PART 2 Potential secondary benefits

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2 Risk reduction of cardiovascular, cancer diseases and of impaired cognitive function

2.1 Cardiovascular disease

1. Folate and cardiovascular disease was considered by the Committee on Medical Aspects of Food and Nutrition Policy (COMA) in 2000 (Department of Health 2000) and updated by the Scientific Advisory Committee on Nutrition (SACN) in 2004 (Department of Health 2004). In 2000, COMA concluded that in the absence of more definitive evidence linking folate directly with cardiovascular disease (CVD) it would not be justifiable to advocate dietary fortification with folic acid solely with the aim of reducing the incidence of CVD. Similarly, the SACN concluded that the evidence was insufficient to draw a causal conclusion from the association of folate and CVD. At present there are a number of large randomised controlled trials testing the hypothesis that folate supplementation will reduce cardiovascular events. These trials need to be completed and data submitted for meta-analysis to estimate more reliably and precisely the effects of folate on cardiovascular disease.

2. The following section of this report will summarise the background of folate and cardiovascular disease and update the SACN information with any key references that were not included in the 2004 report and key references that have been published since the SACN report.

Folate and homocysteine

3. Increased intake of folate effectively lowers total plasma homocysteine concentration (tHcy), and low folate status results in elevated tHcy (Homocysteine Lowering Trialists' Collaboration 1998). Maximal tHcy lowering is observed within the range of 0.4-0.8 mg/day (Wald *et al.* 2001; van Oort *et al.* 2003).

4. There are a number of other modifiable and non-modifiable factors associated with tHcy.

Genetic defects

5. Deficiencies of the principal enzymes in the metabolism of homocysteine predispose to elevated plasma homocysteine levels. The most common form of genetically raised tHcy results from inheritance of a variant of methylene tetrahydrofolate reductase (MTHFR) gene. The C677T mutation in the gene for MTHFR is common with 10% of the Australian population homozygous for the gene and 40% heterozygous (Van Bockxmeer *et al.* 1997). It results in a thermolabile form of the enzyme and a higher plasma level of tHcy among homozygote and heterozygotes with low plasma folate (Jacques *et al.* 1996). The TT polymorphism reduces production of the methyl donor 5-methyl tetra hydrofolate and increases tHcy by about 20% (Engbersen *et al.* 1995; Frosst *et al.* 1995; Klerk *et al.* 2002).

6. Severe deficiency of cystathione beta synthase (CβS) is the classical severe form of homocystinuria (Engbersen *et al.* 1995). It occurs in only 1 in 100,000 live births and results in an increase in tHcy of up to 40-fold. A vascular event such as stroke or myocardial infarction occurs before the age of 30 in about half of the untreated homozygotes (Mudd *et al.* 1985).

Other determinants of homocysteine

7. Homocysteine is also lowered with dietary intake of vitamin B2(Engbersen *et al.* 1995; Frosst *et al.* 1995; Jacques *et al.* 2002; McNulty *et al.* 2002), vitamin B6 and vitamin B12 (Homocysteine Lowering Trialists' Collaboration 1998).

8. Other causes of increased homocysteine include various disease states such as renal failure, hypothyroidism, diabetes mellitus, severe psoriasis, acute lymphoblastic leukaemia, breast cancer, ovarian cancer and pancreatic cancer (Engbersen *et al.* 1995; Frosst *et al.* 1995; Bostom *et al.* 1999; Hankey *et al.* 1999; Ganji *et al.* 2003). Drugs related to homocysteine metabolism are also reported to induce elevated tHcy especially including methotrexate, phenytoin, carbamazepine and thiazide diuretics (Refsum *et al.* 1989; Ueland *et al.* 1989).

9. Plasma levels of homocysteine increase with age (Bree *et al.* 2001; Ganji *et al.* 2003), and are higher in males than females (Lussier-Cacan *et al.* 1996; Ganji *et al.* 2003). Other lifestyle factors such as smoking (Nygard *et al.* 1995; Bree *et al.* 2001; Ganji *et al.* 2003; Nurk *et al.* 2004), physical activity (Nygard *et al.* 1997), consumption of alcohol (Ganji *et al.* 2003) and coffee (Nygard *et al.* 1997; Bree *et al.* 2001; Verhoef *et al.* 2002) have also been reported to have an effect on tHcy in the general population.

Homocysteine and cardiovascular disease

10. The SACN publication reported on two meta-analyses of observational studies concluding 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 MTHFR C677T polymorphism had a significantly higher risk of coronary heart disease, particularly in individuals with low folate status (Klerk *et al.* 2002). Another meta-analysis, which combined both the prospective 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).

11. Since the SACN report another large meta analysis has been published. In the largest meta-analysis to date of studies examining the association between MTHFR and stroke (111 studies), Casas et al (Casas *et al.* 2005) found that people who are homozygous for the MTHFR C677T polymorphism have a significantly greater mean tHcy (weighted mean difference 1.93 μmol/L 95% CI 1.38-2.47), and risk of stroke (odds ratio 1.26, 1.14-1.40) than people who are homozygous for the wild type (CC). The greater risk of stroke conferred by MTHFR-677TT is in proportion to the difference in tHcy that can be attributed to the polymorphism. Furthermore, the estimate of risk obtained from the meta-analysis of genetic association studies is similar to that obtained from previous meta-analyses of non-genetic observational studies (Homocysteine Studies Collaboration 2002; Klerk *et al.* 2002; Wald *et al.* 2002). Because the two types of studies have different sources of error, their consistency supports a causal role for tHcy.

Biological plausibility

12. The biological plausibility was summarised in the SACN report. It has been suggested that elevated tHcy may induce endothelial dysfunction Chambers *et al.*, 2000 - a risk factor for cardiovascular disease (Engbersen *et al.* 1995; Frosst *et al.* 1995; Chambers *et al.* 2000; 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 (Title *et al.* 2000; Doshi *et al.* 2001) and smokers (O'Grady *et al.* 2002), forearm blood flow,, but not arterial elasticity, in smokers (Mangoni 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 not shown any effect on flow mediated dilation (Pullin *et al.* 2001).

Experimental studies and randomised controlled trials of the effects of tHcy-lowering and cardiovascular disease

13. Experimental studies and randomised controlled trials indicate that reducing tHcy with folate produces favourable effects on markers of cardiovascular disease (see Table 1).

Table 1. Summary of experimental studies of the effect of homocysteine-lowering therapy on measures of vascular disease

Study	Population	Intervention	Design	Results
Carotid artery intima thickness				
(Peterson *et al.* 1998)	carotid atherosclerosis; tHcy > 14 µmol/L n=38	FA + B6 + B12	'before-after' study	vitamin therapy associated with reduction in rate of progression of plaque area
(Hackam *et al.* 2000)	carotid atherosclerosis n=101	FA + B6 + B12	'before-after' study	vitamin therapy associated with reduction in rate of progression of plaque area
(Marcucci *et al.* 2003)	renal transplant recipients n=56	FA + B6 + B12 vs placebo	randomised double-blind	vitamin therapy significantly reduced intima-media thickness, significantly increased intima-media thickness in placebo group
Endothelium-dependent vasodilation of forearm vessels				
(Woo *et al.* 1999)	healthy volunteers; tHcy > 75th centile n=17	FA vs placebo	randomised double-blind crossover	folic acid significantly increased endothelium dependent flow
(Bellamy *et al.* 1999)	healthy volunteers, tHcy > 13 µmol/L n=18	FA vs placebo	randomised double-blind crossover	folic acid significantly enhances endothelial dependent vascular function
(Wilmink *et al.* 2000)	healthy volunteers n=20	FA vs placebo	randomised double-blind	folic acid pre-treatment prevented lipid-induced reduction in endothelial dependent vascular function
(Pullin *et al.* 2001)	healthy volunteers n=126	FA vs placebo	randomised double-blind	no change in endothelial-dependent FMD
Exercise electrocardiography				
(Vermuelen *et al.* 2000; Vermeulen *et al.* 2004)	healthy siblings of patients with premature atherothrombotic disease n=167	FA + B6 vs placebo	randomised double-blind	folic acid + B6 reduced rate of abnormal exercise ECG
Coronary artery restenosis and revascularisation				
(Schnyder, *et al.*, 2002)	patients following successful coronary angioplasty n=553	FA + B6 + B12 vs placebo	randomised double-blind	vitamin therapy reduced rate of coronary restenosis
(Lange *et al.* 2004)	patients following successful	FA + B6 + B12 vs	randomised double-blind	vitamin therapy had adverse effects on risk of restenosis

coronary stenting placebo
n=636

FA = Folic acid, B6 = vitamin B6, B12= vitamin B12, RCT = randomised controlled trial
FMD = flow mediated dilation

Serious clinical vascular events

14. The first large intervention trial to report on the effects of folate supplementation on serious clinical vascular events 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 significant 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).

15. The VISP trial evaluated the effect of tHcy lowering by folate, vitamin B6 and vitamin B12 on vascular outcome events such as stroke, heart disease and death. After 2 years of follow-up there was a 2μmol/L (smaller than expected) difference in tHcy between the high-dose and low-dose group, but there was no significant difference in the cumulative incidence of recurrent cerebral infarction (RR 1.0 95% CI: 0.8-1.3), in any coronary event (RR 0.9 95% CI: 0.7-1.2) or death (RR 0.9 95% CI: 0.7-1.1) (Toole *et al.* 2004). Mandatory fortification of foods with folate in North America is likely to have reduced the statistical power of the VISP study. Indeed there was a lower than anticipated rate of recurrent strokes in both study groups which will have lowered the statistical power.

16. The GOES study, a secondary prevention trial comprising 593 patients with coronary heart disease and receiving statin therapy reported no clinical benefit following a 2-year intervention with low dose folate (0.5 mg/day) (Liem *et al.* 2003). The relative risk for all cause mortality and a composite of vascular events was 1.05 (95% CI, 0.63-1.75). This study was underpowered and the dose of folate used may have been too low to demonstrate any beneficial effect other than tHcy lowering.

17. There are at least eight ongoing clinical trials which are testing the homocysteine hypothesis (see Table 2). A number of these studies (WACS and HOPE-2) were initiated before the introduction of mandatory fortification of foods with folate in the US and Canada and will therefore be underpowered to test the hypothesis they were originally designed for. These trials need to be completed and individual data be submitted for meta-analysis to estimate more reliably and precisely the effect of tHcy lowering on the risk of vascular disease.

Table 2. Trials of homocysteine-lowering vitamin supplements in people with prior cardiovascular disease

Trial (Country)	**Fortified population (-/+)**	**Prior disease**	**Scheduled number to be randomized**	**Scheduled duration of treatment (years)**	**Homocysteine-lowering regimen (mg/d)**		
					Folic acid	B12	B6
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

Dose response relationship

18. Wald et al reviewed eight studies of adult participants (mean age range between 40 and 65 years) that reported the effect of specified doses of folate up to 1 mg/day on serum folate. For every 100 μg/day rise in folate, serum folate increased by about 2.5 μg/L (Wald *et al.* 2001). More recently van Oort et al conducted a dose response trial of subjects receiving a daily dose of 50, 100, 200, 400, 600 or 800 μg folate /day and reported similar increases in serum folate (van Oort *et al.* 2003). They calculated the dose response curve for change in tHcy and reported supplementation with folate at 400 μg /day decreased homocysteine by approximately 22%. From the Homocysteine Studies Collaboration a 25% lower than usual tHcy was associated with an 11% (OR, 0.89; 95% CI, 0.83-0.96) lower ischaemic heart disease risk and 19% (OR, 0.81; 95% CI 0.69-0.95) lower stroke risk (The Homocysteine Studies Collaboration).

19. Mandatory fortification with folate in the US at a level of 140 μg/100 g flour is expected to increase the mean intake of folate 70 to 120 μg per day. Even a small increase in intake of folate (as low as 50 to 100 μg /day) is expected to decrease tHcy by approximately 10% and may have a beneficial effect on reducing the incidence of vascular disease (Wald *et al.* 2001; Wald *et al.* 2002; van Oort *et al.* 2003). At the American Heart Association 44th Annual Conference researchers from the Centers for Disease Control estimated that 31,000 stroke-associated deaths and 17,000 deaths related to ischaemic heart disease may have been prevented in the US since mandatory fortification was implemented (Yang *et al.* 2004).

Summary and assessment using FSANZ hierarchy of evidence

20. The features in favour of a causal relationship between tHcy and CVD are that the meta-analyses of prospective observational and genetic studies do not share the same potential sources of error, but all reported highly significant results consistent with a strong, dose-related, independent and biologically plausible association between increasing tHcy (from low folate status) and increasing risk of CVD. It is unlikely that the agreement of results in the genetic studies and prospective studies is due to confounding as the groupings for the genetic studies arise from a genetic mutation effectively allocated at random. Furthermore, randomised controlled trials indicate that reducing tHcy by means of folate supplementation produces favourable effects on multiple surrogate markers of cardiovascular disease.

21. However, there are several features that do not support a causal relationship. There is inconsistency in the results of epidemiological studies obtained by different methods with smaller associations or no association in studies with more rigorous methodological design. The finding of a stronger association in case-control studies than cohort studies suggests that elevated tHcy may be an acute-phase reactant that rises after the vascular event in response to tissue damage or tissue repair (Dudman 1999). In addition at present there is a lack of reliable evidence from randomised controlled trials that lowering tHcy with folate prevents clinical vascular events such as stroke and heart disease. To conclude, there is ***probable evidence*** that increased intake of folate protects against CVD. However the numerous ongoing randomised controlled trials need to be completed and individual data be submitted for meta-analysis before any firm conclusions can be made about a causal relationship between folate and CVD.

2.2 Cancer diseases

Folate and Colorectal Cancer Risk

22. A review of folate and colorectal cancer (CRC) risk was not included in the UK COMA Report (2000). Only findings on folate supplement use and colon cancer from the Nurses' Health Study (Giovannucci *et al.* 1998) were reported. The 2000 report reiterated the 1998 COMA report's (Health 1998) finding of 'insufficient evidence for any specific links between folate intake and the development of cancer'.

23. Studies on folate and CRC risk included in the SACN report are listed in Table 3 below (rows shaded in grey).

24. It is not clear as to how studies were assessed for inclusion in the SACN report, however it appears that only cohort studies or nested case-control studies were considered. Most of those included are large, reasonably well conducted studies that contribute to the body of evidence regarding dietary folate and CRC, with the exception of two studies (Glynn *et al.* 1996; Kato *et al.* 1999) that included a smaller number of cases and questionable sample sizes in their quartile analyses.

25. Most of the studies cited in the SACN report observed a null association between highest level of folate intake and CRC, versus the lowest level intake of folate. However, significant reductions in risk ranging from 18 to 60%, were reported by three major studies. The Nurses' Health Study reported an inverse association between folate intake and colon cancer in women with low methionine intakes (Giovannucci *et al.* 1998) and those with a family history of colon cancer (Fuchs *et al.* 2002). The NHANES I Epidemiology Follow Up Study (Su *et al.* 2001) reported a significantly reduced colon cancer risk for men consuming the highest level of dietary folate. A pooled analysis of the Nurses' Health Study and the Health Professionals' Follow Up Study (Wei *et al.* 2004) reported significant reductions in colon cancer risk for men and women consuming the highest level of total folate. Only one study (the Iowa Womens' Health Study) reported a significant inverse relationship between serum folate and colorectal cancer (Kato *et al.* 1999). All of these studies, with the exception of the pooled analysis (Wei *et al.* 2004), detected a trend in risk reduction across quartiles or quintiles of folate intake or serum folate.

26. To update the UK SACN Report (2004), published studies on dietary and circulating folate and colorectal cancer were identified by searching PubMed using the following MeSH terms; folic acid, folate, colorectal neoplasms, colonic neoplasms, rectal neoplasms. Studies were limited to those conducted on humans and published in English from 1995 onwards. Other original articles were identified by manual searches of reference lists.

27. No new studies suitable for assessing dietary and circulating folate and CRC risk were identified. Some studies cited in the SACN report have since published results stratified according to diet-gene interactions (van Engeland *et al.* 2003; Chen *et al.* 2004; Brink *et al.* 2005), however with the exception of Brink et al., these are not suitable for inclusion in this review. Brink et al., (2005) examined relationships between folate intake and CRC risk according to K-ras tumor mutation

status (and all types combined) in a subsample of the Netherlands Cohort Study (see Table 3, unshaded rows). It should be noted that this analysis utilised considerably fewer cases (430 vs 760 colon; 150 vs 411 rectal) than an earlier analysis on the entire cohort by Konings et al (2002), as the subsample included only those cases and controls for which tumor tissue was available. In the smaller study by Brink et al, no significant relationships between folate intake and colon cancer risk were reported for men (231 cases) or women (199 cases). However, a significant inverse relationship between folate intake and rectal cancer risk was observed in men (99 cases) and conversely; a significantly increased risk of rectal cancer with increasing folate intakes, in women (51 cases). Konings et al., reported similar findings, however the increased rectal cancer risk for women (based on 152 cases) was not statistically significant. Hence, the finding by Brink of an increased rectal cancer risk in women may be due to chance. This study is discussed further in Part 3.

Table 3. 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)	47,951 men Health Professionals Follow-up Study	40-75	6	285 colon	0.86 (0.54 to 1.36) for highest vs. lowest quintile of total folate intake (diet & supps) 0.74 (0.47 to 1.17) for multivitamin use for 10 or more years compared to nonusers	p=0.30 NS	Age, smoking, physical activity, BMI, aspirin use, multivitamin use, total energy, fat, red meat, vitamin C and E, dietary intake, family history of CRC, history of polyps/endoscopy
(Glynn et al 1996)	NHC 89,538 med smokers Alpha-Tocopherol Beta-Carotene cohort	50-69	5.8	430 colon 150 rectal 249 non-cases	0.51 (0.20 to 1.31) for highest vs. lowest quartile of total folate (diet & supps) intake for colon cancer 2.12 (0.43 to 10.54) for highest vs. lowest quartile of total folate intake for rectal cancer 0.99 (0.40 to 2.46) for highest vs. lowest quartile of serum folate concentrations for colon cancer 2.94 (0.84 to 10.33) for highest vs. lowest qrtl of serum folate conc'ns for rectal cancer.	p=0.13 p=0.56 p=0.83 p=0.10	Total energy intake, physical activity, energy adjusted folate intake, vitamins A, C and E, fibre, protein, dairy, cal/d, red meat, alcohol intake, smoking, BMI

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.* 1998)	88,756 women Nurses' Health Study	30-55	15	442 colon	0.48 (0.33 to 0.71) for highest vs. lowest quartile of total folate intake (diet & supps) 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<0.001 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	Mean 62	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 for CRC 0.88 (0.46-1.69) for highest vs. lowest quartile of total folate intake (diet & supps) for CRC	p=0.04 p=0.67	Education, race, religion, physical activity, aspirin use, family, alcohol, smoking, energy, macronutrient, fibre, vitamin A, C and E intakes, Quetelet index
(Su *et al.* 2001)	10,183 general population NHANES I Epidemiology Follow-up Study	25-74	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	p=0.03 p=0.70	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 total folate intake (diet & supps) in women with a family history of colon cancer 0.81 (0.62 to 1.07) for highest vs. lowest quartile of total 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 3123 non cases	0.73 (0.46 to 1.17) for highest vs. lowest quintile of dietary folate intake for colon cancer in men 0.68 (0.39 to 1.20) for highest vs. lowest quintile of dietary folate intake for colon cancer in women 0.66 (0.35 to 1.21) for	p=0.03 p=0.18 p=0.03	Age, energy intake, family history, alcohol, vitamin C, iron and dietary fibre intake

Study (Reference)	Study population	Age range (yrs)	Mean Follow Up (yrs)	No of cases	Adjusted relative risk (95% CI)	Trends	Adjusted for
					highest vs. lowest quintile of dietary folate intake for rectal cancer in men 1.24 (0.58 to 2.70) for highest vs. lowest quintile of dietary folate intake for rectal cancer in women	[illegible] p=0.55	
(Flood *et al.* 2002)	45,264 women Breast Cancer Detection Project Follow-up Study, USA	40-93	8.5	490 colon and rectal	0.86 (0.65 to 1.13) for highest vs. lowest quintile of dietary folate intake for CRC 1.01 (0.75 to 1.35) for highest vs. lowest quintile of total folate intake (diet & supps) for CRC	p=0.14 p=0.67	NSAID use, smoking, education, BMI, physical activity, [illegible]
(Harnack *et al.* 2002)	41,836 women Iowa Women's Health Study	55-69	13	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=0.84	Age, BMI, OC use, smoking, dietary energy, calcium, and vitamin B6
(Terry *et al.* 2002)	NCS 56,837 women Canadian National Breast Screening Study	40-59	[illegible]	[illegible] colon [illegible] rectal 5,179 non-cases	[illegible] for highest vs. lowest quintile of dietary folate intake for colon cancer 0.7 (0.3 to 1.8) for highest vs. lowest quintile of dietary folate intake for rectal cancer	p=0.25 p=0.86	Age, smoking, BMI, physical activity, education, fat and energy intakes
(Wei *et al.*, 2004) (NB: this extends follow up on Giovannucci et al., 1995 and 1998, and Fuchs et al., 2002)	87,733 women in Nurses Health Study and 46,632 men in Health Professionals Followup Study	30-55 40-75	20 14	1,139 colon 269 rectal	0.82 (0.66 to 1.03) for highest vs. lowest quartile of total folate intake (diet & supps) for colon cancer in women 0.72 (0.45 to 1.16) for highest vs. lowest quartile of total folate intake for colon cancer in men 0.82 (0.68 to 0.99) for highest vs. lowest quartile of total 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 [illegible], processed meat, alcohol, calcium, height, smoking, history of endoscopy and gender in combined cohort

Study (Reference)	Study population	Age range (yrs)	Mean Follow Up (yrs)	No of cases	Adjusted relative risk (95% CI)	Trends	Adjusted for
					1.32 (0.86-2.05) for highest vs. lowest quartile of total folate intake for rectal cancer in women 0.67 (0.26-1.72) for highest vs. lowest quartile of total folate intake for rectal cancer in men 1.18 (0.80-1.74) for highest vs. lowest quartile of total folate intake for rectal cancer in men and women	p=0.94 p=0.43 p=0.83	
(Levi *et al.* 2000)	CC Hospital, Switzerland	27-74		223 colon and rectal 491 controls	1.54 (0.8-3.1) for highest vs lowest tertile of dietary folate for colorectal cancer	NS	Age, gender, education, smoking, alcohol, BMI, total energy and fiber, physical activity
(Slattery *et al.* 1997)	CC Multi-centre populations, USA	30-79		1993 colon 2410 controls	1.2 (0.8-1.6) for highest vs lowest quintile of dietary folate intake for colon cancer in men 0.9 (0.6-1.3) for highest vs lowest quintile of dietary folate intake for colon cancer in women	p=0.70 p=0.38	Age, BMI, physical activity, aspirin use, family history, total energy and calcium intake
(Boutron-Ruault *et al.* 1996)	Hospital CC Burgundy, France	30-79		106 colon 65 rectal 309 controls	1.0 (0.5-2.0) for highest vs. lowest tertile of dietary folate intake for colorectal cancer	p=0.95	caloric intake, age, sex, BMI, alcohol, vitamin B6
(Ma *et al.* 1997)	NCC, males Physicians' Health Study	40-84		202 colorectal 326 controls	1.78 (0.93-3.42) for plasma folate < 3ng/mL (deficient folate level) vs plasma folate 3ng/mL (adequate folate level) for colorectal cancer.	NS	Age, smoking, alcohol, multivitamin use, BMI, physical activity, aspirin use
(La Vecchia *et al.* 2002)	CC Hospitals in 6 regions of Italy	20-74		1225 colon 728 rectal	0.65 (0.50-0.85) for highest vs lowest quintile of dietary folate intake for CRC	p<0.01	Age, SES, gender, smoking, alcohol, BMI, physical activity, family

Study (Reference)	Study population	Age range (yrs)	Mean Follow Up (yrs)	No of cases	Adjusted relative risk (95% CI)	Trends	Adjusted for
				4154 controls	in men 0.70 (0.53-0.93) for highest vs lowest quintile of dietary folate intake for CRC in women 0.81 (0.66-1.01) for highest vs lowest quintile of dietary folate intake for colon cancer (sexes comb.) 0.59 (0.45-0.77) for highest vs lowest quintile of dietary folate intake for rectal cancer (sexes comb.)	p<0.01 p<0.03 p<0.01	history, total energy and fiber intakes
(Brink *et al.* 2005)	Netherlands Cohort Study (NCC)	Mean 61	7.3	448 colon 160 rectal	0.87 (0.66-1.14) for 100mg/day increase in folate intake for colon cancer in men 0.98 (0.62-1.56) for 100mg/day increase in folate intake for colon cancer in women 0.58 (0.36-0.93) for 100mg/day increase in folate intake for rectal cancer in men 1.85 (1.13-3.02) for 100mg/day increase in folate intake for rectal cancer in women	N/A	N/A
(Le Marchand *et al.* 2002)	CC	57-74		727 colon and rectal	0.9 (0.6-1.3) for highest vs lowest quartile of dietary folate for colorectal cancer	p=0.43	Age, gender, ethnicity, smoking, alcohol, education, aspirin use, physical activity, family history, BMI, total energy and non starch polysacch. intake from veg, calcium from food and supplements

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

28. Five case-control studies (Boutron-Ruault *et al.* 1996; Slattery *et al.* 1997; Levi *et al.* 2000; La Vecchia *et al.* 2002; Le Marchand *et al.* 2002) and a nested case-control study (Ma *et al.* 1997) not included in the SACN report were identified. These are shown in Table 3 in unshaded rows. Inclusion of two of these studies is questionable owing to poor response rates (Boutron-Ruault *et al.* 1996; Le Marchand *et al.* 2002) and a modest sample size (Boutron-Ruault *et al.* 1996). Only La Vecchia et al reported a significant inverse association between folate intake, colorectal cancer and rectal cancer, with trends in risk reduction across quintiles of folate intake. The remainder

reported null findings.

29. A meta-analysis of folate intake and CRC risk was published in early 2005 (Sanjoaquin *et al.* 2005). This included all of the studies in Table 3 that presented results as relative risks or odds ratios comparing the highest quartile or quintile of folate intake to the lowest, i.e., all studies except Ma et al and Fuchs et al (Ma *et al.* 1997; Fuchs *et al.* 2002) also included one study published before 1995 (Freudenheim *et al.* 1991).

30. Results of the meta-analyses are shown in Table 4. No significant heterogeneity was found between cohort studies, and the summary estimate of CRC risk in cohort studies indicated a 25% reduction in risk for the highest quintile of dietary folate. There was significant heterogeneity between case-control studies reporting dietary folate intake and CRC risk. Meta-regression analysis indicated that within cohort studies, the inverse relationship between dietary folate and risk (Table 4) was stronger for colon than rectal cancer (p=0.03), and marginally stronger than total folate (p=0.06), but was the same for men and women. Among case-control studies, the estimated effects were similar for men and women, type of cancer and source of folate.

Table 4. Summary estimates for folate and colorectal cancer from the meta-analysis by Sanjoaquin *et al.*, 2005:

	Summary Estimate of Relative Risk (95% CI)	
	Dietary Folate	**Total Folate (Diet & Supps)**
Cohort Studies		
Highest quintile of folate intake vs lowest quintile	0.75 (0.64 - 0.89) $\chi2$ = 4.96; 7 df; p=0.67	0.95 (0.81-1.11) $\chi2$ = 4.57; 4 df; p=0.33
Case-Control Studies		
Highest quartile of folate intake vs lowest quintile	0.76 (0.60-0.96) $\chi2$ = 23.10; 9 df; p<0.01	0.81 (0.62-1.05) $\chi2$ = 2.93; 3 df; p=0.50

31. The MTHRF polymorphism (C677T) correlates with reduced MTHRF activity, resulting in reduced plasma folate and increased plasma homocysteine (Ma *et al.* 1997). This leads to the reasonable deduction that 677TT may be a risk factor of colorectal cancer. However the Health Professionals' Follow Up Study (Chen *et al.* 1996), the Physicians' Health Study (Ma *et al.* 1997) and a large, multi-centred US case-control study (Slattery *et al.* 1999) have reported significantly reduced colorectal cancer risks in homozygotes for this polymorphism (677TT) compared to either wild type (677CC) or heterozygotes (677CT) and wild type combined.

32. As mentioned in the SACN report, low folate intake coupled with high alcohol consumption has been associated with a higher risk of colon cancer (Giovannucci *et al.* 1995; Glynn *et al.* 1996; Giovannucci *et al.* 1998; Su *et al.* 2001). In addition, La Vecchia et al, 2002 found a lower risk of CRC in women with high folate and low alcohol intakes. A few studies have examined interactions between the MTHRF-C677T polymorphism, methyl-poor diets (high alcohol-low folate diets) and CRC. The protective effect of homozygosity for MTHRF-677TT seems to be lost when the diet is high in alcohol and low in folate (Chen *et al.* 1996; Ma *et al.* 1997), however this requires confirmation with further research.

Summary and assessment of FSANZ hierarchy of evidence

33. Eighteen published papers were reviewed for this report of which, six reported significant inverse

relationships between dietary or serum folate and CRC, plus trends in risk reduction across increasing levels of folate intake or serum folate (Giovannucci *et al.* 1998; Kato *et al.* 1999; Su *et al.* 2001; Fuchs *et al.* 2002; La Vecchia *et al.* 2002; Wei *et al.* 2004). It is important to note that three of these significant studies (Giovannucci *et al.* 1998; Fuchs *et al.* 2002; Wei *et al.* 2004) are based on data from the very large Nurses' Health Study. Of these, Wei et al (2004) (Wei *et al.* 2004) reports on extended follow up and a pooled analysis of the Nurses' Health Study and the Health Professionals' Follow-Up Study. The Nurses' Health Study was previously reported on by Giovannucci et al (1995 and 1998) (Giovannucci *et al.* 1995; Giovannucci *et al.* 1998) and Fuchs et al (2002)(Fuchs *et al.* 2002). The Nurses' Health Study is a cohort of 88,756 women. This indicates that many of the reviewed studies may have been underpowered to detect a significant relationship between dietary or circulating folate and CRC risk. The misclassification of folate intakes is also likely to have contributed to a lack of detectable differences.

34. One new study on a cohort subsample reported a significantly increased risk of rectal cancer in women with increasing folate intakes (Brink *et al.* 2005), however this is not supported by an analysis of the entire cohort (Konings *et al.* 2002), which indicates that this may be a chance finding due to a smaller sample size. Wei et al (2004) reported a non-significant increase in risk for rectal cancer in women. As the majority of studies report either colon cancer risk or colorectal cancer risk combined, it is not possible at this stage to make a thorough assessment of whether folate intake is protective against rectal cancer or not, particularly in women.

35. The recently published meta-analysis of CRC supports a protective effect from increased folate intakes, and a stronger inverse relationship between folate intake and colon cancer, specifically (Sanjoaquin *et al.* 2005). Only one (La Vecchia *et al.* 2002) of the seventeen studies reviewed reported a significant protective effect from increased folate intake on rectal cancer.

36. No randomised controlled trials have been published, and most of the published results are based on cohort studies.

37. This review, coupled with findings of the meta-analysis, concludes that there is :

- ***probable evidence*** that increasing folic acid intake could reduce the risk of colon cancer, and
- ***insufficient evidence*** that increasing folic acid intake could reduce the risk of rectal cancer.

Folate and Breast Cancer Risk

38. The 2000 COMA report concluded that there was insufficient evidence for any specific links between folate intake and the development of cancer. There was no discussion of a possible association between folic acid intake and breast cancer risk.

39. The 2004 SACN report considered the folic acid – breast cancer association. Table 5 shows details of the studies identified in the 2004 report (rows shaded in grey); studies published since the SACN report (or otherwise not included in it) are presented in the unshaded rows.

40. To update the UK SACN Report (2004), published studies on folate and breast cancer were identified by searching PubMed using the following MeSH terms; folic acid, folate, breast neoplasms. Studies were limited to those conducted on humans and published in English from 1995 onwards. Other original studies were identified in review articles.

Table 5. 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
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	NS	[illegible] MC	[illegible]	[illegible]	[illegible]

(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)	[illegible] Iowa Women's Health Study	[illegible]	[illegible]	[illegible]	1.19 (0.98 to 1.45) for lowest 10th percentile vs. those above the 50th percentile of dietary folate intake 1.40 ([illegible]) for lowest 10th percentile vs. those above the 50th percentile of dietary folate intake in alcohol drinkers with no family history of breast cancer 2.26 (1.59 to 3.21) for lowest 10th percentile vs. those above the 50th percentile of dietary folate intake in women with family history of breast cancer	p = 0.20 p = 0.55 p = 0.00[illegible]	Age, energy intake, education, age at menarche, age at menopause, oral contraceptive use, HRT, parity, age at first birth, BMI, waist-to-hip ratio, [illegible] and physical activity As above + alcohol

(Sellers *et al.* 2002)	34,393 Iowa Women's Health Study	55-69	14	1,875	1.43 (1.02 to 2.02) for lowest 10th percentile of dietary folate intake and > 4g alcohol intake per day vs. non-drinkers above the 50th percentile of dietary folate intake 2.10 (1.18-3.85) for lowest 10th percentile of dietary folate intake and > 4g alcohol intake per day vs. non-drinkers above the 50th percentile of dietary folate intake **for women with oestrogen receptor negative tumours.**		Age, energy intake, methionone 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
(Cho *et al.* 2003)	90,655 Nurses' Health Study II	26-46	8	714	1.03 (0.81-1.32) for highest vs. lowest quintile of folate intake	p=0.95	Age, total energy intake, parity, age at first birth, family history of breast cancer/disease, OCP use, alcohol intake, smoking, BMI, weight gain/loss, age at menarche, height
(Beilby *et al.* 2004)	CC Western Australia	30-84	-	141 109 MC	0.23 (0.09-0.54) for highest vs. lowest quartile of serum folate concentration	p=0.00 1	Age at menarche, parity, alcohol and fat intake, MTHFR C677T genotype.
(Shrubsole *et al.* 2004)	CC China	25-64	-	1144 1236 MC	ORs all ~ 0.5 for highest vs lowest quartile of folate intake; ORs vary slightly by MTHFR genotype.	Overall p<0.05	Age, age at first birth, waist-to-hip ratio, energy intake, meat, physical activity, B6, B12 & methionone intake
(Chen *et al.* 2005)	CC New York	NS	-	1481 1518	0.61 (0.41-0.93) for highest vs. lowest quintile of dietary folate intake **(only in women who did not use supplements)**	p=0.06	Age, total energy intake, family history of breast cancer /disease, BMI
(Hussien *et al.* 2005)	Cross-sectional N Ireland	NS	-	64 30	Non-significant difference in mean red cell folate between breast cancer cases and benign breast disease controls	NS	Crude analysis only.

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

control study.

41. The 2004 SACN report included the assessment of evidence from prospective studies – including cohort and nested case-control studies – only. The reasons for this were not given, but it should be noted that between 1995 and 2003, seven case-control studies (Freudenheim *et al.* 1996; Thorand *et al.* 1998; Potischman *et al.* 1999; Negri *et al.* 2000; Rohan *et al.* 2000; Levi *et al.* 2001; Adzersen 2003) and a case-cohort study (Rohan *et al.* 2000) on the effect of folate on breast cancer risk were published. Two of these studies (Thorand *et al.* 1998; Potischman *et al.* 1999) found no association, while three studies found an overall protective effect (Freudenheim *et al.* 1996; Ronco *et al.* 1999; Adzersen 2003) and three found a protective effect particularly among alcohol drinkers (Negri *et al.* 2000; Rohan *et al.* 2000; Levi *et al.* 2001). These studies have not been added to Table 5.

42. Of the prospective studies of folate intake and breast cancer identified in the 2004 SACN report, two reported null results (Wu *et al.* 1999; Feigelson *et al.* 2003), while three reported a protective effect of folate in alcohol drinkers only (Zhang *et al.* 1999; Sellers *et al.* 2001; Zhang *et al.* 2003). The other study reported a protective effect among women with a family history of breast cancer and in women who drink alcohol.* As discussed in the SACN report, alcohol has been shown to act as a folate (or methyl group) antagonist (Halsted *et al.* 2002). Thus, it is biologically plausible that folate could protect against breast cancer in women at higher risk because of their alcohol intake.

43. Two prospective studies published prior to the SACN report were not included in that report: one from the Iowa Women's Health Study (Sellers *et al.* 2002) and one from the Nurses' Health Cohort II (Cho *et al.* 2003). The details and a summary of results from these two studies have been added to Table 5. Briefly, the latter study reported no association, while the study by Sellers et al. reported an increased risk of breast cancer in women with low folate and high alcohol intake, particularly if they have an oestrogen receptor negative tumour. Note that the table now includes three studies from the Iowa Women's Health Study (by Sellers et al.), which examine various subgroups within the same cohort. The lack of independence of the results of these studies must be taken into account when assessing the weight of evidence.

44. Four studies have been published on the association between folate intake and breast cancer since the SACN report; details of these studies have been added to Table 5 (unshaded rows). These include three case-controls studies (Beilby *et al.* 2004; Shrubsole *et al.* 2004; Chen *et al.* 2005) and a cross-sectional study (Hussien *et al.* 2005). The study by Shrubsole et al. (Shrubsole *et al.* 2004) reported a protective effect of folate in a Chinese population of non-users of alcohol. The Australian study (Beilby *et al.* 2004) also reported a protective effect, but the response among controls was poor, making it difficult to assess the level of bias introduced by likely differences in distribution of SES and other relevant lifestyle factors among cases and controls. The third case-control study found a protective effect only among non-users of supplements, but did not adjust for alcohol consumption even though it was noted that subjects' provision of a blood sample was related to use of alcohol (Chen *et al.* 2005). The cross-sectional study compared red cell folate levels among women with breast cancer and women with benign breast disease (Hussien *et al.* 2005). The analysis was unadjusted, and the difference in geometric means was non-significant.

45. As mentioned in the SACN report, it has been suggested that the MTHFR C677T polymorphism may modify the association between the folate intake and risk of breast cancer, because of its crucial role in the folate metabolic pathway. Accordingly, the most recent studies have examined this putative gene-environment interaction (Beilby *et al.* 2004; Le Marchand *et al.* 2004; Shrubsole

* This category added to Table 5 for current report (Sellers *et al.*, 2004).

et al. 2004; Chen *et al.* 2005). The latter two studies provide some evidence that high folate intake may be more protective in women with the homozygous polymorphism (677TT), while the other two studies show no interactive effects. Further research is required before any firm conclusions can be drawn.

Summary and assessment of FSANZ hierarchy of evidence

46. Since 1995, 20 epidemiological studies have been published on the association between folate intake and risk of breast cancer. Of the eight prospective studies, three were null and five reported a protective effect of folate only among women in higher strata of alcohol intake. Of the remaining 12 studies, two were null, three reported a protective effect of folate only among women in higher strata of alcohol intake, and seven reported an overall protective effect.

47. After taking account of the quality of data provided by these studies, we conclude that there is ***possible evidence*** that high levels of folate intake protect against breast cancer, particularly among women at greater risk because of higher alcohol consumption.

2.3 Cognitive Function

48. Dementia is a common disabling condition of older people. The Australian Institute of Health and Welfare report has estimated that this condition is the second leading cause of years of life lost due to disability. It is the sixth major causes of disability-adjusted-life-years lost in Australia, and on this measure are ranked ahead of asthma and diabetes mellitus (Van Der Weyden 1999- REF). Because of the ageing of the Australian population there is almost certainly going to be a dramatic increase in the numbers affected such that by 2050 the number of people with dementia will rise to over 700,000 (Economics. February 2005)

49. Dementia is a syndrome due to disease of the brain in which there is a disturbance of multiple higher cortical functions including memory and other cognitive processes. For the syndrome of cognitive decline to fulfil the criteria for dementia, this decline must be severe enough to impair personal activities of daily living, and, for a confident clinical diagnosis, this impairment should have been evident for at least six months (Henderson *et al.* January 1998). The prevalence of dementia in older people doubles for every 5.1 years of age (Jorm *et al.* 1987). Three meta-analyses all point to the prevalence rising from approximately 1% in the age group 60 to 64 years to 25% in the age group 85+ years (Henderson *et al.* January 1998).

50. The entity of 'cognitive impairment not dementia' is more common than dementing processes (16.8% versus 8%), but it is not as clearly associated with burden to the affected individuals and their carers (Graham *et al.* 1997). The specific conditions identified within this category of cognitive impairment included delirium, alcohol use, drug intoxication, depression, psychiatric disease, memory impairment associated with the ageing process and mental retardation. Nevertheless, the general conclusion is that the problems of cognitive health in old age extend beyond the issue of dementia.

Common Types of Dementia and Cognitive Impairment in Australia

51. The mixture of types of dementia varies in different societies. In Australia, Alzheimer's disease (AD) is the predominant form of dementia, although vascular dementia (VD) is also common, both by itself and in conjunction with other causes of dementia. Waite *et al,* (Waite *et al.* 1997) found that among a group of community-dwelling elderly people in Sydney 43% of the individuals with dementia had AD, 13% had AD in combination with another disease, another 13% had VD, and 11% had VD mixed with another type of dementia. In patients attending a Memory Clinic in Melbourne, 68% were thought to have AD and 28% vascular dementia (Ames *et al.* 1992). In a

Sydney Memory Clinic 73% were thought to have AD, with 15% having VD and 8% having a combination of AD and VD (Brodaty 1990). An autopsy study in Western Australia found that 45% of patients with dementia had AD, and another 28% had AD with another brain disease (Ojeda *et al.* 1986). Although AD is the commonest cause of dementia in Australia, VD may be the major cause of dementia in Japanese and Chinese residents (Jorm 1991). It is now clear that dementia associated with Lewy Bodies, is also common, that it can be reliably distinguished clinically from AD and that it may account for over 10% of all cases of dementia.

52. The transitional state between normal ageing and mild dementia has been classified by different titles and methodologies. The most recent, popular addition has been mild cognitive impairment (MCI). Originally described by Petersen et al, (Petersen 1999), criteria included:

    - A concern about the patient's memory, expressed by either the patient, their family, or the patient's physician
    - Normal activities of daily living
    - Normal general cognitive function
    - Abnormal memory for age, or other specific cognitive domain (Petersen 2001)
    - Not demented

53. One of the major reasons given for development of this nosological entity was so as to be able to efficiently identify older people who were at greater risk for the development of dementia and thus allow the examination of interventions which may prevent the development of dementia. It is apparent that clinic based populations are more likely to demonstrate higher conversion rates to dementia than those studies which used population based sampling techniques. A systematic review (Bruscoli 2004) which examined 19 studies reported between 1991 and 2001, found that the conversion rate for clinic attendees was twice that of community living volunteers, 15% versus 7.5% respectively. Given that community volunteers are self selected, the true rate for general community subjects may even be lower.

54. Although the purpose of identification of MCI was to allow testing of dementia prevention strategies, no such interventions have been successfully identified. As dementia is a major health issue for older people, the eventual targeting of such interventions only for older people with MCI, would suggest that these strategies would have to be either costly, or marred by side effects, because otherwise a universal public health approach would be indicated. Also, the prospects of harming older people are significant, by labeling them with a diagnostic entity, with as yet no proven interventions. This labeling may precipitate adverse psychological consequences as well as raising medico-legal issues such as casting doubt on their capacity to perform complex functional activities, e.g driving. There is, as yet, no consensus on how to operationalise the criteria to maximize the validity as a dementia pre-syndrome.

Types of Dementia - Categorical or Dimensional Diagnosis

55. Until recently there have been considered to be two major forms of dementia - AD and VD. It is now understood that, in older people, these diagnoses are by no means clear cut. In a representative sample of 85 year olds in Sweden, infarcts were more common in people with dementia than people with normal cognition and there were more subjects with VD than with AD (Skoog *et al.* 1993). Many people with dementia have some elements of both AD and VD. Those who have strokes are more likely to develop dementia if they have Alzheimer pathology before the stroke, and many people with AD also have damage due to disease of small vessels, which appears on neuro-imaging as lucencies in the white matter (leukoariosis). The presence of cerebral atrophy also predicts the risk of dementia following lacunar infarction (Loeb *et al.* 1992). Certainly the two

common types of dementia are not mutually exclusive; patients may have either predominantly Alzheimer pathology with some vascular damage, or predominantly vascular dementia with some evidence of Alzheimer pathology. This is hardly surprising as both processes are associated with advancing age, and older individuals are therefore clearly at risk for both cerebrovascular disease and AD. Indeed, vascular damage may be predisposed by Alzheimer type pathology.

56. Ageing is the most important risk factor for AD. As individuals age, some cognitive abilities decline (Laursen 1997- REF). This is particularly marked for tasks involving reaction time and memory processing. It is unclear whether these changes form a continuum with the clinical presentation of people with AD. Cerebral microinfarcts are also a frequent accompaniment of ageing. Whether these microinfarcts produce significant cognitive impairment is debatable. AD and VD may share other risk factors in addition to ageing such as systolic blood pressure. Raised midlife systolic BP has been shown to be associated with cognitive decline, decreased brain volume, and increased white matter hyperintensities. Risk factors that are common to both conditions and that are remediable are obvious targets for the development of preventive strategies.

Risk factors for dementia

57. Proven risk factors for AD include ageing, family history of AD, Down syndrome and the E4 genotype of apolipoprotein E. Taken together, the known genetic mutations account for less than 5% of all cases. The presence of the E4 allele of the apolipoprotein E (apoE), on the other hand, has been associated with the common sporadic and late-onset dementias (Anonymous. 1996). Other possible risk factors include head trauma, exposure to aluminium, history of depression, fingerprint patterns, hypothyroidism, occupational exposure to electromagnetic radiation, lack of physical activity, poor educational achievement, lack of ongoing intellectual or social stimulation, obesity, lack of moderate alcohol intake, hypertension, diabetes mellitus and smoking. Risk factors for VD include old age, hypertension, family history, diabetes mellitus, hypercholesterolaemia and smoking.

Are Low Folate and High Homocysteine Levels Risk Factors for Dementia?

58. There is some experimental work that hyperhomocysteinemia actually produces cognitive dysfunction. For example, rats treated with parenteral homocysteine for 3 weeks early in life still demonstrated poor cognitive function in experimental tasks at the age of 60 days (Engbersen et al. 1995; Frosst et al. 1995; Streck et al. 2004). Published human studies identified by PubMED searches using the following MESH terms, folate folic acid, homocysteine, cognition, cognitive disorders and dementia, English language from 1995.

Cross-Sectional Studies

59. Initially, an inverse correlation was found between spatial copying skills and Hcy in 68 subjects (Riggs *et al.* 1996). Another group reported elevated Hcy levels in a general psychogeriatric population, affecting both demented and non-demented patients, the latter group mainly suffering from depression (Nilsson *et al.* 1996). Since then there have been numerous studies. Studies that have found an association for high Hcy or low folate with dementia and cognitive impairment include (Prins *et al.* 2002; Miller *et al.* 2003; Nagga *et al.* 2003; Sachdev *et al.* 2003; Almeida *et al.* 2004; Engelborghs *et al.* 2004; Garcia *et al.* 2004; Quadri *et al.* 2004; Ravaglia *et al.* 2004; Wright *et al.* 2004; Adunsky 2005; Aleman *et al.* 2005; Schafer *et al.* 2005). Studies that have failed to find a statistically significant association include Ariogul (Ariogul *et al.* 2005), Bunce et al (Teunissen *et al.* 2003; Bunce *et al.* 2004), Teunissen *et al* (Teunissen *et al.* 2003) and Bunce et al.(Bunce *et al.* 2004; Ariogul *et al.* 2005; Bunce *et al.* 2005)

Case Control Studies

60. In a small case-control study (50 subjects per group) (Joosten *et al.* 1997) demonstrated significant differences in Hcy in AD patients compared to hospital controls despite the hospital controls having a large number of patients with vascular disease. Similarly, another small case-control study (30 subjects per group) (McCaddon *et al.* 1998) demonstrated significant differences between AD patients and community controls in B12, folate and HC. In the landmark optimally designed case-control study (Clarke *et al.* 1998) showed that serum Hcy levels were significantly higher, and folate and B12 lower, in patients with dementia of Alzheimer's type and in patients with confirmed AD. The odds ratio of confirmed AD associated with a HC level in the top tertile compared with bottom tertile was 4.5 [95% CI: 2.2, 9.2]. Similar calculations yielded an odds ratio (OR) of 3.3 [1.8, 6.3] for serum folate and an odds ratio of 4.3 [2.1, 8.8] for serum B12. The lack of a relationship with duration of symptoms argues against this being a consequence of poor nutrition secondary to disordered eating habits with increasing cognitive impairment. These small positive studies contrast with the nested case control studies from the Rotterdam Study (Kalmijn *et al.* 1999). Although there appeared to be an initial negative correlation between baseline cognitive score (MMSE) and homocysteine, OR for highest versus lowest tertile 1.7 [0.70, 4.15], this was not present after adjustment for age, sex and education. Having defined those people with cognitive decline as having more than 1 point annual decline on MMSE, there was no apparent difference between those subjects who declined and the controls, although the subjects with greater cognitive impairment were more likely to be lost to follow-up.

61. Since then five case control studies have found an inverse association between elevated levels of Hcy and cognitive impairment or dementia (McIlroy *et al.* 2002; McCaddon *et al.* 2003; Religa *et al.* 2003; Mizrahi *et al.* 2004; Ravaglia *et al.* 2004). The one study with a statistically non-significant association was small, 43 AD patients and 37 control subjects (Miller *et al.* 2002). The odds ratio in that study for elevated plasma Hcy was 2.2 (95% CI; 0.31, 16)

Cohort Studies

62. The arguments for reverse causality in this area are very strong, ie that as older people become more cognitively impaired their eating habits may alter, their general nutrition may deteriorate and this may specifically affect micronutrients. For this reason cohort studies of normal older subjects with extended periods of follow-up from baseline assessment are desirable. The question of whether Hcy levels "track" i.e. baseline levels correlate strongly with follow-up levels is unclear. A summary of the available cohort studies is presented in Table 6.

63. These data suggest an increased risk of accelerated cognitive impairment, leading to dementia and AD. The data that are available suggest that this is independent of obvious vascular damage to the brain, eg in the study by (Dufouil *et al.* 2003) adjusting for number of white matter intensities seen on magnetic resonance imaging (a marker of vascular damage to the brain) did not substantially alter the effect of Hcy.

Randomised Trials and Systematic Reviews

64. A systematic review has been performed summarizing the four available randomised controlled trials of folate. One trial enrolled healthy women, and the other three recruited people with mild to moderate cognitive impairment or dementia (Malouf *et al.* 2003). The trials were unable to be pooled and there was no evidence of benefit but the trials were small and short term. For example, in the most recent trial available. 149 subjects with MCI or dementia were randomised using a 2 x 2 x 2 factorial design to receive aspirin, vitamin E and B vitamins or matching placebos Vital Trial Collaborative Group 2003. Treatment duration was for 12 weeks. No effect of treatment was found for cognitive outcomes although Hcy was lowered in the B vitamin group.

Summary and assessment of FSANZ hierarchy of evidence

65. Although there has been a massive increase in observational data suggesting an association between low folate levels and high Hcy levels and the presence of cognitive decline, dementia and AD, there is no current evidence of an effect of Hcy lowering strategies in reducing cognitive decline. For the above reasons there is only ***possible evidence*** of an association between folate and risk of cognitive decline.

Table 6. Cohort studies of association of circulating folate and homocysteine concentrations with risk of dementia and cognitive impairment

Study Reference	Study Population	Hcy or Folate	Outcome	Age	Mean follow-up (yrs)	No of cases	Adjusted relative risk (95% CI)	Adjusted for
(McCaddon *et al.* 2001)	23	Hcy	Cognitive decline	74	5 years	N/A	Hcy level predicted the rate of change in MMSE and ADAS-Cog Scores	Age, sex, education, renal function, vitamin B status smoking and hypertension,
(Wang *et al.* 2001)	370	Folate	Incident dementia And AD	Over 75 years	3 years	78 dementia 60 AD	< 10 nmol/l vs > 10 nmol/l Folate level Dementia Unadjusted RR 1.7 (1.0, 3.0) Adjusted 1.6 (0.9, 2.9) AD Unadjusted RR 1.8 (1.0-3.4) Adjusted RR 1.7 (0.9, 3.2)	Age, sex and education
(Seshadri *et al.* 2002)	1092 Subjects	Both	Incident dementia and AD	Mean 76 ± 6 ±	8 years (median)	111 dementia 83 AD	Dementia RR 1.4 (CI: 1.1, 1.9) per SD log adjusted Hcy AD RR 1.8 (CI: 1.3, 2.5) per SD log adjusted Hcy For Folate data not shown but stated not independently related to the risk of dementia	Age, sex and ApoE ε4.
(Teunissen *et al.* 2003)	144 subjects	Both	Cognitive decline	Mean 55	6 years	N/A	Hcy or folate did not correlate	Age, sex and education

Study Reference	Study Population	Hcy or Folate	Outcome	Age	Mean follow-up (yrs)	No of cases	Adjusted relative risk (95% CI)	Adjusted for
				years			with change in cognition	
(Nilsson *et al.* 2003)	535 subjects	Hcy	Dementia	Mean 85	6 years	?	Not given	Not reported
(Luchsinger *et al.* 2004)	909 cross-sectionally 128 had prevalent dementia 679 followed longitudinal y	Hcy	Incident AD	Mean 77.2 + 6.3	679 people followed for 3206 person years	109 incident cases	Cross-sectional Baseline Unadjusted OR 2.0 (CI: 1.2, 3.4) Adjusted OR 1.3 (CI: 0.7 2.3) Longitudinal Hazard` Ratio (Highest quartile to lowest) 2.0 (CI 1.2, 3.5) Adjusted 1.4 (CI0.8, 2.4)	Age, sex, education, ApoE ε4.
(Dufouil *et al.* 2003)	1241 subjects	Both but only Hcy results presented	Cognitive decline as determined by MMSE score decrease of 3 points or more	Mean 67.o + 3.0	2 years	134 (10.7%) subjects demonstrated cognitive decline	Compared > 15 µmol/l to the group < 10 µmol/l Unadjusted OR 2.8 (1.3, 6.0) Adjusted OR 2.8 (1.2, 6.2)	Age, sex, education, baseline cognition, BMI, alcohol, smoking, hypertension, hypercholesterolemia, glycemic status, history of vascular disease, folate and B12 levels
(Kado *et al.* 2005)	499 Subjects	Both	Cognitive decline	74.3 + 2.7	5 years	N/A	Cognitive decline determined by greatest quartile vs the rest Folate Compared the bottom quartile of folate to rest Adjusted RR 1.71 (1.13, 2.37) Hcy	Age, sex, education, baseline cognitive function, baseline physical function, smoking

Study Reference	Study Population	Hcy or Folate	Outcome	Age	Mean follow-up (yrs)	No of cases	Adjusted relative risk (95% CI)	Adjusted for
							Compared the greatest quartile to the rest Adjusted RR 1.44 (0.91, 2.09)	

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