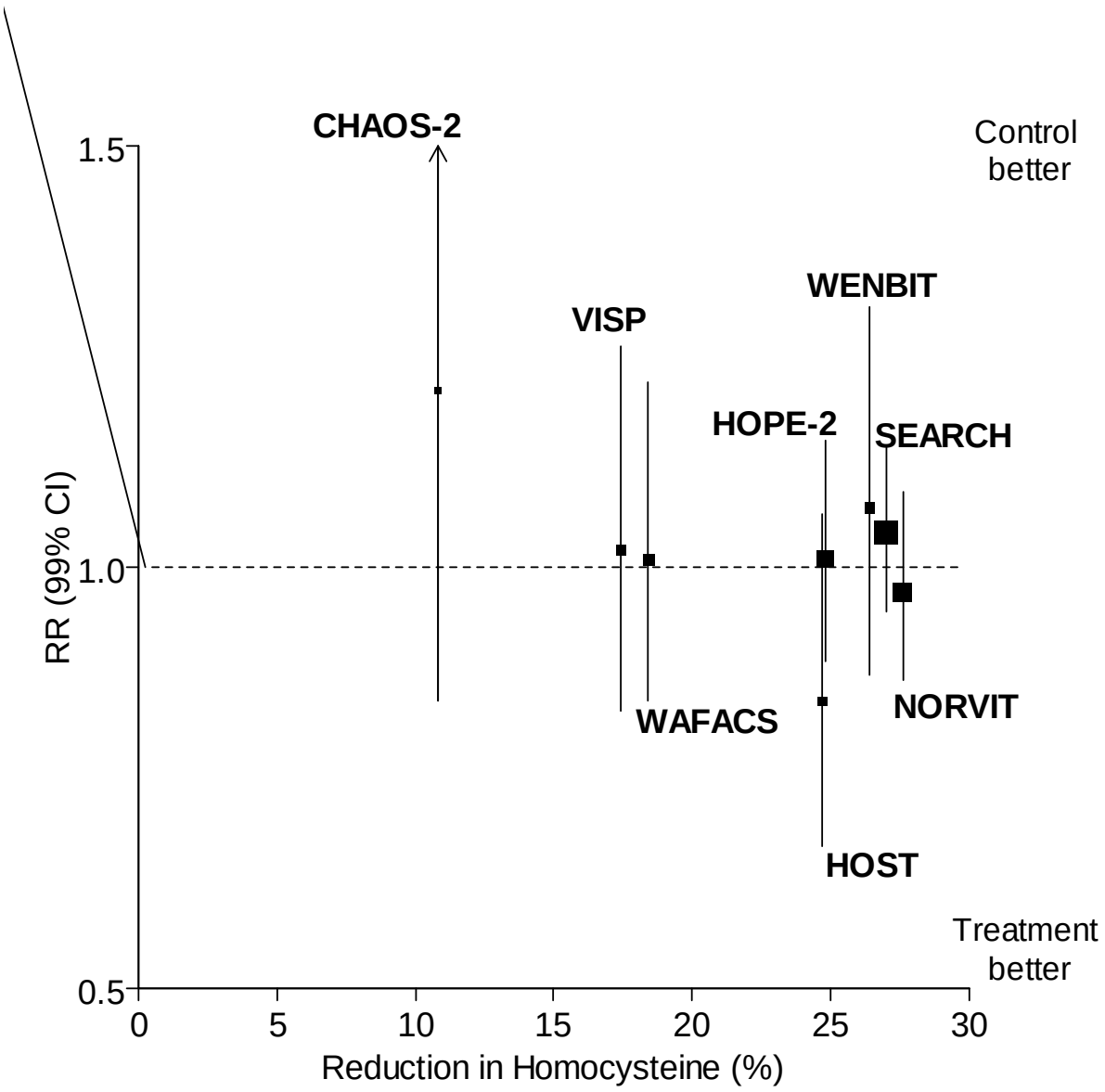


Figure 4: Effects of folic acid on CANCER EVENTS, in different categories

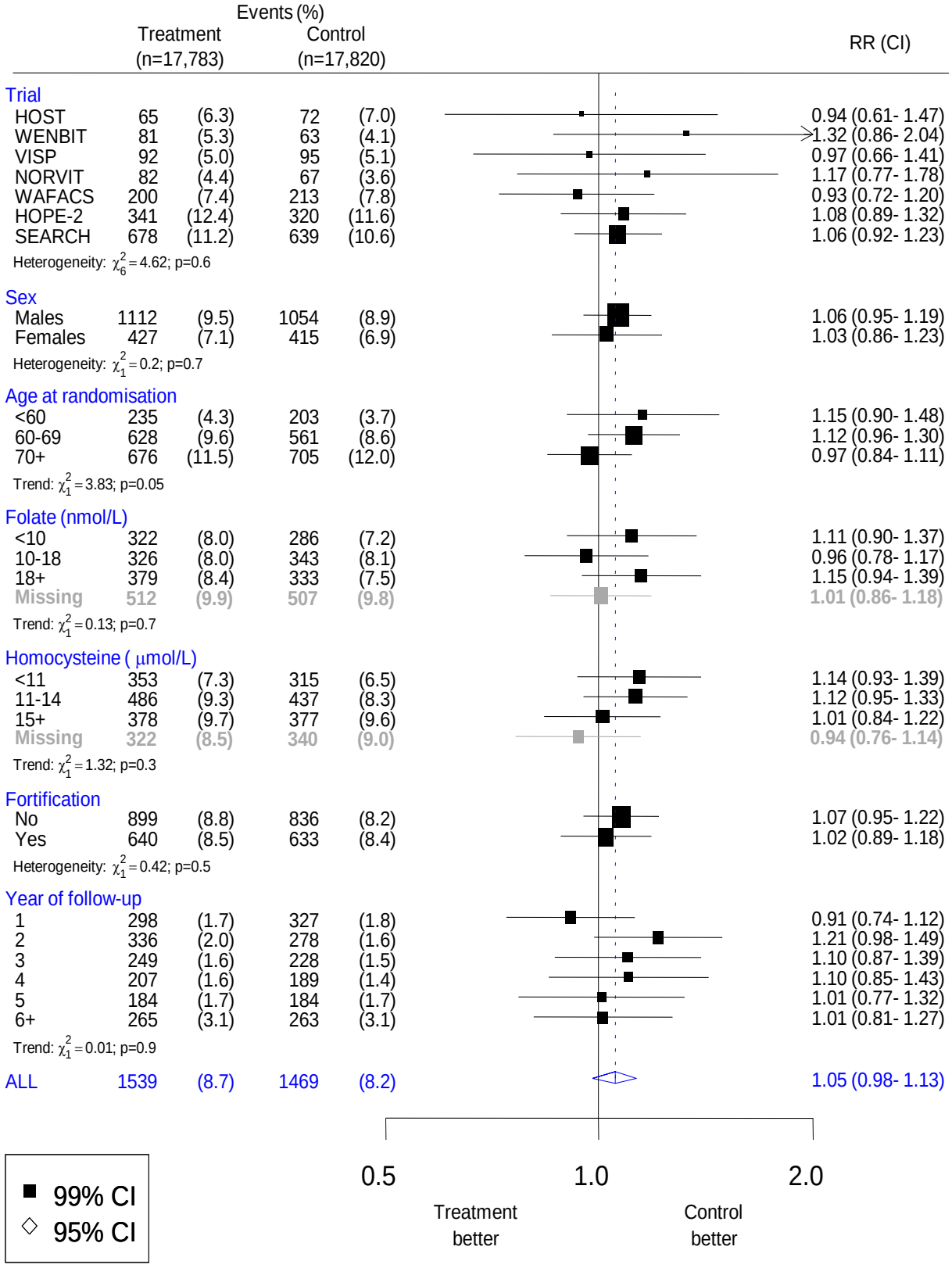


Figure 5: Effects of folic acid on CANCER EVENTS by type

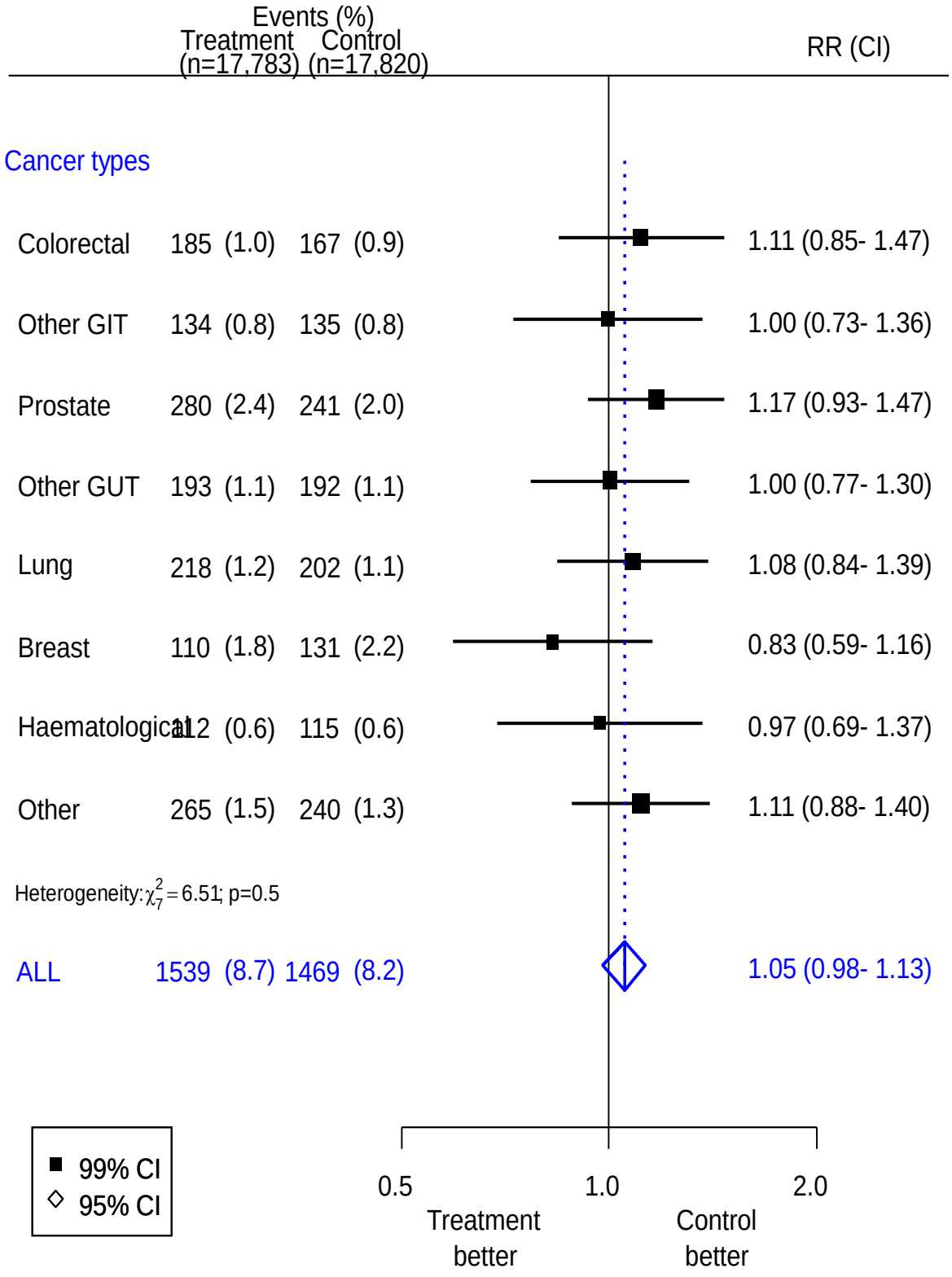


Figure 6: Effects of folic acid on cause-specific MORTALITY

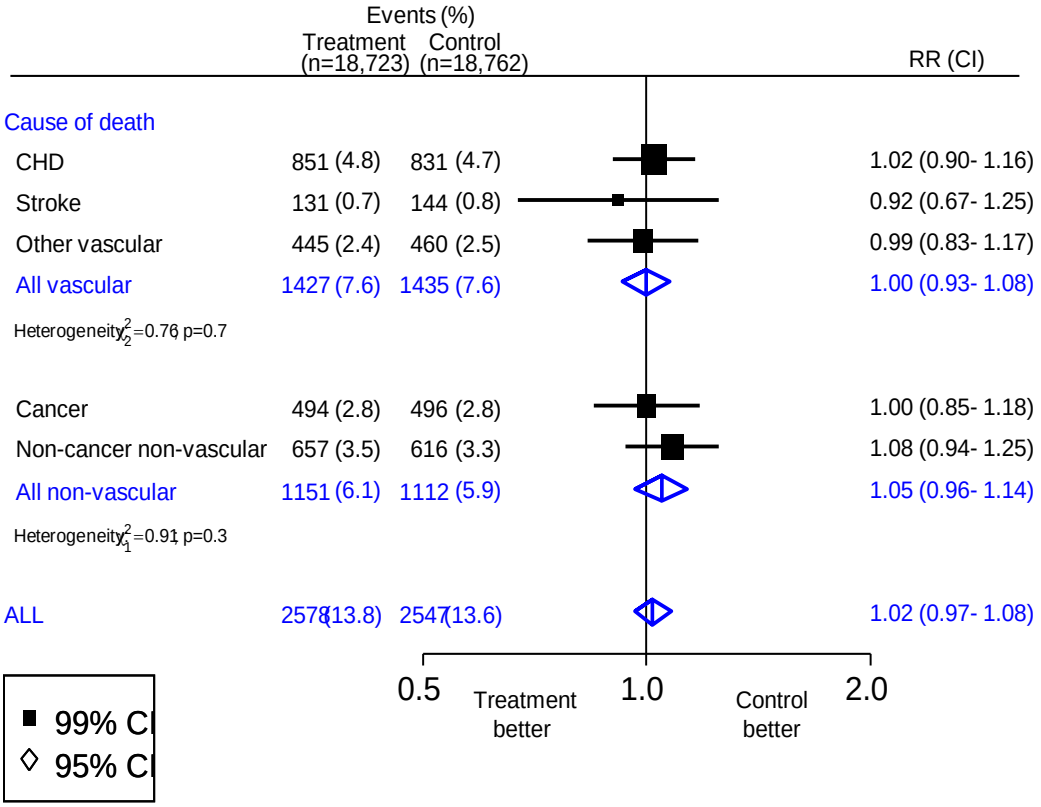
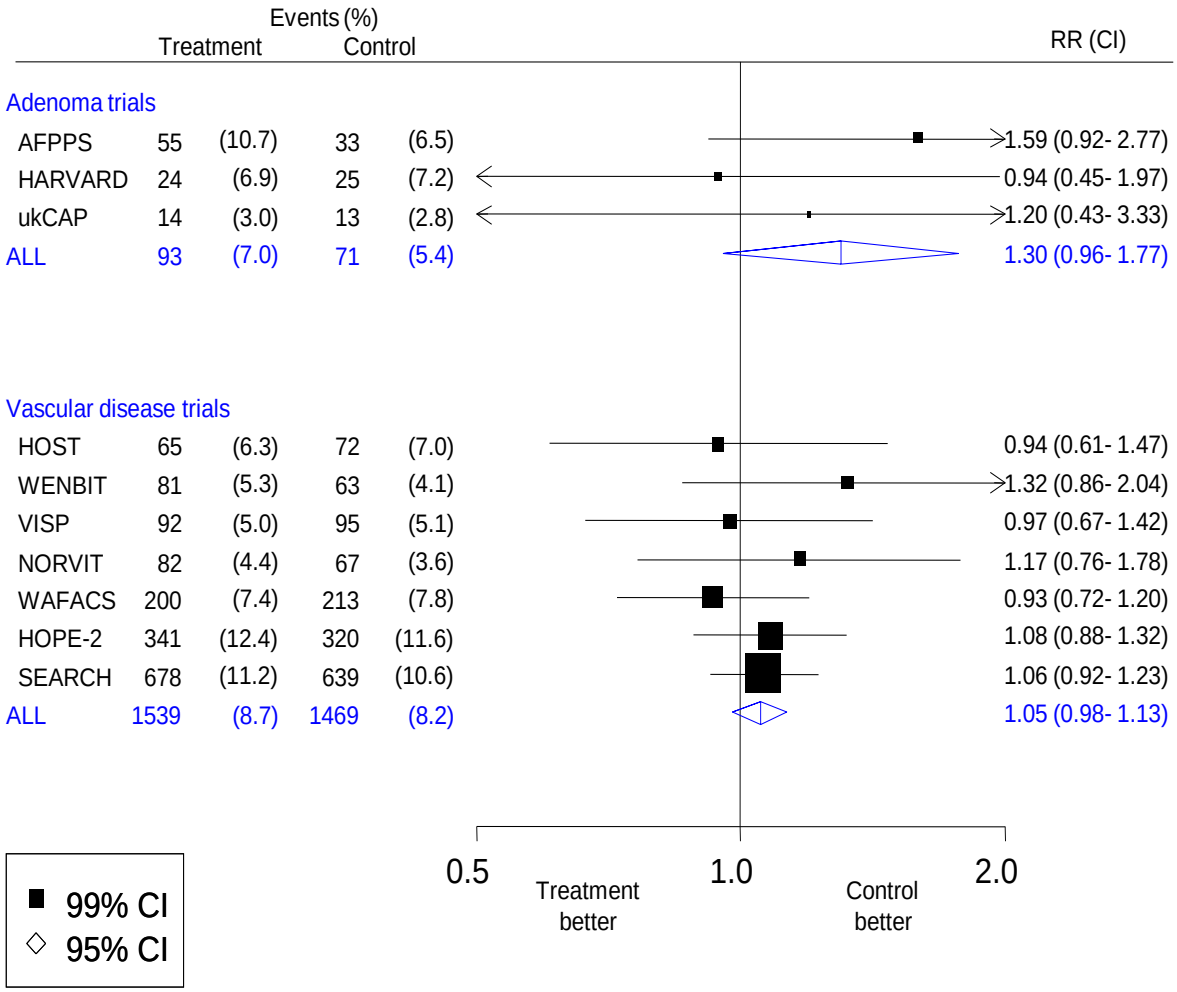
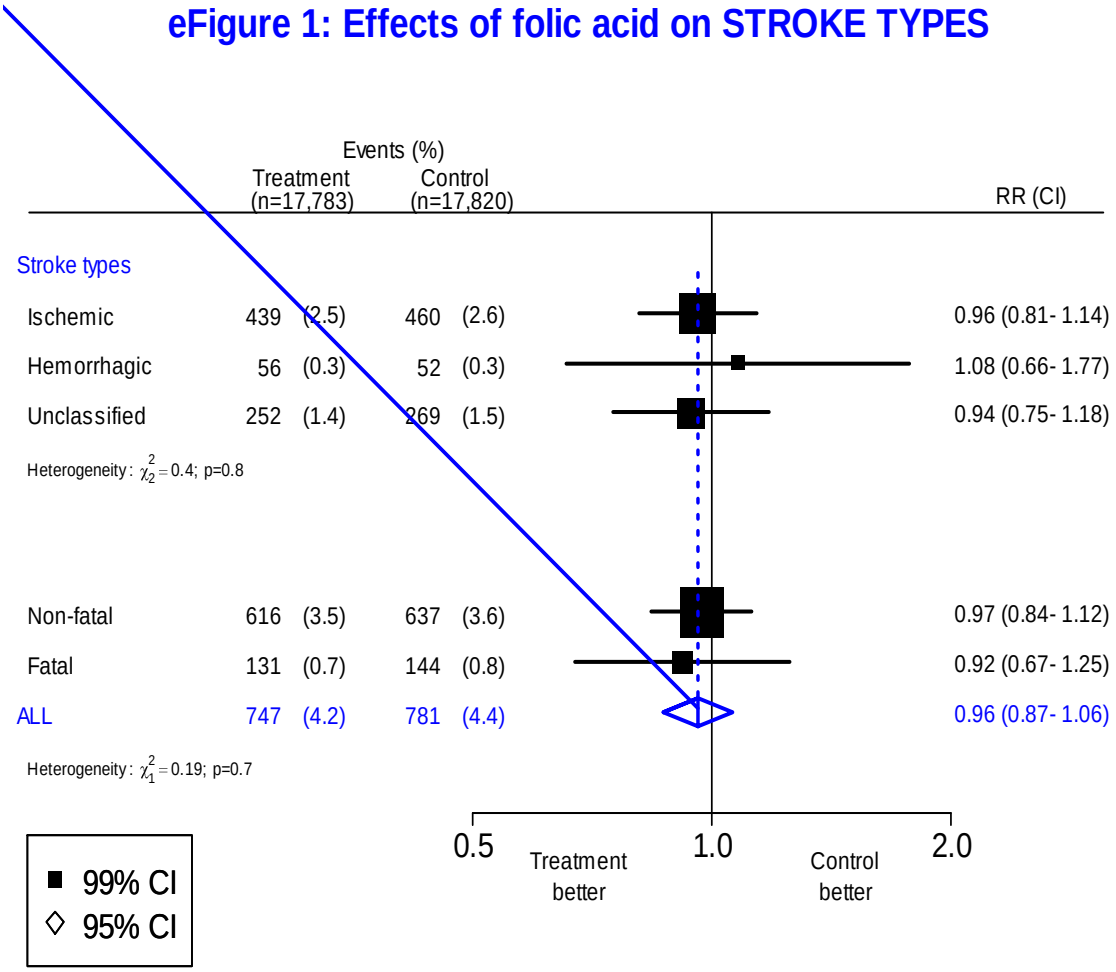


Figure 7 - Effects of folic acid on CANCER EVENTS by trial



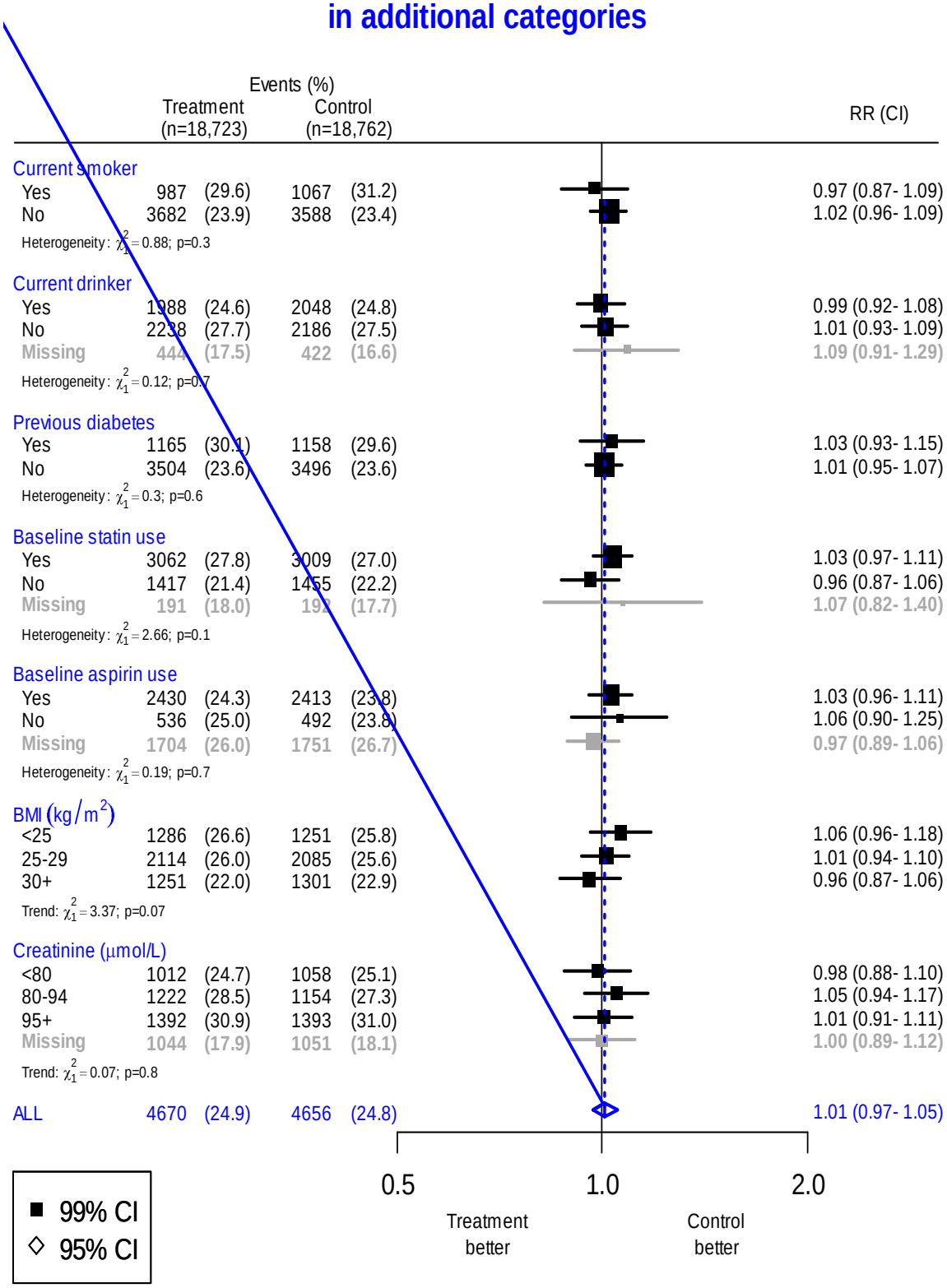
k:/vep/bvtt/analyses/august2009/figures/BVTT_CANCER_TRIAL_ALL.R 07AUG2009 11:21

eFigure 1: Effects of folic acid on STROKE TYPES

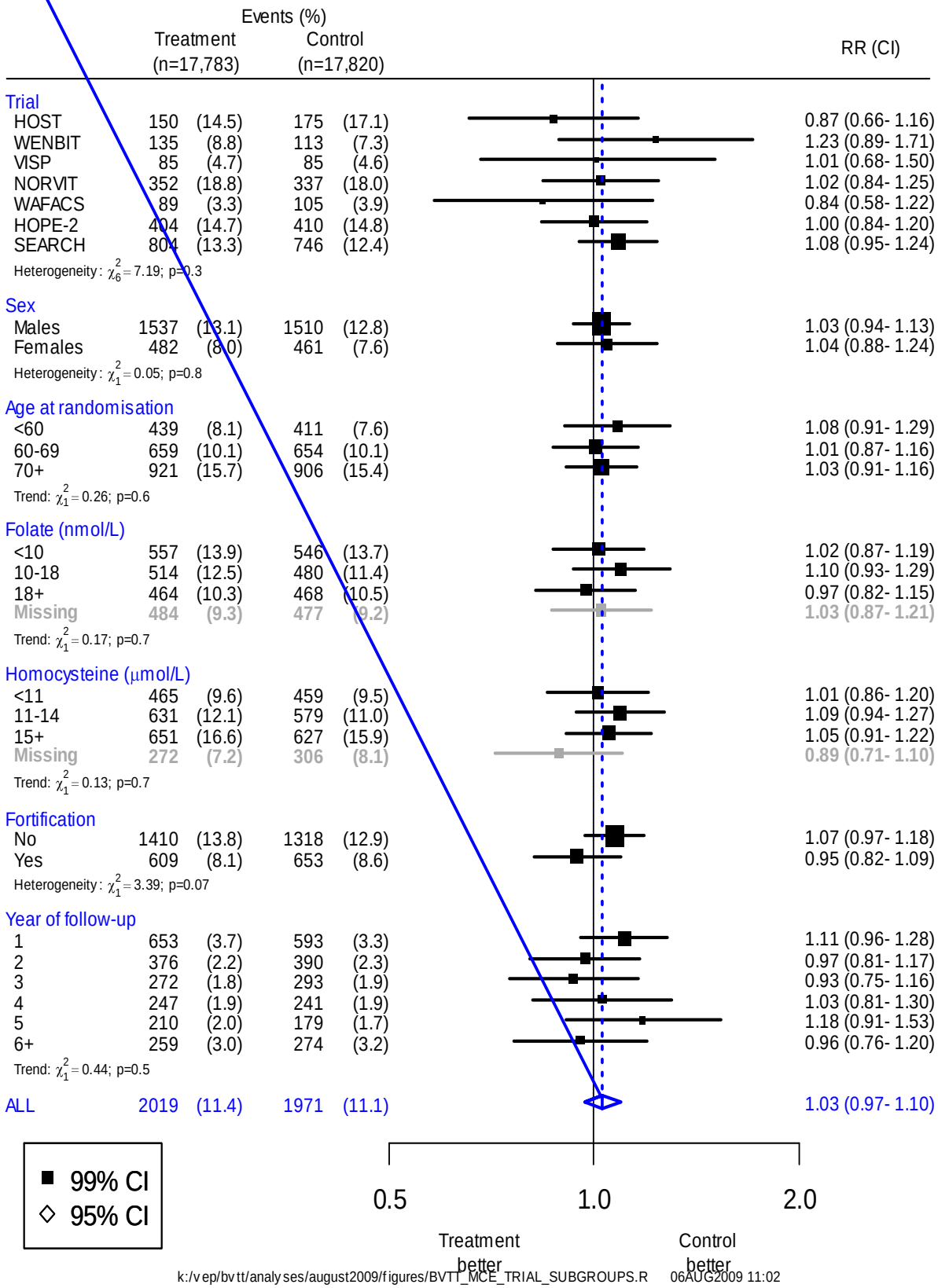


k:/vep/bvtt/analyses/august2009/figures/BVTT_STROKE_SUBTYPES.R 06AUG2009 11:02

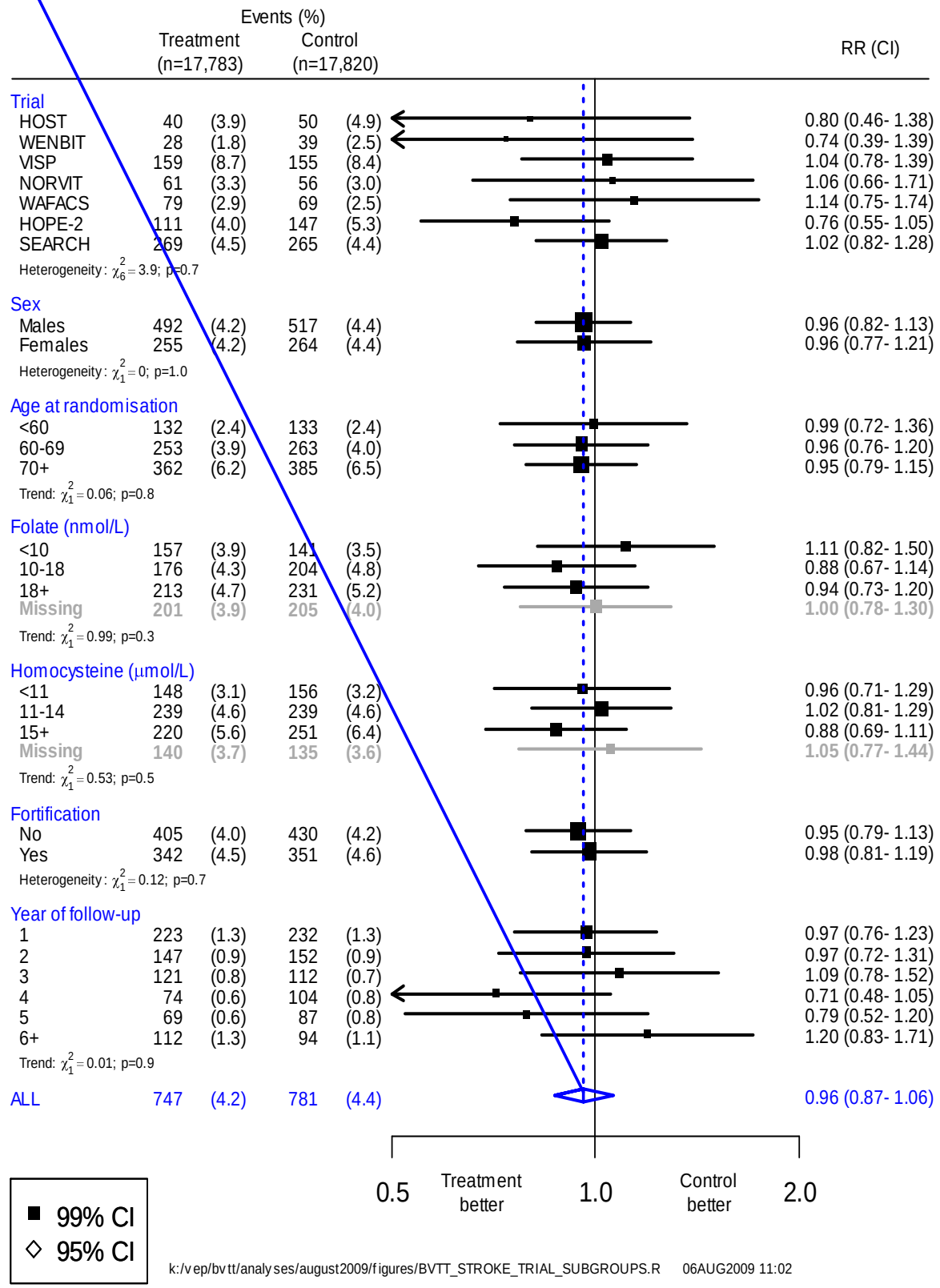
eFigure 2: Effects of folic acid on MAJOR VASCULAR EVENTS, in additional categories



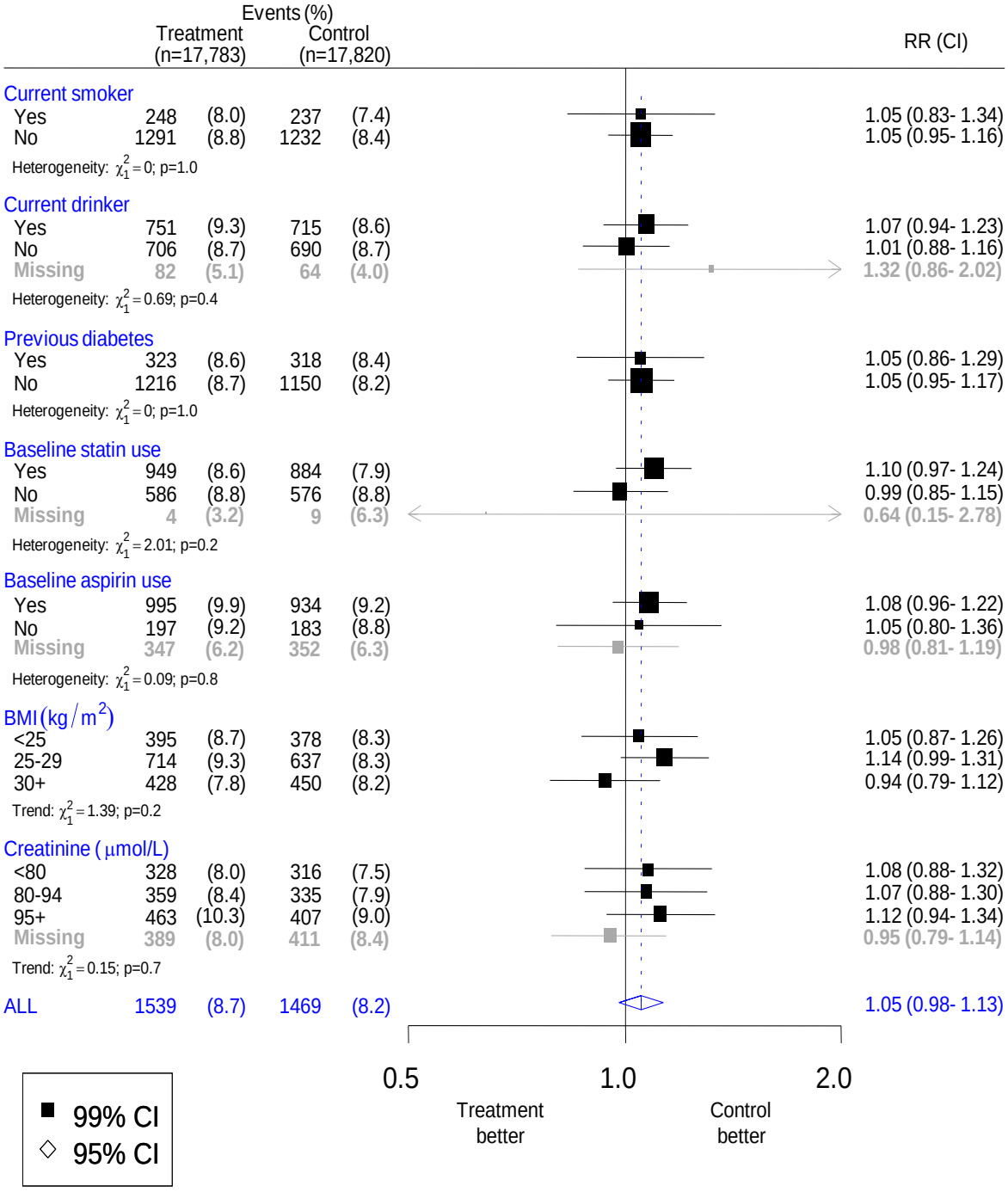
eFigure 3: Effects of folic acid on MAJOR CORONARY EVENTS, in different categories



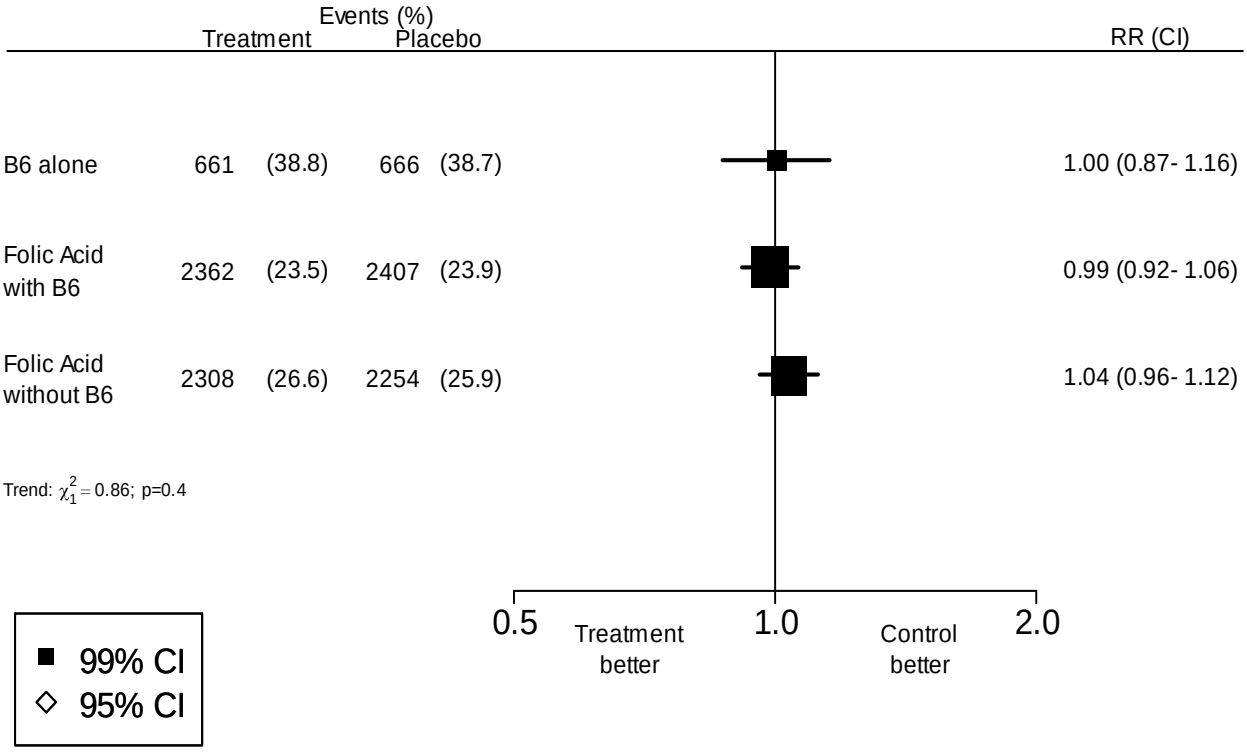
eFigure 4: Effects of folic acid on STROKE EVENTS, in different categories



eFigure 5: Effects of folic acid on CANCER incidence, in additional categories

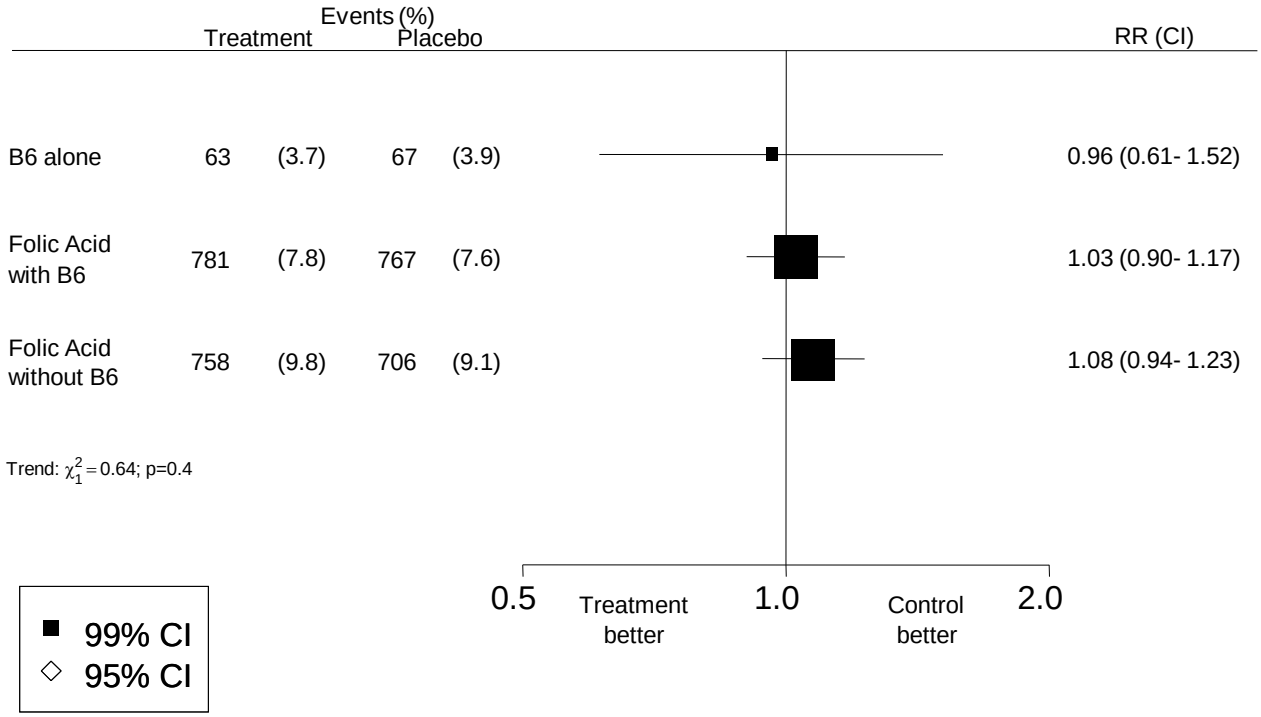


eFigure 6: Effects of vitamin B6 on MAJOR VASCULAR EVENTS



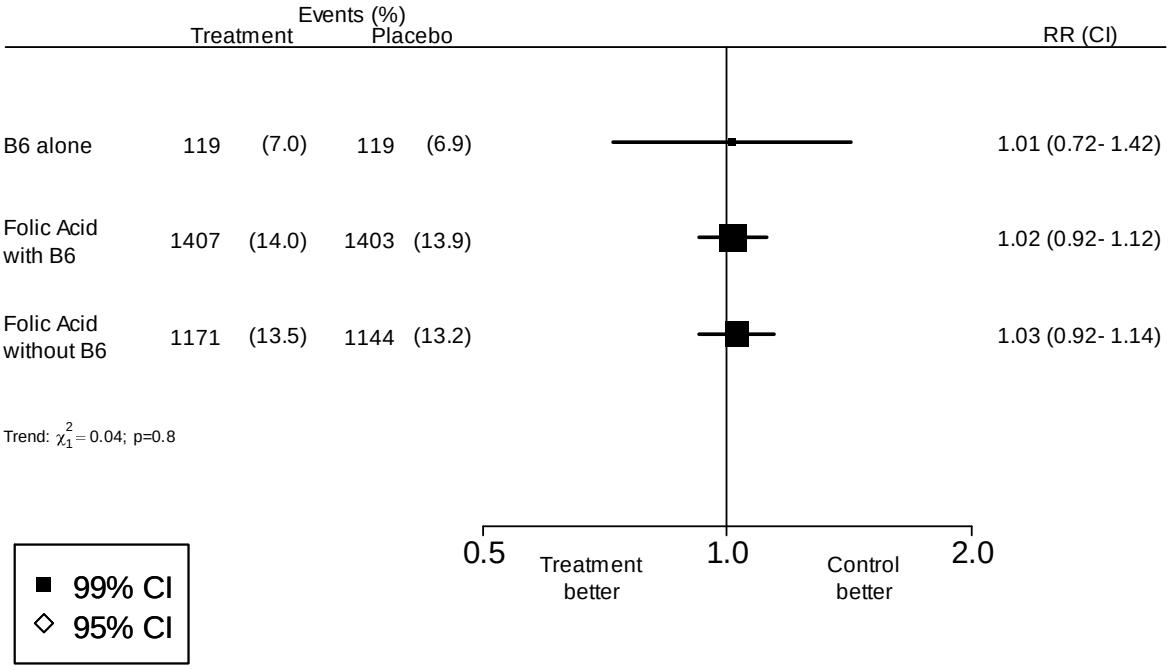
k:/vep/bvtt/analyses/august2009/figures/BVTT_MVE_B6.R 06AUG2009 11:02

eFigure 7: Effects of vitamin B6 on CANCER incidence



k:/vep/bvtt/analyses/august2009/figures/BVTT_CANCER_B6.R 06AUG2009 11:02

eFigure 8: Effects of vitamin B6 on TOTAL MORTALITY



k:/vep/bvtt/analyses/august2009/figures/BVTT_Mortality_B6.R 06AUG2009 11:02