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Scientific Advisory Committee on Nutrition

Paper for discussion

Folates and disease prevention: an update

At its horizon-scanning meeting in September 2003 SACN requested an update on the evidence that had arisen since the COMA report, Folic Acid and the Prevention of Disease (Department of Health, 2000). A Subgroup was established in February 2004 to consider the evidence and make recommendations to the Committee. The Subgroup is requested to:

- Consider the evidence that has arisen since the COMA report, Folic Acid and the Prevention of Disease (Department of Health, 2000)
- Advise on any gaps in the evidence base, with particular reference to the issue of folic acid masking vitamin B_{12}-deficiency
- Consider when and how to review the previous COMA risk assessment

This paper provides an update of the evidence regarding the health benefits and risks of dietary folate intake.

Background

1. The Committee on Medical Aspects of Food and Nutrition Policy (COMA) published a report on Folic Acid and the Prevention of Disease (Department of Health, 2000), concluding that the universal folic acid fortification of flour would have a significant effect in preventing neural tube defect (NTD) affected conceptions and births. A formal public consultation took place in 2000, and in March 2002 the Food Standards Agency with the Health Departments convened a major stakeholder meeting to discuss issues relating to folic acid fortification.

2. The Agency's Board considered the fortification of flour with folic acid at its May 2002 open meeting (Food Standards Agency, 2002a; Board paper attached at Annex I), and subsequently provided its advice to the Health Ministers, who are responsible for making a decision on folic acid fortification, in July 2002 (Food Standards Agency, 2002b; Board advice attached at Annex II).

3. The Health Ministers replied in June 2004 (letter attached at Annex III) and agreed with the Agency's advice not to recommend mandatory fortification at present, due to outstanding concerns about vitamin B_{12}-deficiency. The letter stated that:

   - The wider impact of folic acid fortification, in particular the benefits and risks to older adults, should be assessed. SACN has agreed to consider this issue.
   - The matter will be reassessed following SACN's consideration and as the evidence becomes available from overseas
   - At the same time, options will be considered to increase the usage of preconceptual supplements, and increase dietary intakes of folate, for example through the Healthy Start and 5 A Day Programmes and to address the concerns relating to prevalence and identification of vitamin B_{12}-deficiency

National Diet and Nutrition Survey of Adults (19-64 years): folate intake and status

4. The National Diet and Nutrition Survey (NDNS) of Adults 19-64 (Henderson *et al.,* 2003; Rushton *et al.,* 2004) provides data on the current folate intakes and status of adults in the UK. Mean daily intakes of folate from food sources were above the RNI (200µg/d) for men and women in each age group. In addition, less than 0.5% of men and 2% of women had low intakes (below the LRNI; 100µg/d). Only 8-16% of women aged 19 to 49 years, however, reached intakes from food and supplements of 400µg/d or above, the level recommended by the Department of Health to reduce the risk of a NTD-affected pregnancy.

5. The mean daily intake of folate, from food sources, ranged from 229-255µg for women aged 19-49 years. Dietary supplements providing folate increased mean intakes to 248-280µg for women aged 19-49 years. Only 14% of women aged 19-24 years, 8% of the 25-34 year group and 16% of the 35-49 group had a folate intake of 400µg/day or more including intake from supplements.

6. Five percent of both men and women had a red cell folate concentration indicative of marginal status with increased risk of deficiency. No more than 1% of any age/sex group had a red cell folate concentration indicating severe folate-deficiency; however, 8% of the youngest women (19 to 24 years) and 4% of those aged 25 to 34 years had marginal folate status.

The use of folic acid supplements in pregnancy

7. The Health Survey of England (Blake *et al*., 2003) provides the latest information on the use of folate supplements, prior to and during pregnancy, by women of childbearing age. The Survey collected information, on folate supplement intake prior to pregnancy, from mothers who had planned their pregnancy (two-thirds of the interviewed sample).

8. Of those mothers who reported planning their pregnancy, over half (55%) reported taking supplements or modifying their diet to increase folate intake. The proportion of mothers taking action to address folate intake increased with age from 32% (16-24 years) to 60% (aged >35 years). Observations highlighted an inequality towards taking action as mothers in most deprived areas seemed less likely (43%) to increase folate intake when compared to mothers from least deprived areas where 70% took action.

9. All mothers were asked about their supplement intake during pregnancy. The Survey found that 79% of mothers increased their folate intake during pregnancy. As with action in planned pregnancies, the proportion of mothers taking action, whilst pregnant, to increase folate intake increased with maternal age.

10. The Infant Feeding Survey (Hamlyn *et al*., 2002), carried out across the UK, is based on retrospective postpartum interviews and provides similar findings to the Health Survey. Of those mothers interviewed 73% (up from 50% in 1995) indicated that they had taken supplements or modified their diets in early pregnancy. However, it is not possible to pinpoint when mothers took action, whether pre/post-conception or when they became aware they were pregnant. There is very little difference, between countries, in the uptake of folic acid supplements. However, compared with other countries, mothers in Scotland were more likely to modify their diets (34% compared to 31% in England & Wales and 28% in Northern Ireland).

11. The review of the Welfare Food Scheme (Department of Health, 2002) considered the merits of free provision of peri-conceptual folic acid supplements to beneficiary population groups. It commented that this might increase uptake, though acknowledged that uptake of supplements by those groups eligible under the scheme is likely to be poor. The Panel suggested that the composition of vitamin supplements, available through the scheme, be reviewed and expressed changes to the current preparation to include folic acid.

Folic acid fortification strategies to reduce NTD incidence in other countries

12. Details of countries that have introduced folic acid fortification strategies are outlined in Table 1. In several countries where mandatory folic acid fortification has been introduced (Canada, Chile and the USA) evidence is available of the incidence of NTD-affected births pre- and post-fortification (see Table 2 for a comparison of current NTD rates).

13. In the USA, a reduction in the incidence of NTDs of 19% was observed in one study (Honein *et al.,* 2001) and in a subsequent study a reduction of 23% was observed (Mathews *et al.,* 2002). Recent information from the USA (Mersereau *et al.,* 2004) reports a 27% decline in NTD-affected pregnancies since mandatory fortification. Using information reported on spina bifida and anencephaly the crude estimated pre-fortification NTD rate was 5.52 in 10,000 compared to a post fortification NTD rate of 3.82 in 10,000 (this data was based on total number of pregnancies, including live/still births, prenatally diagnosed cases and elective terminations). In Canada, a study in Quebec observed a 32% reduction in the incidence of NTD-affected births post-fortiication (de Wals *et al.,* 2003). Three other studies

in Canada observed reductions in NTDs of 51% (Ray *et al.,* 2002) 54% (Persad *et al.,* 2002) 78% (Liu et al., 2004) in Ontario, Nova Scotia and Newfoundland respectively. Overall, for Canada, a reduction of 23% has been observed (Health Canada, 2003). A study of the incidence of NTDs in South America reported a 31% reduction in NTD-affected births in Chile only, corresponding to the introduction of folic acid fortification (Catilla *et al.,* 2003).

Table 1. Folic acid fortification strategies

Country	Fortification position	Information on fortification level
Belgium	Voluntary	For the product to be labelled as fortified with folic acid the daily portion has to contain 15 to 200 % of 200µg
UK	Voluntary	Breakfast cereals and products 8-643µg/100g; flora spread products 1mg/100g.
Germany	Voluntary	Breakfast cereals and products – levels unavailable
France	Voluntary	Breakfast cereals and products aimed at children or women – levels unavailable Goats milk at 4.5µg/100g
Ireland	Voluntary	
	Mandatory – Due to consult on approach.	Mandatory level likely to be considered – 160-200µg/100g (most likely vehicle flour)
Greece	Voluntary	Unavailable
Hungary	Voluntary	Previously fortified @ 60µg/100g bread
Czech Republic	Mandatory	Information on foods unavailable – level 200µg/100g
Iceland	Voluntary	Breakfast cereals and products 30-700µg/100g, flour and rice 30-100µg/100g
Canada	Mandatory (1998)	Mandatory - 150µg/100g white flour, 200µg/100g enriched pasta Voluntary - 150 – 220 µg/100g cornflour
New Zealand	Voluntary (1996)	Voluntary – includes breakfast cereal, some bread & marmite – levels unavailable
	Mandatory – Due to consult on approach.	
Australia	Voluntary (1996)	
	Mandatory – starting to investigate issues	
USA	Mandatory (1998)	140µg/100g grain in food as consumed
Chile	Mandatory (2000)	220µg/100g flour
Israel	Voluntary (Legislation pending)	Wheat flour (rate unknown) B_{12} also added
Brazil	Legislation pending	150µg/100g wheat & maize flour
Bolivia, Colombia, Paraquay Ecuador	Mandatory fortification	Wheat flour - 60-300µg/100g

Table 2. Comparison of NTD rates in countries

Country	NTD rate (per 10,000) live births (unless otherwise stated)
Chile (Castillo *et al.,* 2003)	
Pre fortification	13.45
Post fortification	8.8
Canada (Health Canada, 2003)	
Pre fortification [total births]	7.6
Post fortification [total births]	5.8
USA (Honein *et al.,* 2001; *Matthews et al., 2002)*	
Pre fortification	3.78
Post fortification	3.05*[2.95]*
(Mersereau *et al.,* 2004)	
Pre fortification [total births]	5.52
Post fortification [total births]	3.82
England & Wales (Office for National Statistics, 2002)	1.2
Scotland (Information and Statistics Division: Scotland, 2002)	3.9
Northern Ireland (The Northern Ireland Child Health System, 2001)	8.3

Total births include live/still births, prenatally diagnosed cases and elective terminations.

Overage of folic acid in fortified foods

14. Measurements of the total folate content in folic acid enriched cereal-grain products in the USA following fortification have shown that a considerable number of these products contain total folate levels that are higher than the amount required by regulations (Rader *et al.,* 2000). As a consequence, typical folic acid intakes in the USA from fortified foods are more then twice the level originally intended, which was an average increase of folic acid intake of approximately 100µg/d (Choumenkovitch *et al.,* 2002; Quinlivan & Gregory, 2003).

Dietary reference values for folate

15. In the UK, the reference nutrient intake (RNI) value for folate is 200µg/d for adults. In the USA, however, the recommended daily allowance (RDA; equivalent to the RNI) for folate is 400µg/d dietary folate equivalents (DFE) for adults (1µg of DFE = 1µg food folate = 0.5µg of folic acid taken on an empty stomach = 0.6µg of folic acid with meals). The primary indicator used to estimate the US RDA was erythrocyte folate in conjunction with plasma folate and tHcy concentrations. In the UK, erythrocyte folate and autopsied liver folate concentrations were the primary indicator. In the US the cut-offs for adequate folate status were 305nmol/l (140ng/ml) erythrocyte folate and 7nmol/l serum folate. In the UK the cut-offs for adequate folate status were 150ng/ml erythrocyte folate and liver levels above 3µg/g.

16. In the US, a metabolic maintenance study (O'Keefe *et al.,* 1995) was given greatest weight in the recommendation for the estimated average requirement of 320µg/d of DFE. O'Keefe *et al.,* (1995) conducted a controlled metabolic study in which five women were fed a diet for

70 days that provided 319 µg/d of DFE (30µg food folates and 170µg folic acid). Three of the five had erythrocyte folate concentrations less than 305nmol/l (140ng/ml) erythrocyte folate; 7nmol/l serum folate and two of the five had tHcy greater then 16µmol/l. This was interpreted as meaning that approximately half would have adequate folate status if 320µg/d of DFE had been consumed. The RDA was set using a coefficient of variation of 10 percent giving a value of 400µg/d of DFE.

17. In the UK, the RNI for adults was based on folate intake and status assessments from Canada (Department of health, 1991) that showed that with a mean dietary intake of food folate of 150-200µg/d the folate content of the liver was consistently greater than 3µg/g. In this population no more than 8-10% had erythrocyte concentrations below 150ng/ml. An RNI of 200µg/d was, therefore, set for adults.

18. The NDNS of adults (Henderson *et al.,* 2003; Rushton *et al.,* 2004) reported that erythrocyte folate concentrations of less than 350nmol/l were found in 5% of the samples from both men and women, while 1% of men and less than 0.5% of women had a serum folate concentration below 7nmol/l.

Vitamin B_{12}-deficiency in the elderly and masking by folic acid

19. Vitamin B_{12} (cobalamin) deficiency caused by either intrinsic factor deficiency or hypochlorhydria mainly affects the elderly. Vitamin B_{12}-deficiency may present as macrocytic anaemia, but it can also cause neuropathy (Lindenbaum *et al.,* 1988). The neurological symptoms may occur in the absence of anaemia in 20-30% of cases (Lindenbaum *et al.,* 1988). The diagnosis of vitamin B_{12}-deficiency is complicated by the limitations of using low serum vitamin B_{12} concentrations: low levels do not always indicate vitamin B_{12}-deficiency and patients with vitamin B_{12}-deficiency do not always have low levels (Lindenbaum *et al.,* 1990; Joosten *et al.,* 1993). Serum or plasma methylmalonic acid (MMA) has been suggested to be a more specific indicator of functional vitamin B_{12} status (Bates *et al.,* 2003), although impaired renal function may affect this measure. Persons with biologically significant vitamin B_{12}-deficiency almost always have elevated plasma concentrations of MMA or homocysteine (Lindenbaum *et al.,* 1990); consequently, the measurement of these metabolites among persons with low or borderline concentrations of vitamin B_{12} may be used to identify those at high risk of vitamin deficiency.

20. Folic acid supplementation can prevent anaemia among persons with vitamin B_{12}-deficiency, but it does not prevent damage to the nerves, spinal cord or brain. Treatment of vitamin B_{12}-deficient patients with folic acid, therefore, may delay diagnosis of the deficiency and result in the worsening of neurological damage (the 'masking' of vitamin B_{12}-deficiency). The masking of vitamin B_{12}-deficiency by folic acid has only been observed at doses of folic acid of 1mg/d or more (Expert Group on Vitamins and Minerals, 2003).

21. The COMA report on folic acid (Department of Health, 2000) adopted a serum vitamin B_{12} concentration of <118pmol/L or a mean corpuscular volume (MCV) > 101fl to define poor vitamin B_{12} status; applying, this to the NDNS of adults aged over 65 years (Finch *et al.,* 1998) gives 5-10% and 2-4% with a low status, respectively. A subsequent analysis of the same cohort (Bates *et al.,* 2003) used serum vitamin B_{12} concentrations <150pmol/l or plasma MMA concentrations >0.5µmol/l to define low status, giving 20% and 24% of the population, respectively. Clarke *et al.,* (2003) reported the proportion of subjects in the Oxford Healthy Ageing Project (OHAP) (aged 65years or over) as between 10% and 20% who were at high risk of vitamin B_{12} deficiency; this was defined as serum vitamin B_{12} concentrations of <150pmol/L and plasma MMA concentrations >0.35µmol/l. A subsequent

analysis (Clarke *et al.,* 2004) combined the OHAP and NDNS studies with the MRC nutrition study (aged 75years or over) and found that the prevalence of vitamin B_{12}-deficiency, whether defined as serum vitamin B_{12} concentrations <150pmol/l or serum vitamin B_{12} concentrations <200pmol/l and homocysteine concentrations >20µmol/l, increased with age in all studies from 5% among people aged 65-74 to 10% or greater among people aged 75 years or more. The authors recommend increased detection and treatment for vitamin deficiencies among the elderly.

22. The serum vitamin B_{12} cut-off levels adopted in the analyses of the NDNS of adults aged over 65 years subsequent to the COMA report on folic acid (Department of Health, 2000) have been criticized as not being low enough to cause clinical disease (Wald *et al.,* 2004). As described above, however, the use of serum vitamin B_{12} concentrations to diagnose vitamin B_{12}-deficiency is limited (Clarke & Grimley Evans, 2004).

23. Mills *et al.,* (2003) compared rates of megaloblastic anemia pre- and post-fortification in the USA, in patients will low serum vitamin B_{12} concentrations. Patients at the Veterans Medical Center in Washington, DC, between 1992 and 2000 (fortification was introduced 1996-1998) that had low serum vitamin B_{12} concentrations (<258pmol/L) were assessed for anaemia. The proportion without anaemia did not increase significantly from the prefortification period (39.2%) to the period of optional fortification (45.5%) and the post mandatory fortification period (37.6%). These findings did not change when the analysis was limited to patients aged over 60 years or when low vitamin B12 was assessed as <150pmol/l.

24. Another post-fortification study in the USA (Johnson *et al.,* 2003) observed a prevalence for B_{12}-deficiency (defined as serum vitamin B_{12} concentrations <258pmol/L and MMA >0.27µmol/l) of 23% in an at risk elderly group (n=103); these subjects were three times more likely to have impaired cognition than those who were not deficient. The study reported only one non-anaemic vitamin B_{12}-deficient subject with elevated MCV; however, it was also noted that coexisting iron deficiency was quite common in the vitamin B_{12}-deficient subjects with several of them exhibiting microcytic anaemia or low-normal MCV. Vitamin B_{12}-deficiency was associated with poor cognition, anaemia and hyperhomocysteinemia. The authors point out that anaemia in the elderly with many chronic illnesses is likely to be multifactorial in origin, and correcting one aspect, such as vitamin B_{12}-deficiency, may not result in a rise in haemoglobin.

25. Two studies have investigated folate and vitamin B12 status in the elderly pre- and post-fortification. In Canada (Ray *et al.,* 2003), a study of women aged 65 years or more in Ontario and British Columbia observed an increase of 64% in mean serum folate concentrations with a concomitant decrease in folate-deficiency (serum folate < 6.0 nmol/l) from 6.3% to 0.88% after fortification. Average vitamin B12 concentrations increased from 280 to 300pmol/l, while the prevalence of combined vitamin B12 insufficiency (serum vitamin B12 < 150pmol/l) and supraphysiological concentrations of serum folate (>45 nmol/l) increased from 0.09% pre-fortification to 0.61% post. The authors conclude that the addition of vitamin B12 to folate fortified foods should be considered.

26. In Chile (Hirsch *et al.,* 2002), a study of elderly people (aged 70 years or more and on a low income) with vitamin B12-deficiency (serum concentrations <165pmol/l) in 27.6% and hyperhomocysteinemia (>14µmol/l) in 31% observed an increase in average serum folate concentrations from 16.2 ± 6.2 to 32.7 ± 7.1 nmol/l after fortification. The folic acid fortification of flour was estimated to add, on average, about 400µg/d to their diet. A moderate reduction in homocysteine concentrations was also observed (12.95 ± 3.7 to 11.43

± 3.6 μmol/l), while serum vitamin B12 concentrations were unchanged. The authors speculated that the masking of vitamin B12-deficiency might occur in their study group and that vitamin B12 should be added to folate fortified foods.

27. It has been suggested that the predominant naturally occurring form of folate, [6S]-5-methyltetrahydrofolate [6S]-5-MTHF, would be unlikely to mask vitamin B12-deficiency (Lamers *et al.,* 2004). The conversion of [6S]-5-MTHF to tetrahydrofolate, which is the precursor of the folate forms involved in DNA synthesis, is vitamin B12-dependent; whereas, folic acid can be converted to tetrahydrofolate independently of vitamin B_{12}. Folic acid can, therefore, maintain DNA synthesis and ameliorate megaloblastic anaemia (Scott & Wier, 1981).

28. Lamers *et al.* (2004) supplemented healthy women for 24 weeks with either 208μg/d, 416μg/d, [6S]-5-MTHF or 400 μg/d folic acid. All doses were equivalent in their ability to lower plasma total homocysteine concentrations; whereas, plasma folate concentrations were significantly lower in group receiving 208μg/d [6S]-5-MTHF as compared to those receiving either 416μg/d [6S]-5-MTHF or 400 μg/d folic acid. It remains an assumption, however, that vitamin B_{12}-deficiency would not be masked in the elderly. Estimating the rate of conversion, and hence "safe" dose, would presumably depend upon a better understanding of the enzyme kinetics and a dose-response relationship. An understanding of the effect in population groups other than young healthy women is also important.

Possible adverse effects of folic acid supplementation

29. Several studies have reported that the consumption of multivitamins or folic acid by women during pregnancy is associated with an increase of almost 40% in multiple births (Czeizel *et al.,* 1994; Werler *et al.,* 1997; Ericson *et al.,* 2001). Multiple pregnancies result in more complications and poorer outcomes than do singleton pregnancies (Kinzler *et al.,* 2000). A randomized controlled trial supplementing women with folic acid to prevent NTD-affected births, however, indicated no increase in multiple births (Kirke *et al.,* 1992).

30. Several large studies investigating whether folic acid fortification or folic acid supplementation is associated with an increased prevalence of multiple births have recently reported. In a population-based cohort study in China of women who either did (n=127,018) or did not (n=114, 997) receive folic acid supplements (400μg/d) during pregnancy, no association was observed between folic acid consumption and multiple births (Li *et al.,* 2003). Two studies in the USA investigated rates of twinning pre- and post-fortification of foods with folic acid. The first, in California, examined the prevalence of twinning among more than 2.5 million births between 1990 and 1999: no change in twinning prevalence was associated with folic acid fortification (Shaw *et al.,* 2003). The second, in Texas, examined over 1 million births between 1 January 1996 and 31 December 1998 (mandatory fortification introduced 1 January 1998); although an increase in twinning frequency was observed this was consistent with an ongoing increase in twinning of 1-4% per year observed prior to fortification began (Waller *et al.,* 2003).

31. High-dose folic acid supplementation during gestation in rats (20-fold the rat requirement – equivalent to about 5mg/d in humans) has been shown to result in significant reductions in body weight and vertex-coccyx length of fetuses. Folic acid administration also induced a higher S-adenosylmethionine: S-adenosylhomocysteine value due to increased S-adenosylmethionine synthesis, as well as lowering dietary protein utilization (Achon *et al.,* 1999, 2000).

Folate and cardiovascular disease

32. This was considered previously by COMA in 2000 (Department of Health, 2000). At that time, the Committee concluded that in the absence of more definitive evidence linking folate directly with cardiovascular disease, it would not be justifiable at present to advocate dietary fortification with folic acid solely with the aim of reducing the incidence of cardiovascular disease.

Plasma homocysteine

33. Plasma total homocysteine (tHcy) levels are inversely associated with measures of folate status – plasma and red blood cell folate levels. The remethylation of homocysteine to methionine by methylene tetrahydrofolate reductase (MTHFR) is dependent on an adequate supply of folate; thus, low folate status results in elevated tHcy concentrations. Maximal lowering of tHcy by folic acid supplementation in healthy subjects is observed within the 0.2-0.4mg/d range (Ward *et al.,* 1997, 2002; van Oort *et al.,* 2003). Several other B vitamins are also required for the remethylation of homocysteine: cobalamin (B_{12}), vitamin B_6 and riboflavin (B_2). The dietary supply of vitamins B_6 (McKinley *et al*. 2001) B2 (McNulty *et al.,* 2002; Jacques *et al.,* 2002) and B_{12} (Quinlivan *et al*. 2002) has also been shown to affect tHcy concentrations. Population subgroups, such as vegetarians and, more so, vegans, in which the dietary supply of vitamin B_{12} is limited tend to have raised tHcy levels, despite an adequate supply of folate (Hermann *et al.,* 2003; Lloyd-Wright *et al.,* 2003).

34. Other factors, besides B vitamins, may also affect tHcy concentrations. Lifestyle factors, such as alcohol consumption, physical activity and smoking (de Bree *et al.,* 2001; Mennen *et al*. 2002, Ganji and Kafai, 2003; Husemoen *et al.,* 2004; Nurk *et al.,* 2004) have been associated with tHcy concentrations. Intervention studies have also demonstrated that coffee consumption can raise (Verhoef *et al*. 2002; Strandhagen *et al.,* 2003) and betaine consumption (Olthof *et al.,* 2003) can lower tHcy concentrations. The modification to dietary patterns (e.g. diet rich in fruits, vegetables, and low-fat dairy products and reduced in saturated and total fat) was shown to be more effective than increasing folate rich foods alone (e.g. diet rich in fruits, vegetables) in lowering tHcy (Appel *et al*. 2000).

35. The 677 C→T MTHFR polymorphism also influences tHcy concentrations (Brattstrom *et al.,* 1998). Its prevalence is related to ethnicity: the homozygous TT genotype is about 10% in Caucasians (though it may be population dependent, and is about 20% or more in some Italian and US Hispanics) and only a few percent in Africans and Afro-Americans (Botto & Yang, 2000). The homozygous form (TT) is associated with elevated tHcy levels of typically 25% (Engbersen et al. 1995).

36. Two meta-analyses of observational studies concluded that: elevated levels of tHcy were a modest independent risk factor for cardiovascular disease in healthy populations (Homocysteine Studies Collaboration, 2002); and individuals with the C677T MTHFR genotype had a significantly higher risk of coronary heart disease, particularly in a low folate status (Klerk *et al.,* 2002). Another meta-analysis, which combined both the prospective and and MTHFR genotype evidence, concluded there was strong evidence for a causal relationship between elevated levels of tHcy and cardiovascular disease (Wald *et al.,* 2002).

37. It has been suggested that elevated tHcy may induce endothelial dysfunction (Chambers *et al.,* 2000) – a risk factor for cardiovascular disease (Widlansky *et al.,* 2003). High doses of folic acid (5-10mg/d) have also been shown to improve flow-mediated dilation in coronary artery disease patients (Doshi *et al.,* 2001; Title *et al.,* 2000) and smokers (O'Grady *et al.,*

2002), forearm blood flow, but not arterial elasticity, in smokers (Mangoni *et al.,* 2002), and volumetric coronary blood flow in hyperhomocysteinemic patients with coronary artery disease (Willems *et al.,* 2002). This effect was shown to be independent of a plasma homocysteine lowering effect (Doshi *et al.,* 2002). Lower doses of folic acid (e.g. 0.4mg/d), which are attainable through the diet, and result in maximal, or near maximal, tHcy reductions have no effect on flow mediated dilation (Pullin *et al.,* 2001; Hirsch *et al.,* 2002).

Prospective epidemiological studies

38. Prospective epidemiological evidence linking dietary levels of folate, and vitamins B_6 and B_{12}, with cardiovascular disease in the general population are described in Table 3.

Table 3. Cohort studies investigating an association of circulating and dietary folate concentrations with risk of coronary heart disease

Study (Reference)	**Study population**	**Age range (yrs)**	**Mean follow-up (yrs)**	**No of cases**	**Adjusted relative risk (95% CI)**	**Trends**	**Adjusted for**
Morrison *et al.,* 1996	5,056 general population	35-79	15	165 fatal CHD	1.69 (1.10 to 2.61) for lowest v highest quartile of serum folate	NS	Sex, age, smoking, diabetes, serum cholesterol and hypertension
Chasan-Taber et al., 1996	NCC 14,916 men	40-84	7.5	333 MI 333 MC	1.4 (0.9 to 2.3) for lowest 20th percentile vs. those above the 20th percentile of serum folate	NS	Diabetes, angina, hypertension, Quetelet's index, total cholesterol, HDL cholesterol, aspirin use.
Folsom *et al.,* 1998	15,792 general population	45-64	3.3	232 fatal and non-fatal CHD	1.01 (0.5 to 2.2) for highest v lowest quintile of plasma folate for men 0.36 (0.1 to 0.98) for highest v lowest quintile of plasma folate for women	p=0.65 Men p=0.003 Women	Sex, age, race, field centre, smoking, total cholesterol, HDL cholesterol, hypertension and diabetes
Ford *et al.,* 1998	2,657 general population	25-74	19	873 CHD	1.04 (0.86 to 1.85) for lowest v highest quintile of serum folate	NS	Sex, age, race, education, smoking, hypertension, serum cholesterol, BMI, physical activity, diabetes and alcohol consumption
Rimm *et al.,* 1998	80,082 female nurses	30-55	14	939 MI and fatal CHD	0.69 (0.55 to 0.87) for highest v lowest quintile of dietary folate intake	P=0.03	Age, time period, smoking, BMI, postmenopausal hormones, aspirin, vitamin E supplements, exercise, hypertension, parental history of CHD, and intake of polyunsaturated, saturated and trans fat, fibre and alcohol

Voutilainen *et al.,* 2001	1,980 men	42-60	10	199 acute coronary events	0.45 (0.25 to 0.81) for highest v lowest quintile of dietary folate intake in subjects with no previous CHD	No previous CHD P=0.008 Previous CHD P=0.453	Age, total, LDL and HDL cholesterol and triglyceride, smoking, physical activity, hypertension, diabetes, CHD in family and nutritional factors.
Hung *et al.,* 2003	2,314 general population	20-90	29	644 fatal CVD of which 372 fatal CHD	Men 1.03 (0.83 to 1.29) and women 1.15 (0.91 to 1.46) for lowest v highest quartile of red blood cell folate	Men p=0.35 Women p=0.21	Age, blood pressure, BMI, serum cholesterol, white cell count, smoking menopause, diabetes, hypertension, alcohol, history of CVD.
He *et al.,* 2004	43,732 men	40-75	14	725 incident strokes, of which 455 were ischemic	0.71 (0.52 to 0.96) for highest v lowest quintile of dietary folate intake. Association for ischemic, but not hemorrhagic, stroke.	P=0.05	Age, smoking, BMI, physical activity, history of hypertension and hypercholesterolemia, aspirin, alcohol, fibre, potassium, vitamin E and total energy
Voutilainen *et al.,* 2004	1027 men	46-64	7.7	144 cardiac events	0.39 (0.18 to 0.83) for highest v lowest tertile of serum folate. No association between tHcy and acute coronary events was observed.	P=0.016	Age, smoking, BMI, systolic blood pressure, serum LDL and HDL cholesterol, serum lycopene, alpha tocopherol and beta carotene

MI, myocardial infarctions; BMI, body mass index; NS, not stated; NCC, nested case-control; MC, matched control.

Randomized controlled trials investigating an effect of folic acid on cardiovascular disease

39. Only a few randomized controlled trials (RCTs) have, as yet, been published on tHcy-lowering treatment and subsequent effects on cardiovascular disease risk.

40. In a trial of patients with atherosclerotic vascular disease (n=101; 51 with initial tHcy plasma levels above, and 50 below 14 micromol/L) supplementation with 2.5 mg folic acid, 0.25 mg B_{12} and 25 mg B_6 for an average of over 2.5 years (range 0.9 –6.0 years) was shown to reduce atherosclerotic plaque progression (Hackam *et al.,* 2000), as assessed by two-dimensional B-mode ultrasound measurement of carotid plaques before and after treatment.

41. A trial in 134 healthy siblings of patients with premature atherothrombotic disease who were supplemented with either placebo or 5mg folic acid and 250mg vitamin B_6 for two years (Vermeulen *et al.,* 2000) measured the development or progression of subclinical atherosclerosis as estimated from exercise eletrocardiography as the primary outcome. Subjects receiving the vitamin treatment showed a decrease in both tHcy levels and abnormal exercise electrocardiography. The choice of primary outcome measure was criticized, however, because of its very low positive-predictive value when used in symptom-free populations (Bostom & Garber, 2000). The trial also demonstrated a lowering effect of vitamin treatment on blood pressure, but failed to demonstrate an effect on measures of vascular function (van Dijk *et al.,* 2001).

42. The effect B vitamin supplementation on restenosis and major adverse events after coronary angioplasty was studied in a trial of patients (n=205) who were supplemented with either 1 mg folic acid, 0.4 mg B_{12} and 10 mg B_6 or placebo for 6 months. A significant decrease in the frequency of restenosis was observed after 6 months of vitamin treatment (19.6% vs. 37.6%) concurrent with a decrease in tHcy (Schnyder *et al.,* 2001).

43. In a follow-up paper (Schnyder *et al.,* 2002) the continued observation was reported of the groups in the original trial plus additional patients (n=553) for another 6 months after the vitamin treatment was stopped; repeated revascularization was lower in the vitamin group (10.8% vs. 22.3%) and a non-significant trend was observed toward fewer deaths and non-fatal myocardial infarctions. This was seen in the absence of vitamin supplementation during the 6 months post supplementation.

44. In a more recent trial (Lange *et al.,* 2004), however, 636 patients, who had undergone successful coronary stenting, were supplemented with either 1.2 mg folic acid, 0.6 mg B_{12} and 48 mg B_6 or placebo for 6 months; in-stent restenosis was then assessed using coronary angiography. B vitamin supplementation had an adverse effect on the risk of restenosis (34.5% vs. 26.5%) and a higher percentage of patients in that group required repeat target-vessel revascularization (15.8% vs. 10.6%). B vitamin supplementation significantly reduced plasma levels of tHcy.

45. A number of prevention trials are underway (see Table 4) and the major folic acid trials are expected to report in 2005/6.

46. The first large trial intervention trial to report was CHAOS-2 (Baker *et al.,* 2002), where 1882 ischemic heart disease patients received either 5mg folic acid or placebo for two years. Despite reducing tHcy concentrations, folic acid supplementation had no effect on the composite end-point of either non-fatal myocardial infarction, cardiovascular death or unplanned revascularization (risk ratio 0.97; 95% CI, 0.72-1.29).

47. Recently VISP reported, examining the effect on recurrent stroke, myocardial infarction and death of lowering tHcy in patients with ischemic stroke reported (Toole *et al.,* 2004). In this trial, the control group received each day 200 μg pyridoxine, 6 μg vitamin B_{12} and 20 μg folic acid and intervention group received 25mg pyridoxine, 0.4mg vitamin B_{12} and 2.5 mg folic acid. A moderate reduction of tHcy in the intervention group was observed, but there was no effect on vascular outcomes during the two years of follow-up (risk ratio 1.0; 95% CI, 0.8-1.3). The trial did, however, observe an association of tHcy with vascular risk.

48. It has been suggested that these early trials were all under powered (Verhoef and Katan, 2004), especially in populations where mandatory folic acid fortification had been introduced, e.g. VISP, HOPE-2, WACS (Bostom *et al.,* 2000), and until later trials report it will not be known whether tHcy lowering reduces the risk of CVD.

Table 4. Trials of homocysteine-lowering vitamin supplements in people with prior CHD, prior stroke or renal disease

Trial (Country)	Fortified population (-/+)	Prior disease	Scheduled number to be randomized	Scheduled duration of treatment (years)	Homocysteine-lowering regimen (mg/d)		
					Folic acid	B_{12}	B_6
CHAOS-2† (UK)	-	CHD	1880	2	5.0	-	-
SU.FOL.0M3 (France)	-	CHD	2000	5	0.5	0.02	3
WENBIT (Norway)	-	CHD	2800	3	0.8	0.4	40
NORVITE (Norway)	-	CHD	3750	3	0.8	0.4	40
SEARCH (UK)	-	CHD	12064	5	2.0	1.0	-
HOPE-2 (Canada)	+	CHD	5520	5.5	2.5	1.0	50
WACS (USA)	+	CHD	5500	7	2.5	1.0	50
Su.Fol.03 (France)	-	Stroke	1000	5	0.5	0.02	3
VITATOPS (Australia)	-	Stroke	8000	3	2.0	0.5	25
VISP† (USA)	+	Stroke	3600	2	2.5	0.4	25
FAVORIT (USA)	+	Renal	4000	5	2.5	0.4	20
VA Trial (USA)	+	Renal	2000	5	40.0	0.5	100

Table courtesy of Dr Robert Clarke; † terminated early.

Folate and cancer

49. This was previously considered by COMA in 1998 (Department of Health, 1998) and 2000 (Department of Health, 2000). At that time, the Committee concluded that there was insufficient evidence for any specific links between folate intake and the development of cancer. Prospective cohort studies investigating an association between dietary folate and cancer risk are described below. No randomized controlled trials investigating an effect of folate on cancer risk have reported at this time.

Prospective epidemiological studies

50. The majority of studies have investigated the relationship between dietary folate and risk of breast and colon cancer (details are given in Tables 5 and 6). Other studies have observed no relationship between dietary folate intake and pancreatic cancer (Skinner *et al.,* 2004; although Soltzenberg-Solomom *et al.,* 2000, observed an inverse relationship in smokers), squamous or basal cell carcinoma of the skin (Fung *et al.,* 2002, 2003; van Dam *et al.,* 2000), bladder cancer (Michaud *et al.,* 2002) cervical cancer (Alberg *et al.,* 2000) and non-Hodgkin's lymphoma (Zhang *et al.,* 2000). A prospective nested case-control study that investigated the relationship between serum folate levels and prostate cancer risk also reported no association (Hultdin *et al.,* 2004).

51. While no relationship between dietary folate intake and lung cancer was observed in one study (Yuan *et al.,* 2003), a nested case-control study (Voorrips et al 2000) did report an inverse relationship.

52. A prospective study of the relationship between dietary folate intake and the incidence of ovarian cancer (Larrson 2004) reported a weak inverse association, although this was not significant. Among women who consumed more than 20g of alcohol per week, however,

there was a strong inverse association, but among women who consumed 20g of alcohol per week or less there was no association.

53. Low folate intake in the presence of alcohol consumption (and in some studies low methionine intake) has been associated with a higher risk of colon and breast cancers in some epidemiological studies (Giovannucci *et al.,* 1995; Glynn *et al.,* 1996; Zhang et al 1999, 2003; Su & Arab 2001; Sellers *et al.,* 2001). Alcohol has been shown to act as a folate (or methyl group) antagonist (Halsted, 1995). Other studies, however, found no association between cancer risk and a combined low folate and high alcohol intake (Flood *et al.,* 2002; Harnack *et al.,* 2002; Feigelson *et al.,* 2003).

54. A recent review of case-control studies investigating the relationship between polymorphic genes involved in folate metabolism and colorectal cancer risk (Little *et al*., 2003; Sharp & Little, 2004) concluded that, in most studies, MTHFR 677 TT and 1298 CC were associated with a moderate reduction in risk for colorectal cancer. It has also been suggested that the MTHFR C677T polymorphism modifies the association between dietary folate and risk for breast cancer (Shrubsole *et al.,* 2004).

Table 5. Cohort studies of association of circulating and dietary folate concentrations with risk of colorectal cancer

Study (Reference)	**Study population**	**Age range (yrs)**	**Mean follow-up (yrs)**	**No of cases**	**Adjusted relative risk (95% CI)**	**Trends**	**Adjusted for**
Giovannucci *et al.,* 1995	14,931 men Health Professionals' Follow-up Study	40-759	6	205 colon	0.86 (0.54 to 1.36) for highest vs. lowest quintile of folate intake 0.74 (0.47 to 1.17) for multivitamin use for 10 or more years compared to nonusers 3.30 (1.58 to 6.88) for high alcohol (>20g/d) low methyl (methionine and folate) diet vs a low alcohol (<5g/d) high methyl diet in non aspirin users.	p=0.30 NS p<0.01	Age, smoking, physical activity, BMI, aspirin use, multivitamin use, total energy, fat, read meat, vitamin D and calcium intake, family history of CRC, history of polyps/endoscopy
Glynn *et al.,* 1996	NCC 29,133 men smokers Alpha-Tocopherol Beta-Carotene cohort	50-69	5-8	91 colon 53 rectal 276 non-cases	0.51 (0.20 to 1.31) for highest vs. lowest quartile of folate intake for colon cancer. 2.12 (0.43 to 10.54) for highest vs. lowest quartile of folate intake for rectal cancer. 0.96 (0.40 to 2.30) for highest vs. lowest quartile of serum folate concentrations for colon cancer. 2.94 (0.84 to 10.33) for highest vs. lowest quartile of serum folate concentrations for rectal cancer.	p=0.15 p=0.66 p=0.83 p=0.10	Total energy intake, physical activity, energy-adjusted folate intakes, vitamins A, C and E, fibre, protein, starch, calcium. iron, alcohol intake, smoking, BMI

Giovannucci *et al.,* 1998	88,756 women Nurses' Health Study	30-55	15	442 colon	0.69 (0.52 to 0.93) for highest vs. lowest quartile of folate intake. 0.48 (0.33 to 0.71) for highest vs. lowest quartile of folate intake in women whose methionine intake <1.8g/d. 0.29 (0.15 to 0.56) for multivitamin use for 15 or more years compared to nonusers	p=0.01 p<0.001 p<0.001	Age, aspirin use, physical activity, BMI, smoking, family history of CRC, and red meat, fibre, methionine and fibre intake
Kato *et al.,* 1999	NCC 15,785 women Women's Health Study	NS	3-9	105 colon and rectal 523 non cases	0.50 (0.26 to 0.96) for highest vs. lowest quartile of serum folate concentrations.	p=0.04	Education, race, religion, physical activity, aspirin use, family history of CRC, alcohol, smoking, energy, macronutrient, fibre, vitamin A, C and E intakes, Quetelet index
Su & Arab *et al.,* 2001	10,183 general population NHANES I Epidemiology Follow-up Study	30-55	20	219 colon	0.40 (0.18 to 0.88) for highest v lowest quartile of dietary folate intake in men 0.74 (0.36 to 1.51) for highest v lowest quartile of dietary folate intake in women 2.22 (1.03 to 4.77) for alcohol drinkers (>1.16 drinks/wk) low methyl (methionine and folate) diet vs non-drinkers, high methyl diet in men, but not women.	p=0.03 p=0.70 p=0.05	Age, race, gender, smoking, BMI, family history of colon cancer, intake of fat, fibre, calcium, vitamin B6, vitamin B12, total energy and alcohol
Fuchs *et al.,* 2002	88,758 women Nurses' Health Study	30-55	16	535 colon	0.48 (0.28 to 0.83) for highest vs. lowest quartile of folate intake in women with a family history of colon cancer 0.81 (0.62 to 1.07) for highest vs. lowest quartile of folate intake in women with no family history of colon cancer	p=0.01 NS	Age, aspirin use, physical activity, BMI, smoking, family history of CRC, postmenopausal oestrogen use, red meat, alcohol, animal fat, vitamins A, C, D, E, methionine and fibre intake

Konings *et al.,* 2002	NCC 120,852 general Netherlands Cohort Study	55-69	7.3	760 colon 411 rectal 3500 non cases	0.73 (0.46 to 1.17) for highest vs. lowest quintile of folate intake for colon cancer in men 0.68 (0.39 to 1.20) for highest vs. lowest quintile of folate intake for colon cancer in women 0.66 (0.35 to 1.21) for highest vs. lowest quintile of folate intake for rectal cancer in men 1.26 (0.58 to 2.76) for highest vs. lowest quintile of folate intake for rectal cancer in women	p=0.03 p=0.18 p=0.03 p=0.55	Age, energy intake, family history, alocohol, vitamin C, iron and dietary fibre intake
Flood *et al.,* 2002	45,264 women Breast Cancer Detection Project Follow-up Study	NS	8.5	490 colon and rectal	0.86 (0.65 to 1.13) for highest vs. lowest quintile of dietary folate intake 0.94 (0.70 to 1.26) for highest vs. lowest quintile of total folate intake (includes supplements)	p=0.14 NS	NSAID use, smoking, education, BMI, physical activity, red meat, alcohol, total fat, vitamins D, grains, methionine and fibre intake
Harnack *et al.,* 2002	41,836 women Iowa Women's Health Study	55-69	5	598 colon 123 rectal	1.12 (0.77 to 1.63) for highest vs. lowest quintile of folate intake for colon cancer 0.89 (0.52 to 1.51) for highest third vs lowest third of folate intake for rectal cancer	p=0.67 p=0.44	Age, BMI, oestrogen use, smoking, dietary energy, calcium, and vitamin E
Terry *et al.,* 2002	NCC 56,837 women Canadian National Breast Screening Study	40-59	10.4	595 cases 5,334 non-cases	0.6 (0.4 to 1.1) for highest vs. lowest quintile of folate intake for colon cancer 0.7 (0.3 to 1.8) for highest vs. lowest quintile of folate intake for rectal cancer	p=0.25 p=0.36	Age, smoking, BMI, physical activity, education, fat and energy intakes
Wei *et al.,* 2004	87,733 women 46,632 men Nurses' Health Study and Health Professionals' Follow-up Study	30-55 40-75	20 14	1.139 colon 339 rectal	0.82 (0.66 to 1.03) for highest vs. lowest quartile of folate intake for colon cancer in women 0.72 (0.45 to 1.16) for highest vs. lowest quartile of folate intake for colon cancer in men 0.82 (0.68 to 0.99) for highest vs. lowest quartile of folate intake for colon cancer in men and women	p=0.04 p=0.57 p=0.06	Age, family history, BMI, physical activity, beef, pork or lamb as main dish, processed meat, alcohol, calcium, height, smoking, history of endoscopy and gender in combined cohort

BMI, body mass index; NSAID, non-steroidal anti-inflammatory drugs; NS, not stated; NCC, nested case-control.

Table 6. Cohort studies of association of circulating and dietary folate concentrations with risk of breast cancer

Study (Reference)	Study population	Age range (yrs)	Mean follow-up (yrs)	No of cases	Adjusted relative risk (95% CI)	Trends	Adjusted for
Zhang *et al.,* 1999	88,818 Nurses' Health Study	30-55	16	3,483	0.93 (0.83 to 1.03) for highest vs. second lowest quintile of folate intake 0.55 (0.39 to 0.76) for highest vs. second lowest quintile of folate intake for alcohol intake more than or equal to 15g/d	P=0.26 P=0.001	Age, total energy intake, parity, age at first birth, family history of breast cancer/disease, alcohol intake, BMI, weight gain/loss, height, age at menopause, HRT use, beta carotene and supplement intake
Wu *et al.,* 1999	NCC 12,450 women	NS	NS	133 133 MC	1.08 (0.50 to 2.37) lowest vs. highest quintile of serum folate concentration	p=0.73	Family history, bilateral ovariectomy, age at menarche/menopause /first birth, number of pregnancies, months of breast feeding, oral contraceptive use, HRT, education, BMI and physical exercise
	NCC 14625 women	NS	NS	110 110 MC	0.79 (0.33 to 1.90) lowest vs. highest quintile of serum folate concentration	p=0.41	
Sellers *et al.,* 2001	34,387 Iowa Women's Health Study	55-69	12	1,586	1.19 (0.90 to 1.58) for lowest 10th percentile vs. those above the 50th percentile of total folate intake 1.59 (1.05 to 2.41) for lowest 10th percentile vs. those above the 50th percentile of folate intake for alcohol intake more than 4g/d	NS NS	Age, education, family history of breast cancer, age at menarche, age at menopause, oral contraceptive use, HRT, parity, age at first birth, BMI, waist-to-hip ratio, height, alcohol, smoking, physical activity and other B vitamins
Zhang *et al.,* 2003	32,826 women	43-69	NS	712 712 MC	0.73 (0.50 to 1.07) highest vs. lowest quintile of serum folate concentration 0.11 (0.02 to 0.59) for highest vs. lowest quintile of serum folate concentration for alcohol intake more than or equal to 15g/d	p=0.06 p=0.01	Parity, age at first birth, family history of breast cancer/disease, alcohol intake, BMI, age at menopause and menarche

Feigelson *et al.,* 2003	66,561 American Cancer Society Cancer Prevention Study II Nutrition Cohort	NS	5	1,303	1.10 (0.94 to 1.29) for highest vs. lowest quartile of folate intake 1.33 (0.94 to 1.88) within the lowest quartile of folate intake for highest quartile of alcohol intake (>15g/d) vs. non drinkers.	NS NS	Age, ethanol, methionine, multivitamin use, race, education, family history, breast lump history, mammographic history, HRT use, parity at first birth. Age at menopause, age at menarche, physical activity, BMI, weight gain, energy intake
Sellers *et al.,* 2004	33,552 Iowa Women's Health Study	55-69	14	1823	1.19 (0.98 to 1.45) for lowest 10th percentile vs. those above the 50th percentile of dietary folate intake 2.26 (1.59 to 3.21) for lowest 10th percentile vs. those above the 50th percentile of dietary folate intake in those with a family history of breast cancer	p=0.20 P=0.005	Age, energy intake, education, age at menarche, age at menopause, oral contraceptive use, HRT, parity, age t first birth, BMI, waist-to-hip ratio, height, smoking and physical activity

HRT, hormone replacement therapy; BMI, body mass index; NS, not stated; NCC, nested case-control; MC, matched control.

Folate and cognitive function

This was previously considered by COMA in 2000 (Department of Health, 2000). At that time, the Committee made no conclusions.

tHcy and cognitive decline or dementia in the elderly

In the elderly tHcy has been shown to be positively associated with age, independently of vitamin status, and negatively associated with folate status, independently of age and other vitamin status (Selhub *et al*., 1993).

In cross-sectional and case-control studies in the elderly, elevated tHcy concentrations, and low folate and vitamin B_{12} status, have been associated with poor cognition (Riggs *et al*., 1996; Selhub *et al*., 2000; Duthie *et al*., 2002), dementia and Alzheimer's disease (Clarke *et al*., 1998; Quadri *et al*., 2004). Hyperhomocysteinemia has also been associated with Parkinson's disease in case-control studies (Kuhn *et al*., 1998).

Prospective epidemiological studies investigating an association between tHcy, B vitamins and cognitive decline or dementia

Several prospective cohort studies have investigated the association between tHcy, B vitamins and cognitive decline or dementia. In the study by Clarke *et al*. (1998) 43 patients with dementia of Alzheimer's type were followed-up for three years and radiological evidence of disease progression was determined each year. Those patients with tHcy > 11µmol/l showed a more rapid progression of Alzheimer's disease than those below this cut-off. The association between blood levels of folate and vitamin B_{12} at the first visit and disease progression showed a similar trend, but these were not statistically significant.

In a study of 23 normal elderly subjects (aged 69-80 years) followed-up for five years, tHcy was found to predict cognitive scores (mini-mental state examination and Alzheimer's disease assessment scale – cognitive component) independently of age, sex, education, renal function, serum folate and vitamin B_{12} concentrations, smoking and hypertension ($p<0.001$) (McCaddon *et al*. 2001).

Low serum concentrations of both folate and vitamin B_{12} at baseline (tHcy was not measured) were associated with twice the risk of subsequently developing Alzheimer's disease at three years follow-up in a cohort of 370 elderly (Wang *et al*. 2001).

A sample of the Framingham Study cohort (1092 subjects, aged 68-97 years and without dementia) were followed-up for a median of eight years during which time 111 developed dementia including 83 with Alzheimer's disease. An increased tHcy concentration was observed to be a risk factor for dementia and Alzheimer's disease independently of age, sex, apolipoprotein E genotype, vascular risk factors other than tHcy and for plasma concentrations of folate, vitamin B_{12} and vitamin B_6 (Seshadri *et al.,* 2002).

A study of subjects drawn from the Maastricht Aging Study (144 aged 30-80 years) and followed-up for six years, investigated the relationship between cognitive performance and tHcy and serum vitamin B_{12} and folate concentrations (Teunissen *et al.,* 2003). Word learning and delayed recall tests (though not cognitive speed and information processing) were negatively associated with tHcy independently of age, sex and education level. No significant associations with serum vitamin B_{12} and folate concentrations were observed at the follow-up.

A recent prospective study combining the Health Professionals' Follow-up Study and the Nurses' Health Study cohorts examined the relationship between folate intake and Parkinson's disease (Chen *et al*., 2004). A total of 47,341 men (aged 40-75 years) and 88,716 women (aged 30-55 years) were followed-up for an average of 12.7 and 17.3 years respectively, during which time 248 men and 167 women developed Parkinson's disease. Baseline intake of folate was not associated with risk of Parkinson's disease, nor were vitamin B_{12} or B_6 intakes.

Randomized controlled trials investigating an effect of folic acid on cognition and dementia

A Cochrane review has been conducted of the four RCTs that have examined the effects of folic acid supplementation, with or without vitamin B12, on elderly healthy and demented people, in preventing cognitive impairment or retarding its progress (Malouf *et al*., 2004). The review concluded there was no beneficial effect of folic acid in either older healthy women or patients with mild to moderate cognitive decline and different forms of dementia. It was also concluded that more studies are needed.

Summary

Conclusions

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