



Agenda Item 1

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 B₁₂-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.

Journal articles that accompany this paper are formatted bold in the text.

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 B₁₂-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 B₁₂-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-fortification (de Wals *et al.*, 2003). Two other studies in Canada observed reductions in NTDs of 51% (Ray *et al.*, 2002) and 54% (Persad *et al.*,

2002) in Ontario and Nova Scotia, 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	
Greece	Mandatory – Due to consult on approach.	Mandatory level likely to be considered – 160-200µg/100g (most likely vehicle flour)
	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, Paraguay 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 <i>et al.</i> , 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 <i>et al.</i> , 2001; Matthews <i>et al.</i> , 2002)	
Pre fortification	3.78
Post fortification	3.05[2.95]
(Mersereau <i>et al.</i> , 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.

Vitamin B₁₂-deficiency in the elderly and masking by folic acid

14. Vitamin B₁₂ (cobalamin) deficiency caused by either intrinsic factor deficiency or hypochlorhydria mainly affects the elderly. Vitamin B₁₂-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 B₁₂-deficiency is complicated by the limitations of using low serum vitamin B₁₂ concentrations: low levels do not always indicate vitamin B₁₂-deficiency and patients with vitamin B₁₂-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 B₁₂ status (Bates *et al.*, 2003), although impaired renal function may affect this measure. Persons with biologically significant vitamin B₁₂-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 B₁₂ may be used to identify those at high risk of vitamin deficiency.
15. Folic acid supplementation can prevent anaemia among persons with vitamin B₁₂-deficiency, but it does not prevent damage to the nerves, spinal cord or brain. Treatment of vitamin B₁₂-deficient patients with folic acid, therefore, may delay diagnosis of the deficiency and result in the worsening of neurological damage (the 'masking' of vitamin B₁₂-deficiency).
16. The COMA report on folic acid (Department of Health, 2000) adopted a serum vitamin B₁₂ concentration of <118pmol/L or a mean corpuscular volume (MCV) > 101fl to define poor vitamin B₁₂ 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 B₁₂ 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 B₁₂ deficiency; this was defined as serum vitamin B₁₂ 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 B₁₂-deficiency, whether defined as serum vitamin B₁₂ concentrations <150pmol/l or serum vitamin B₁₂ 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.

17. The serum vitamin B₁₂ 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 B₁₂ concentrations to diagnose vitamin B₁₂-deficiency is limited (Clarke & Grimley Evans, 2004).
18. **Mills *et al.*, (2003)** compared rates of megaloblastic anemia pre- and post-fortification in the USA, in patients with low serum vitamin B₁₂ concentrations. Patients at the Veterans Medical Center in Washington, DC, between 1992 and 2000 (fortification was introduced 1996-1998) that had low serum vitamin B₁₂ 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.
19. Another post-fortification study in the USA (**Johnson *et al.*, 2003**) observed a prevalence for B₁₂-deficiency (defined as serum vitamin B₁₂ 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 B₁₂-deficient subject with elevated MCV; however, it was also noted that coexisting iron deficiency was quite common in the vitamin B₁₂-deficient subjects with several of them exhibiting microcytic anaemia or low-normal MCV. Vitamin B₁₂-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 B₁₂-deficiency, may not result in a rise in haemoglobin.
20. 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.

21. 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.
22. The masking of vitamin B₁₂-deficiency is unique to folic acid. The predominant naturally occurring form of folate, [6S]-5-methyltetrahydrofolate [6S]-5-MTHF, is unlikely to mask vitamin B12-deficiency. The conversion of [6S]-5-MTHF to tetrahydrofolate, which is the precursor of folate forms involved in DNA synthesis, is vitamin B12-dependent; whereas, folic acid can be converted to tetrahydrofolate independently of vitamin B₁₂. Folic acid can, therefore, maintain DNA synthesis and ameliorate megaloblastic anaemia (Scott & Wier, 1981).
23. It has been demonstrated that [6S]-5-MTHF is equivalent to folic acid in its ability to raise folate status and lower plasma total homocysteine concentrations (Lamers *et al.*, 2004). [6S]-5-MTHF could, therefore, provide an alternative to folic acid for use in the fortification of foods or supplements.

Folic acid supplementation or fortification and multiple births

24. 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).
25. 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).

Folate and cardiovascular disease

26. 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

27. 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 (B₁₂), vitamin B₆ and riboflavin (B₂). The dietary supply of vitamins B₆ (McKinley *et al.* 2001) B₂ (McNulty *et al.*, 2002; Jacques *et al.*, 2002) and B₁₂ (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 B₁₂ is limited tend to have raised tHcy levels, despite an adequate supply of folate (Hermann *et al.*, 2003; Lloyd-Wright *et al.*, 2003).
28. 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).
29. 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).
30. 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, however, concluded that elevated levels of tHcy were a strong independent risk factor for cardiovascular disease (**Wald *et al.*, 2002**).
31. 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

32. Prospective epidemiological evidence linking dietary levels of folate, and vitamins B₆ and B₁₂, 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 <i>et al.</i>, 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
Folsom <i>et al.</i>, 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 <i>et al.</i>, 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 <i>et al.</i>, 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 <i>et al.</i>, 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 <i>et al.</i>, 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 <i>et al.</i>, 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 <i>et al.</i>, 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.

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

33. Only a few randomized controlled trials (RCTs) have, as yet, been published on tHcy-lowering treatment and subsequent effects on cardiovascular disease risk.
34. 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 B₁₂ and 25 mg B₆ 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.
35. 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 B₆ for two years (Vermeulen *et al.*, 2000) measured the development or progression of subclinical atherosclerosis as estimated from exercise electrocardiography 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).
36. 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 B₁₂ and 10 mg B₆ 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).
37. 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.

38. 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 B₁₂ and 48 mg B₆ 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.
39. A number of prevention trials are underway (see Table 4) and the major folic acid trials are expected to report in 2005/6.
40. 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).
41. 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 B₁₂ and 20 µg folic acid and intervention group received 25mg pyridoxine, 0.4mg vitamin B₁₂ 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.
42. 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

43. 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

44. 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).

45. 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.

46. A prospective study of the relationship between dietary folate intake and the incidence of ovarian cancer (Larsson 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.

47. 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; Zhang *et al.* 1999; 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).

48. A recent review of case-control studies investigating the relationship between polymorphic genes involved in folate metabolism and colorectal cancer risk (Sharp & Little, 2004; Little *et al.*, 2003) 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).

49. Several prospective, nested case-control studies have investigated the relationship between serum folate levels or dietary folate intake and colorectal cancer: most (Glynn *et al.*, 1996; Kato *et al.*, 1999; Terry *et al.*, 2002; Konings *et al.*, 2002), though not all (Wu *et al.*, 1999) have reported an inverse relationship. A prospective, nested case-control study that

investigated the relationship between serum folate levels and breast cancer risk also reported an inverse relationship (Zhang *et al.*, 2003).

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 <i>et al.</i> , 1995	14,931 men Health Professionals' Follow-up Study	40-75	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, red meat, vitamin D and calcium intake, family history of CRC, history of polyps/endoscopy
Giovannucci <i>et al.</i> , 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
Su & Arab <i>et al.</i> , 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

<i>Fuchs et al., 2002</i>	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
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
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.

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 <i>et al.</i> , 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
Sellers <i>et al.</i> , 2001	34,387 Iowa Women's Health Study	55-69	12	1,586	1.19 (0.90 to 1.58) for lowest 10 th percentile vs. those above the 50 th percentile of total folate intake 1.59 (1.05 to 2.41) for lowest 10 th percentile vs. those above the 50 th 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
Feigelson <i>et al.</i> , 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 <i>et al.</i> , 2004	33,552 Iowa Women's Health Study	55-69	14	1823	1.19 (0.98 to 1.45) for lowest 10 th percentile vs. those above the 50 th percentile of dietary folate intake 2.26 (1.59 to 3.21) for lowest 10 th percentile vs. those above the 50 th 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.

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 B₁₂ 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 > 11 µmol/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 B₁₂ 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 B₁₂ concentrations, smoking and hypertension (p<0.001) (McCaddon *et al.* 2001).

Low serum concentrations of both folate and vitamin B₁₂ 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 B₁₂ and vitamin B₆ (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 B₁₂ 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 B₁₂ 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 B₁₂ or B₆ 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

References

Alberg AJ, Selhub J, Shah KV, Viscidi RP, Comstock GW & Helzlsouer KJ. The risk of cervical cancer in relation to serum concentrations of folate, vitamin B12, and homocysteine. *Cancer Epidemiol Biomarkers Prev.* 2000, **9**:761-764.

Appel LJ, Miller ER 3rd, Jee SH, Stolzenberg-Solomon R, Lin PH, Erlinger T, Nadeau MR & Selhub J. Effect of dietary patterns on serum homocysteine: results of a randomized, controlled feeding study. *Circulation* 2000, **102**:852-857

Baker F, Picton D, Blackwood S, Hunt J, Erskine M, Dyas M, Ashby M, Siva A & Morris MJ. Blinded comparison of folic acid and placebo in patients with ischemic heart disease: an outcome trial. *Circulation* 2002, **106**:3642 (abstr)

Bates CJ, Schneede J, Mishra G, Prentice A & Mansoor MA. Relationship between methylmalonic acid, homocysteine, vitamin B12 intake and status and socio-economic indices, in a subset of participants in the British National Diet and Nutrition Survey of people aged 65 y and over. *Eur J Clin Nutr.* 2003, **57**:349-357.

Blake M, Herrick K & Kelly Y. *Health Survey for England 2002: Maternal and Infant Health.* London. TSO, 2003.

Bostom AG & Garber C. Endpoints for homocysteine-lowering trials. *Lancet* 2000, **355**:511-512

Bostom AG, Selhub J, Jacques PF & Rosenberg IH. Power Shortage: clinical trials testing the "homocysteine hypothesis" against a background of folic acid-fortified cereal grain flour. *Ann Intern Med.* 2001, **135**:133-137.

Botto LD & Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital abnormalities: a HuGE review. *Am J Epidemiol* 2000, **151**, 862-877

Brattstrom L, Wilcken DE, Ohrvik J & Brudin L. Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta-analysis. *Circulation* 1998, **98**:2520-2526

Chambers JC, Ueland PM, Obeid OA, Wrigley J, Refsum H & Kooner JS. Improved vascular endothelial function after oral B vitamins: An effect mediated through reduced concentrations of free plasma homocysteine. *Circulation* 2000, **102**: 2479-2483

Castilla EE, Orioli IM, Lopez-Camelo JS, Dutra Mda G & Nazer-Herrera J; Latin American Collaborative Study of Congenital Malformations (ECLAMC). Preliminary data on changes in neural tube defect prevalence rates after folic acid fortification in South America. *Am J Med Genet.* 2003, **123A**:123-128.

Chen H, Zhang SM, Schwarzschild MA, Hernan MA, Logroscino G, Willett WC & Ascherio A. Folate intake and risk of Parkinson's disease. *Am J Epidemiol.* 2004, **160**:368-375.

Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L & Ueland PM. Folate, vitamin B₁₂, and serum total homocysteine levels in confirmed Alzheimer's disease. *Arch Neurol* 1998;**55**:1449-55.

Clarke R, Refsum H, Birks J, Evans JG, Johnston C, Sherliker P, Ueland PM, Schneede J, McPartlin J, Nexo E & Scott JM. Screening for vitamin B-12 and folate deficiency in older persons. *Am J Clin Nutr.* 2003, **77**:1241-1247.

Clarke R, Grimley Evans J, Schneede J, Nexo E, Bates C, Fletcher A, Prentice A, Johnston C, Ueland PM, Refsum H, Sherliker P, Birks J, Whitlock G, Breeze E & Scott JM. Vitamin B12 and folate deficiency in later life. *Age Ageing.* 2004, **33**:34-41.

Clarke R & Grimley Evans J. Vitamin B-12 and folate deficiency in elderly persons. *Am J Clin Nutr.* 2004, **79**: 338-9.

Czeizel AE, Metneki J & Dudas I. The higher rate of multiple births after periconceptional multivitamin supplementation: an analysis of causes. *Acta Genet Med Gemellol (Roma).* 1994, **43**:175-184.

de Bree A, Verschuren WM, Blom HJ & Kromhout D. Lifestyle factors and plasma homocysteine concentrations in a general population sample. *Am J Epidemiol.* 2001, **154**:150-154

de Wals P, Rusen ID, Lee NS, Morin P & Niyonsenga T. Trend in prevalence of neural tube defects in Quebec. *Birth Defects Res.* 2003, **67**:919-923.

Department of Health. *Nutritional aspects of the development of cancer. Report on health and social subjects 48.* London: The Stationary Office, 1998.

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

Department of Health. *Scientific Review of the Welfare Food Scheme. Report on health and social subjects 51.* London: TSO, 2002.

Doshi SN, McDowell IF, Moat SJ, Lang D, Newcombe RG, Kredan MB, Lewis MJ & Goodfellow J. Folate improves endothelial function in coronary artery disease: an effect mediated by reduction of intracellular superoxide? *Arteriosclerosis Thromb Vascular Biol* 2001, **21**:1196-1202.

Doshi SN, McDowell IF, Moat SJ, Payne N, Durrant HJ, Lewis MJ & Goodfellow J. Folic acid improves endothelial function in coronary artery disease via mechanisms largely independent of homocysteine lowering. *Circulation* 2002, **105**, 22-26.

Duthie SJ, Whalley LJ, Collins AR, Leaper S, Berger K & Deary IJ. Homocysteine, B vitamin status, and cognitive function in the elderly. *Am J Clin Nutr.* 2002, **75**:908-913.

Ericson A, Kallen B & Aberg A. Use of multivitamins and folic acid in early pregnancy and multiple births in Sweden. *Twin Res.* 2001, **4**:63-66.

Engbersen AM, Franken DG, Boers GH, Stevens EM, Trijbels FJ & Blom HJ. Thermolabile 5,10-methylenetetrahydrofolate reductase as a cause of mild hyperhomocysteinemia. *Am J Hum Genet* 1995, **56**:142-150

Finch S, Doyle W, Lowe C, Bates CJ, Prentice A, Smithers G & Clarke PC. National Diet and Nutrition Survey: people aged 65 years and over. Volume 1: report of the diet and nutrition survey. London: The Stationary Office, 1998.

Flood A, Caprario L, Chatterjee N, Lacey JV Jr, Schairer C & Schatzkin A. Folate, methionine, alcohol, and colorectal cancer in a prospective study of women in the United States. *Cancer Causes Control*. 2002, **13**:551-561.

Folsom AR, Nieto FJ, McGovern PG, Tsai MY, Malinow MR, Eckfeldt JH, Hess DL & Davis CE. Prospective study of coronary heart disease incidence in relation to fasting total homocysteine, related genetic polymorphisms, and B vitamins: the atherosclerosis risk in communities (ARIC) study. *Circulation* 1998, **98**: 204-210

Food Standards Agency (2002a). Folic acid and the prevention of disease. http://www.food.gov.uk/multimedia/pdfs/FSA02_05_02.pdf

Food Standards Agency (2002b). Agency advice to health ministers on folic acid and the prevention of disease. http://www.food.gov.uk/multimedia/pdfs/folicacid_disease_annexa.pdf

Ford ES, Byers TE & Giles WH. Serum folate and chronic disease risk: findings from a cohort of United States adults. *Int J Epidemiol* 1998, **27**: 592-598

Fung TT, Hunter DJ, Spiegelman D, Colditz GA, Speizer FE & Willett WC. Vitamins and carotenoids intake and the risk of basal cell carcinoma of the skin in women (United States). *Cancer Causes Control*. 2002, **13**:221-230.

Fung TT, Spiegelman D, Egan KM, Giovannucci E, Hunter DJ & Willett WC. Vitamin and carotenoid intake and risk of squamous cell carcinoma of the skin. *Int J Cancer*. 2003, **103**:110-115.

Ganji V & Kafai MR. Demographic, health, lifestyle, and blood vitamin determinants of serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey, 1988-1994. *Am J Clin Nutr* 2003, **77**:826-833.

Giovannucci E, Rimm EB, Ascherio A, Stampfer MJ, Colditz GA & Willett WC. Alcohol, low-methionine--low-folate diets, and risk of colon cancer in men. *J Natl Cancer Inst*. 1995, **87**:265-273.

Giovannucci E, Stampfer MJ, Colditz GA, Hunter DJ, Fuchs C, Rosner BA, Speizer FE & Willett WC. Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. *Ann Intern Med*. 1998, **129**:517-524.

Glynn SA, Albanes D, Pietinen P, Brown CC, Rautalahti M, Tangrea JA, Gunter EW, Barrett MJ, Virtamo J & Taylor PR. Colorectal cancer and folate status: a nested case-control study among male smokers. *Cancer Epidemiol Biomarkers Prev*. 1996, **5**:487-494.

Hackam DG, Peterson JC & Spence JD. What level of plasma homocyst(e)ine should be treated? Effects of vitamin therapy on progression of carotid atherosclerosis in patients with homocyst(e)ine levels above and below 14 micromol/L. *Am J Hypertens*. 2000, **13**:105-110.

Halstead C. *Alcohol and folate interaction: clinical implication*. In: Bailey LB ed. *Folate in health and disease*. New York, NY: Marcel Dekker, 1995:313-328.

Hamlyn B, Brooker S, Oleinikova K & Wands S. *Infant Feeding 2000. A survey conducted on behalf of the Department of Health, the Scottish Executive, the National Assembly for Wales and the Department of Health, Social Services and Public Safety in Northern Ireland*. London: TSO, 2002.

Harnack L, Jacobs DR Jr, Nicodemus K, Lazovich D, Anderson K & Folsom AR. Relationship of folate, vitamin B-6, vitamin B-12, and methionine intake to incidence of colorectal cancers. *Nutr Cancer*. 2002, **43**:152-158.

Health Canada (2003). Canadian Perinatal Health Report 2003. Canadian Perinatal Surveillance System. www.hc-sc.gc.ca/pphb-dgspsp/publicat/cphr-rspc03/pdf/cphr-rspc03_e.pdf

He K, Merchant A, Rimm EB, Rosner BA, Stampfer MJ, Willett WC & Ascherio A. Folate, vitamin B6, and B12 intakes in relation to risk of stroke among men. *Stroke* 2004, **35**:169-174.

Herrmann W, Schorr H, Obeid R & Geisel J. Vitamin B-12 status, particularly holotranscobalamin II and methylmalonic acid concentrations, and hyperhomocysteinemia in vegetarians. *Am J Clin Nutr*. 2003, **78**:131-136.

Hirsch S, de la Maza P, Yanez P, Glasinovic A, Petermann M, Barrera G, Gattas V, Escobar E & Bunout D. Hyperhomocysteinemia and endothelial function in young subjects: effects of vitamin supplementation. *Clin Cardiol* 2002, **25**:495-501

Hirsch S, de la Maza P, Barrera G, Gattas V, Petermann M & Bunout D. The Chilean flour folic acid fortification program reduces serum homocysteine levels and masks vitamin B-12 deficiency in elderly people. *J Nutr*. 2002, **132**:289-291

Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta- analysis. *JAMA* 2002, **288**:2015-2022

Honein MA, Paulozzi LJ, Mathews TJ, 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.

Hultdin J, van Guelpen B, Bergh A, Hallmans G & Stattin P. Plasma folate, vitamin B₁₂ and homocysteine and prostate risk: a prospective study. *In J Cancer*. 2004, *accepted for publication*.

Hung J, Beilby JP, Knuiman MW & Divitini M. Folate and vitamin B-12 and risk of fatal cardiovascular disease: cohort study from Busselton, Western Australia. *BMJ* 2003, **326**: 131-136

Husemoen LL, Thomsen TF, Fenger M & Jorgensen AT. Effect of lifestyle factors on plasma total homocysteine concentrations in relation to MTHFR(C677T) genotype. *Eur J Clin Nutr*. 2004, **58**:1142-1150

Information and Statistics Division: Scotland. *Scottish Perinatal and Infant Mortality & Morbidity Report: Information 2002*.

http://www.isdscotland.org/isd/info3.jsp?pContentID=2506&p_applic=CCC&p_service=Content.show&

Jacques PF, Kalmbach R, Bagley PJ, Russo GT, Rogers G, Wilson PW, Rosenberg IH & Selhub J. The relationship between riboflavin and plasma total homocysteine in the Framingham Offspring cohort is influenced by folate status and the C677T transition in the methylenetetrahydrofolate reductase gene. *J Nutr.* 2002, **132**:283-288

Johnson MA, Hawthorne NA, Brackett WR, Fischer JG, Gunter EW, Allen RH & Stabler SP. Hyperhomocysteinemia and vitamin B-12 deficiency in elderly using Title IIIc nutrition services. *Am J Clin Nutr.* 2003, **77**:211-220.

Joosten E, van den Berg A, Riezler R, Naurath HJ, Lindenbaum J, Stabler SP & Allen RH. Metabolic evidence that deficiencies of vitamin B-12 (cobalamin), folate, and vitamin B-6 occur commonly in elderly people. *Am J Clin Nutr.* 1993, **58**:468-476.

Kato I, Dnistrian AM, Schwartz M, Toniolo P, Koenig K, Shore RE, Akhmedkhanov A, Zeleniuch-Jacquotte A & Riboli E. Serum folate, homocysteine and colorectal cancer risk in women: a nested case-control study. *Br J Cancer.* 1999, **79**:1917-1922.

Kinzler WL, Ananth CV & Vintzileos AM. Medical and economic effects of twin gestations. *J Soc Gynecol Investig.* 2000, **7**:321-327.

Kirke PN, Daly LE & Elwood JH. A randomised trial of low dose folic acid to prevent neural tube defects. The Irish Vitamin Study Group. *Arch Dis Child.* 1992, **67**:1442-1446.

Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ & Schouten, EG and the MTHFR studies collaboration group. MTHFR 677 C T polymorphism and risk of coronary heart disease. *JAMA* 2002, **288**: 2023-2031

Konings EJ, Goldbohm RA, Brants HA, Saris WH & van den Brandt PA. Intake of dietary folate vitamers and risk of colorectal carcinoma: results from The Netherlands Cohort Study. *Cancer.* 2002, **95**:1421-1433.

Kuhn W, Roebroek R, Blom H, van Oppenraaij D, Przuntek H, Kretschmer A, Buttner T, Woitalla D, Muller T. Elevated plasma levels of homocysteine in Parkinson's disease. *Eur Neurol.* 1998, **40**:225-227.

Lamers Y, Prinz-Langenohl R, Moser R & Pietrzik K. Supplementation with [6S]-5-methyltetrahydrofolate or folic acid equally reduces plasma total homocysteine concentrations in healthy women. *Am J Clin Nutr.* 2004, **79**:473-478

Lange H, Suryapranata H, De Luca G, Borner C, Dille J, Kallmayer K, Pasalary MN, Scherer E & Dambrink JHE. Folate therapy and in-stent restenosis after coronary stenting. *New Engl J Med.* 2004, **350**:2673-2681

Larsson SC, Giovannucci E & Wolk A. Dietary folate intake and incidence of ovarian cancer: the Swedish Mammography Cohort. *J Natl Cancer Inst.* 2004, **96**:396-402.

Li Z, Gindler J, Wang H, Berry RJ, Li S, Correa A, Zheng JC, Erickson JD & Wang Y. Folic acid supplements during early pregnancy and likelihood of multiple births: a population-based cohort study. *Lancet*. 2003, **361**:380-384.

Lindenbaum J, Heaton EB, Savage DG, Brust JC, Garrett TJ, Podell ER, Marcell PD, Stabler SP & Allen RH. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. *N Engl J Med*. 1988, **318**:1720-1728.

Lindenbaum J, Savage DG, Stabler SP & Allen RH. Diagnosis of cobalamin deficiency: II. Relative sensitivities of serum cobalamin, methylmalonic acid, and total homocysteine concentrations. *Am J Hematol*. 1990, **34**:99-107.

Little J, Sharp L, Duthie S & Narayanan S. Colon cancer and genetic variation in folate metabolism: the clinical bottom line. *J Nutr*. 2003, **133**(suppl):3758S-3766S.

Lloyd-Wright Z, Hvas AM, Moller J, Sanders TA & Nexø E. Holotranscobalamin as an indicator of dietary vitamin B12 deficiency. *Clin Chem*. 2003, **49**:2076-2078.

Malouf M, Grimley Evans J & Areosa SA. Folic acid with or without vitamin B12 for cognition and dementia (Cochrane Review). In: *The Cochrane Library*, Issue 3. 2004. Chichester, UK: John Wiley & Sons, Ltd.

Mangoni AA, Sherwood RA, Swift CG & Jackson SH. Folic acid enhances endothelial function and reduces blood pressure in smokers: a randomized controlled trial. *J Intern Med*. 2002, **252**, 497-503.

Mathews TJ, Honein MA, Erickson JD. Spina bifida and anencephaly prevalence--United States, 1991-2001. *MMWR Recomm Rep*. 2002, **51**:9-11.

McCaddon A, Hudson P, Davies G, Hughes A, Williams JH, Wilkinson C. Homocysteine and cognitive decline in healthy elderly. *Dement Geriatr Cogn Disord*. 2001, **12**:309-313

McKinley MC, McNulty H, McPartlin J, Strain JJ, Pentieva K, Ward M, Weir DG & Scott JM. Low-dose vitamin B-6 lowers fasting plasma homocysteine levels in healthy elderly persons who are folate and riboflavin replete. *Am J Clin Nutr* 2001, **73**, 759-764.

McNulty H, McKinley MC, Wilson B, McPartlin J, Strain JJ, Weir DG & Scott JM. Impaired functioning of thermolabile methylenetetrahydrofolate reductase is dependent on riboflavin status: implications for riboflavin requirements. *Am J Clin Nutr* 2002, **76**: 436-441

Mennen LI, de Courcy GP, Guillard JC, Ducros V, Bertrais S, Nicolas JP, Maurel M, Zarebska M, Favier A, Franchisseur C, Hercberg S & Galan P. Homocysteine, cardiovascular disease risk factors, and habitual diet in the French Supplementation with Antioxidant Vitamins and Minerals Study. *Am J Clin Nutr* 2002, **76**:1279-1289

Mersereau P, Kilker K, Carter H, Fassett E, Williams GJ, Flores A, Prue C, Williams L, Mai C & Mulinare J. CDC Spina Bifida and Anencephaly before and after folic acid mandate – United States, 1995-1996 and 1999-2000. *MMWR*. 2004, **53**:362-365

Michaud DS, Pietinen P, Taylor PR, Virtanen M, Virtamo J & Albanes D. Intakes of fruits and vegetables, carotenoids and vitamins A, E, C in relation to the risk of bladder cancer in the ATBC cohort study. *Br J Cancer*. 2002, **87**:960-5.

Mills JL, Von Kohorn I, Conley MR, Zeller JA, Cox C, Williamson RE & Dufour DR. Low vitamin B-12 concentrations in patients without anemia: the effect of folic acid fortification of grain. *Am J Clin Nutr*. 2003, **77**:1474-1477.

Morrison HI, Schaubel D, Desmeules M & Wigle DT. Serum folate and risk of fatal coronary heart disease. *JAMA* 1996, **275**: 1893-1896

Nurk E, Tell GS, Vollset SE, Nygard O, Refsum H, Nilsen RM & Ueland PM. Changes in lifestyle and plasma total homocysteine: the Hordaland Homocysteine Study. *Am J Clin Nutr*. 2004, **79**:812-819.

Office for National Statistics (updated 2002). Congenital Anomaly Statistics Notifications 2001: England and Wales. <http://www.statistics.gov.uk/STATBASE/xsdataset.asp?More=Y&vlnk=5827&All=Y&B2.x=69&B2.y=10>

O'Grady HL, Leahy A, McCormick PH, Fitzgerald P, Kelly CK & Bouchier-Hayes DJ. Oral folic acid improves endothelial dysfunction in cigarette smokers. *J Surg Res* 2002, **106**:342-345.

Persad VL, Van den Hof MC, Dube JM & Zimmer P. Incidence of open neural tube defects in Nova Scotia after folic acid fortification. *CMAJ*. 2002, **167**:241-245.

Pullin CH, Ashfield-Watt PA, Burr ML, Clark ZE, Lewis MJ, Moat SJ, Newcombe RG, Powers HJ, Whiting JM & McDowell IF. Optimization of dietary folate or low-dose folic acid supplements lower homocysteine but do not enhance endothelial function in healthy adults, irrespective of the methylenetetrahydrofolate reductase (C677T) genotype. *J Am Coll Cardiol* 2001, **38**:1799-1805.

Quadri P, Fragiacomio C, Pezzati R, Zanda E, Forloni G, Tettamanti M & Lucca U. Homocysteine, folate, and vitamin B-12 in mild cognitive impairment, Alzheimer disease, and vascular dementia. *Am J Clin Nutr*. 2004, **80**:114-122.

Quinlivan EP, McPartlin J, McNulty H, Ward M, Strain JJ, Weir DG & Scott JM. Importance of both folic acid and vitamin B12 in reduction of risk of vascular disease. *Lancet* 2002, **359**: 227-228

Ray JG, Meier C, Vermeulen MJ, Boss S, Wyatt PR & Cole DE. Association of neural tube defects and folic acid food fortification in Canada. *Lancet*. 2002, **360**:2047-2048.

Ray JG, Vermeulen MJ, Langman LJ, Boss SC & Cole DE. Persistence of vitamin B12 insufficiency among elderly women after folic acid food fortification. *Clin Biochem*. 2003, **36**:387-391.

Riggs KM, Spiro A, Tucker K & Rush D. Relations of vitamin B-12, vitamin B-6, folate, and homocysteine to cognitive performance in the Normative Aging Study. *Am J Clin Nutr* 1996, **63**:306-314.

Rimm EB, Willett WC, Hu FB, Sampson L, Colditz GA, Manson JE, Hennekens C & Stampfer MJ Folate and vitamin B6 from diet and supplements in relation to risk of coronary heart disease among women. *JAMA* 1998, **279**: 359-364

Ruston D, Henderson L, Gregory J, Bates CJ, Prentice A, Birch M, Swan G & Farron M. The National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 4: Nutritional Status (anthropometry and blood analytes), blood pressure and physical activity, London: TSO, 2004

Schnyder G, Roffi M, Flammer Y, Pin R, & Hess OM. Effect of homocysteine-lowering therapy with folic acid, vitamin B12 and vitamin B6 on clinical outcome after percutaneous coronary intervention. *JAMA* 2002, **288**: 973-979

Schnyder G, Roffi M, Pin R, Flammer Y, Lange H, Eberli FR, Meier B, Turi ZG & Hess OM. Decreased rate of coronary restenosis after lowering of plasma homocysteine levels. *N Engl J Med*. 2001, **345**: 1593-1600

Scott JM & Weir DG. The methyl group trap. *Lancet* 1981, **2**:337-340.

Sellers TA, Kushi LH, Cerhan JR, Vierkant RA, Gapstur SM, Vachon CM & Olson JE, Therneau TM, Folsom AR. Dietary folate intake, alcohol, and risk of breast cancer in a prospective study of postmenopausal women. *Epidemiology*. 2001, **12**:420-428.

Sellers TA, Vierkant RA, Cerhan JR, Gapstur SM, Vachon CM, Olson JE, Pankratz VS, Kushi LH & Folsom AR. Interaction of dietary folate intake, alcohol, and risk of hormone receptor-defined breast cancer in a prospective study of postmenopausal women. *Cancer Epidemiol Biomarkers Prev*. 2002, **11**:1104-1107.

Sellers TA, Grabrick DM, Vierkant RA, Harnack L, Olson JE, Vachon CM & Cerhan JR. Does folate intake decrease risk of postmenopausal breast cancer among women with a family history? *Cancer Causes Control*. 2004, **15**:113-120.

Selhub J, Jacques PF, Wilson PW, Rush D & Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *JAMA*. 1993, **270**:2693-2698.

Selhub J, Bagley LC, Miller J & Rosenberg IH. B vitamins, homocysteine, and neurocognitive function in the elderly. *Am J Clin Nutr*. 2000, **71**(suppl):614S-620S

Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, Wilson PW & Wolf PA. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med*. 2002, **346**:476-483.

Sharp L & Little J. Polymorphisms in Genes Involved in Folate Metabolism and Colorectal Neoplasia: A HuGE Review. *Am J Epidemiol*. 2004, **159**:423-443.

Shaw GM, Carmichael SL, Nelson V, Selvin S & Schaffer DM. Food fortification with folic acid and twinning among California infants. *Am J Med Genet*. 2003, **119A**:137-140.

Shrubsole MJ, Gao YT, Cai Q, Shu XO, Dai Q, Hebert JR, Jin F & Zheng W. MTHFR polymorphisms, dietary folate intake, and breast cancer risk: results from the Shanghai Breast Cancer Study. *Cancer Epidemiol Biomarkers Prev*. 2004 **13**:190-6

Skinner HG, Michaud DS, Giovannucci EL, Rimm EB, Stampfer MJ, Willett WC, Colditz GA & Fuchs CS. A prospective study of folate intake and the risk of pancreatic cancer in men and women. *Am J Epidemiol.* 2004, **160**:248-258.

Stolzenberg-Solomon RZ, Pietinen P, Barrett MJ, Taylor PR, Virtamo J & Albanes D. Dietary and other methyl-group availability factors and pancreatic cancer risk in a cohort of male smokers. *Am J Epidemiol.* 2001, **153**:680-687.

Su LJ & Arab L. Nutritional status of folate and colon cancer risk: evidence from NHANES I epidemiologic follow-up study. *Ann Epidemiol.* 2001, **11**:65-72.

Teunissen CE, Blom AH, Van Boxtel MP, Bosma H, de Bruijn C, Jolles J, Wauters BA, Steinbusch HW & de Vente J. Homocysteine: a marker for cognitive performance? A longitudinal follow-up study. *J Nutr Health Aging.* 2003, **7**:153-159.

Terry P, Jain M, Miller AB, Howe GR & Rohan TE. Dietary intake of folic acid and colorectal cancer risk in a cohort of women. *Int J Cancer.* 2002, **97**:864-867.

The Northern Ireland Child Health System 2001. Northern Ireland Department of Health, Social Services and Public Safety – Personal Communication.

Title LM, Cummings PM, Giddens K, Genest JJ, Jr & Nassar BA. Effect of folic acid and antioxidant vitamins on endothelial dysfunction in patients with coronary artery disease. *J Am Coll Cardiol.* 2000, **36**, 758-765.

Toole JF, Malinow MR, Chambless LE, Spence JD, Pettigrew LC, Howard VJ, Sides EG, Wang CH & Stampfer M. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. *JAMA* 2004, **291**:565-575.

van Dam RM, Huang Z, Giovannucci E, Rimm EB, Hunter DJ, Colditz GA, Stampfer MJ & Willett WC. Diet and basal cell carcinoma of the skin in a prospective cohort of men. *Am J Clin Nutr.* 2000, **71**:135-41.

van Dijk RA, Rauwerda JA, Steyn M, Twisk JW & Stehouwer CD. Long-term homocysteine-lowering treatment with folic acid plus pyridoxine is associated with decreased blood pressure but not with improved brachial artery endothelium-dependent vasodilation or carotid artery stiffness: a 2-year, randomized, placebo-controlled trial. *Arterioscler Thromb Vasc Biol.* 2001, **21**:2072-2079.

van Oort FV, Melse-Boonstra A, Brouwer IA, Clarke R, West CE, Katan MB & Verhoef P. Folic acid and reduction of plasma homocysteine concentrations in older adults: a dose-response study. *Am J Clin Nutr* 2003, **77**:1318-1323

Verhoef P & Katan MB. A healthy lifestyle lowers homocysteine, but should we care? *Am J Clin Nutr.* 2004, **79**:713-714

Verhoef P, Pasman WJ, Van Vliet T, Urgert R & Katan MB. Contribution of caffeine to the homocysteine-raising effect of coffee: a randomized controlled trial in humans. *Am J Clin Nutr* 2002, **76**: 1244-1248

Vermeulen EG, Stehouwer CD, Twisk JW, van den Berg M, de Jong SC, Mackaay AJ, van Campen CM, Visser FC, Jakobs CA, Bulterjis EJ & Rauwerda JA. Effect of homocysteine-lowering treatment with folic acid plus vitamin B6 on progression of subclinical atherosclerosis: a randomized, placebo-controlled trial. *Lancet* 2000, **355**:517-522.

Voorrips LE, Goldbohm RA, Brants HA, van Poppel GA, Sturmans F, Hermus RJ & van den Brandt PA. A prospective cohort study on antioxidant and folate intake and male lung cancer risk. *Cancer Epidemiol Biomarkers Prev.* 2000, **9**:357-65.

Voutilainen S, Rissanen TH, Virtanen J, Lakka TA & Salonen JT. Kuopio Ischemic Heart Disease Risk Factor Study Group. Low dietary folate intake is associated with an excess incidence of acute coronary events. *Circulation* 2001, **103**:2674-2680

Voutilainen S, Virtanen JK, Rissanen TH, Alfthan G, Laukkanen J, Nyyssonen K, Mursu J, Valkonen VP, Tuomainen TP, Kaplan GA & Salonen JT. Serum folate and homocysteine and the incidence of acute coronary events: the Kuopio Ischaemic Heart Disease Risk Factor Study. *Am J Clin Nutr.* 2004, **80**:317-323.

Wald DS, Law M & Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *BMJ.* 2002, **325**:1202-1206

Wald NJ, Law M, & Hoffbrand VA. Vitamin B-12 and folate deficiency in elderly persons. *Am J Clin Nutr.* 2004, **79**:338.

Wang HX, Wahlin A, Basun H, Fastbom J, Winblad B & Fratiglioni L. Vitamin B₁₂ and folate in relation to the development of Alzheimer's disease. *Neurology.* 2001, **56**:1188-1194.

Waller DK, Tita AT & Annegers JF. Rates of twinning before and after fortification of foods in the US with folic acid, Texas, 1996 to 1998. *Paediatr Perinat Epidemiol.* 2003, **17**:378-383.

Ward M, McNulty H, McPartlin J, Strain JJ, Weir DG & Scott JM. Plasma homocysteine, a risk factor for cardiovascular disease is lowered by physiological doses of folic acid. *Q. J. Med.* 1997, **90**:519-524

Ward M, Strain JJ, McPartlin J, Scott JM & McNulty H. Plasma homocysteine is a reliable functional indicator of folate status. *Proc Nutr Soc,* 2002, **61**, 93A.

Wei EK, Giovannucci E, Wu K, Rosner B, Fuchs CS, Willett WC & Colditz GA. Comparison of risk factors for colon and rectal cancer. *Int J Cancer.* 2004, **108**:433-442.

Werler MM, Cragan JD, Wasserman CR, Shaw GM, Erickson JD & Mitchell AA. Multivitamin supplementation and multiple births. *Am J Med Genet.* 1997, **71**:93-96.

Widlansky ME, Gokce N, Keaney JF Jr & Vita JA. The clinical implications of endothelial dysfunction. *J Am Coll Cardiol.* 2003, **42**:1149-1160.

Willems FF, Aengevaeren WR, Boers GH, Blom HJ & Verheugt FW. Coronary endothelial function in hyperhomocysteinemia: improvement after treatment with folic acid and cobalamin in patients with coronary artery disease. *J Am Coll Cardiol.* 2002, **40**, 766-772

Wu K, Helzlsouer KJ, Comstock GW, Hoffman SC, Nadeau MR & Selhub J. A prospective study on folate, B12, and pyridoxal 5'-phosphate (B6) and breast cancer. *Cancer Epidemiol Biomarkers Prev.* 1999, **8**:209-217.

Yuan JM, Stram DO, Arakawa K, Lee HP & Yu MC. Dietary cryptoxanthin and reduced risk of lung cancer: the Singapore Chinese Health Study. *Cancer Epidemiol Biomarkers Prev.* 2003, **12**:890-898.

Zhang SM, Hunter DJ, Rosner BA, Giovannucci EL, Colditz GA, Speizer FE & Willett WC. Intakes of fruits, vegetables, and related nutrients and the risk of non-Hodgkin's lymphoma among women. *Cancer Epidemiol Biomarkers Prev.* 2000, **9**:477-85.

Zhang SM, Willett WC, Selhub J, Hunter DJ, Giovannucci EL, Holmes MD, Colditz GA & Hankinson SE. Plasma folate, vitamin B6, vitamin B12, homocysteine, and risk of breast cancer. *J Natl Cancer Inst.* 2003, **95**:373-380.