Scientific Advisory Committee on Nutrition

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Draft - Update on folic acid

Scientific consultation: 16 February to 2 March 2017

Contents

1) Introduction

Purpose

1. This report has been prepared in response to a request by Food Standards Scotland (FSS) for the Scientific Advisory Committee on Nutrition (SACN) to provide advice on whether its previous recommendations regarding mandatory folic acid fortification (2006; 2009) still apply. This report updates the previous reviews of potential adverse effects of folic acid on specific health outcomes and its recommendations on folic acid fortification (2006; 2009).

Folate and folic acid

2. The term *folate* describes a family of B-group vitamins. There are large numbers of naturally occurring folates that are found in a wide variety of foods. Rich sources include liver, yeast extract and green leafy vegetables (e.g., spinach and kale).

3. Folic acid is a synthetic form of folate. It is widely used for food fortification and in supplements because it is more stable in foods than natural folates and is better absorbed. In the UK, fortification of food products with folic acid is on a voluntary basis. Foods which are voluntarily fortified with folic acid include breakfast cereals and reduced fat spreads.

4. Although folates occur naturally in a wide range of foods, they are present in relatively low amounts. This means that it is hard to achieve high folate intakes from consumption of naturally occurring folates alone. In addition, the absorption of naturally occurring folates is approximately 50% lower than that of folic acid.

Folate and neural tube defects

5. Conclusive evidence from randomised controlled trials (RCTs) has shown that folic acid supplementation during the early stages of pregnancy can reduce the risk of the fetus developing neural tube defects (NTDs) (Czeizel & Dudas, 1992; MRC Vitamin Study Research Group, 1991). The two most common NTDs are anencephaly and spina bifida.

6. All women who could become pregnant are therefore advised to take a daily supplement of folic acid (400 µg) prior to conception and until the 12th week of pregnancy (Department of Health, 1992). Folic acid supplements of 5 mg/d are recommended to prevent the occurrence of NTDs in the offspring of men or women with spina bifida themselves or women with a previous pregnancy affected by NTD (Department of Health, 1992). Diabetes UK recommends that all women with diabetes planning a pregnancy should also take a folic acid supplement of 5mg/d[1].

7. Findings from a large cross-sectional UK study of women (n=466,860) attending an antenatal screening for Down syndrome and NTDs (between 1999 and 2012) suggest that the current recommendations are not being followed (Bestwick et al., 2014). The proportion of women taking folic acid supplements prior to pregnancy declined from 35% in 1999-2001 to 31% in 2011-12; only 6% of women aged under 20y took folic acid supplements before pregnancy compared with 40% aged 35-39y. In addition, it has been estimated that only 55% of pregnancies in Britain are planned (Wellings et al., 2013).

[1] https://www.diabetes.org.uk/Guide-to-diabetes/Living_with_diabetes/Pregnancy/

8. Significant reductions in the birth prevalence of NTDs have been reported in countries with mandatory folic acid fortification policies. For example in the USA, where mandatory folic acid fortification was introduced in 1998, an immediate and stable 28% reduction in prevalence was observed for anencephaly and spina bifida post-fortification (1999-2011) (Williams et al., 2015). Internationally, a systematic review (179 studies) and meta-analysis (123 studies) reported that prevalence of spina bifida (1985-2010) was lower in geographical regions with mandatory (35 per 100,000 live births, stillbirths and terminations of pregnancy) versus voluntary (52 per 100,000 live births, stillbirths and terminations of pregnancy) fortification (Atta et al., 2016). This shows that the policy of mandatory folic acid fortification has been effective in reducing NTD risk in those countries where it has been introduced.

Background

9. In 2000, the Committee on Medical Aspects of Food and Nutrition Policy concluded that universal fortification of flour with folic acid would significantly reduce the number of conceptions and births affected by NTDs (Department of Health, 2000). In July 2002, the Food Standards Agency (FSA) advised Health Ministers not to introduce mandatory fortification at that time, due to outstanding concerns that folic acid might mask the symptoms of vitamin B12 deficiency in older people.

10. In 2006, SACN recommended mandatory fortification of flour with folic acid to improve the folate status of women most at risk of NTD-affected pregnancies in the UK (SACN, 2006). However, it recognised that mandatory fortification combined with voluntary fortification and inappropriate supplement use would also increase the proportion of people in the UK at risk of exceeding folic acid intakes above the Guidance/Tolerable Upper Intake Level (GL/UL)[2] of 1 mg/day. SACN stipulated, therefore, that mandatory fortification should only be introduced alongside restrictions on voluntary fortification of foods with folic acid. This was to ensure no increase in the numbers of people with an intake of folic acid above the GL/UL and no substantial increase in the mean intake or folate status of the UK population.

11. In June 2007, based on SACN's advice, the FSA recommended mandatory fortification of wheat flour with folic acid, alongside controls on voluntary fortification and guidance on supplement use, to UK Health Ministers.

12. In October 2007, the Chief Medical Officer requested SACN to consider two studies (Cole et al., 2007; Mason et al., 2007) suggesting potential adverse effects of high folic acid intake on colorectal cancer risk. Preliminary data from both studies had been considered in the SACN (2006) report which concluded that the relationship between folic acid and increased or reduced cancer risk was unclear. As a precaution, SACN had recommended that there should not be a substantial increase in the average population intake of folic acid or in the numbers consuming intakes above the GL/UL for folic acid.

[2] In the UK, the Expert Group on Vitamins and Minerals (2003) set a GL of 1 mg/d of folic acid for adults. GLs are based on limited data and provide an approximate indication of intakes that would not be expected to cause adverse effects. There was insufficient evidence for adverse effects of folic acid to establish a safe upper level which represents the amount of a nutrient that can consumed over a lifetime without significant risk to health. In the USA and Europe, a UL of 1 mg/d of folic acid was set for adults (Food and Nutrition Board, 1998; Scientific Committee on Food, 2000). The UL represents the highest level of a nutrient that is likely to pose no risk of adverse health effects for almost all individuals in the general population.

13. The main studies considered in SACN's further assessment of the evidence on folic acid and cancer were: Cole et al. (2007); Mason et al. (2007); and results of a then unpublished[3] meta-analysis of RCTs on the effects of B vitamins (including folic acid) on risk of cardiovascular disease, which also reported on cancer risk. This meta-analysis and the study by Cole et al. (2007) were also considered by the Committee on Carcinogenicity[4] together with a paper (Figueiredo et al., 2009) reporting secondary findings from the study by Cole et al. (2007).

14. Following a detailed consideration of the above data, SACN concluded that there were still uncertainties regarding folic acid and cancer risk. Its 2006 recommendation had taken these uncertainties into account by minimising exposure to high intakes of folic acid. SACN reiterated its previous recommendation for mandatory fortification of flour with folic acid but stipulated that it should only be introduced if it was accompanied by controls on voluntary fortification, guidance on supplement use and appropriate population monitoring. In addition, people aged over 50y and those with a previous history of colorectal adenomas were advised not to consume supplements containing folic acid above the recommended nutrient intake for folate of 200 µg/d (SACN, 2009).

15. In October 2015, SACN wrote to UK Health Ministers to alert them to evidence from other countries on the safety of folic acid fortification at the levels proposed in 2006 and the associated improvements in folate status and falls in the prevalence of NTDs. SACN also expressed concern that, in the absence of mandatory fortification, the low folate status of women of reproductive age in the UK may have been exacerbated by reduced levels of voluntary fortification by some sectors of the food industry in compliance with SACN's 2006 and 2009 recommendations.

16. In February 2016, FSS informed SACN that in the absence of any progress regarding mandatory folic acid fortification in the UK, Scottish Ministers had indicated a wish to proceed unilaterally with mandatory folic acid fortification of flour in Scotland. FSS was, therefore, requesting advice from SACN on whether its 2006/2009 risk assessments remained valid.

17. In addition to the request from FSS, SACN had received submissions expressing concern about potential risks of folic acid fortification.

18. In this context, SACN agreed to conduct a review of relevant evidence published since its 2006/2009 risk assessments.

Terms of reference

19. A small group of SACN Members, with relevant expertise, was established in April 2016 to take this work forward. The terms of reference were to:

    - Conduct a review of major new evidence (with an emphasis on systematic reviews and meta-analyses) published since SACN's 2006 and 2009 reports on folate and folic acid, to assess whether its previous advice stands.
    - Provide comments in relation to the modelling exercise carried out by the FSA for SACN's 2006 report and any updated modelling exercise undertaken by FSS.

[3] Subsequently published: Vollset et al (2013).

[4] The Committee on Carcinogenicity provides independent advice to government departments and agencies on the potential carcinogenicity of chemicals used in pesticides, pharmaceuticals and other products.

2) Methodology

20. Since SACN's full risk assessment in 2006, five reports on folic acid have been published by the following organisations: European Food Safety Authority (EFSA) (2014), the Food and Drug Administration (FDA) (2016), Food Safety Authority of Ireland (FSAI) (2016), National Toxicology Program (NTP) (2015); and the Norwegian Scientific Committee for Food Safety (VKM) (2015). These reports were considered for their potential utility as a reference resource and basis for this review. Although the reports provided useful background and context, none were considered to be sufficiently comprehensive to serve as an alternative to undertaking SACN's current review. A summary of their key findings and conclusions is provided in Annex 1.

21. In this report, SACN's review of the evidence was restricted to potential adverse effects of folic acid which had been identified as areas of concern in SACN's previous risk assessments, in other reports looking at potential adverse effects associated with folic acid and in submissions to SACN. These were: masking or exacerbation of low vitamin B12 status; cognitive decline in older individuals; and cancer (prostate, breast, colorectal and overall). In addition, the appearance of unmetabolised folic acid (UMFA) in the systemic circulation has raised concerns regarding long-term effects of exposure to folic acid. Therefore, evidence on potential adverse effects of UMFA was also considered.

22. In order to complete the review in a timely fashion, only systematic reviews and meta-analyses were considered. For most of the health outcomes considered, several meta-analyses were identified (summarised in Tables 1-21, Annex 2). In general, if a number of systematic reviews/meta-analyses address the same health outcome then, dependent on the search inclusion criteria, they would be expected to include many of the same individual studies (published up to that time). The most recent meta-analysis would therefore be expected to include the largest number of studies and cases of disease and, consequently, provide the most reliable summary of the evidence.

23. Individual studies were considered in relation to evidence concerning UMFA (summarised in Tables 22-25, Annex 2) because the evidence base was less extensive than for the potential adverse health outcomes under consideration.

24. In assessing the evidence, effects or associations in studies were judged to be statistically significant if the results reported a p-value <0.05 or a confidence interval that excluded a null effect.

25. Details of the literature search are provided in Annex 3 and the individual studies included in the meta-analyses are provided in Annex 4.

3) Folate metabolism

26. Folates comprise an aromatic pteridine ring linked to p-aminobenzoic acid and one or more glutamate residues. Naturally-occurring folates are broken down in the gut by folate conjugase in the mucosal brush borders from folate polyglutamates to the monoglutamate form. Upon absorption, the monoglutamates are converted in the mucosal cells to 5-methyl tetrahydrofolic acid (5-MTHF), which is usually the only form found in plasma. 5-MTHF is taken up by cells but cannot be retained intracellularly unless it is metabolised to tetrahydrofolate (THF). THF can be conjugated into polyglutamate forms, which prevents it leaving the cell.

27. Folic acid is not conjugated and is therefore better absorbed. Before it can become active, folic acid has to be reduced to THF and methylated. The reduction is catalysed by dihydrofolate reductase (DHFR). Reduction of folic acid by DHFR is the rate limiting step in the biotransformation of folic acid to 5-MTHF and activity of this enzyme is low in human tissue. The majority of a physiological dose of folic acid is transported in the portal vein to the liver, which is the main site of metabolism in humans (Patanwala et al., 2014). Low activity of DHFR in the gut mucosa and the liver may explain the appearance of unmetabolised folic acid in the systemic circulation following consumption of folic acid in food and supplements (Kelly et al., 1997; Bailey et al., 2010). The implications for health, of the presence of unmetabolised folic acid in the systemic circulation, are considered in section 4 (paragraphs 93-114).

28. For a detailed review of folate metabolism, see Tibbetts & Appling (2010).

4) Potential adverse effects of folic acid fortification

29. The purpose of reviewing the evidence for potential adverse effects of folic acid on specific health outcomes was to assess whether there was any new evidence that might cause SACN to alter its previous advice (2006; 2009) regarding mandatory folic acid fortification.

30. The range of evidence considered included intervention studies of folic acid supplementation (including those that had considered folic acid together with other B vitamins or multivitamins) and observational studies of dietary folate intake (from foods), total folate intake (diet and supplements) and folate status. Although FSS's request for advice related to folic acid as the proposed fortificant, prospective studies of dietary and total folate intake were also considered because the biological effect is likely to depend on the total intake of natural and synthetic folates rather than folic acid *per se.*

31. In addition to the general confounders in studies of diet and disease (including, for example, smoking, alcohol intake, physical activity levels), a number of other factors need to be considered in the assessment of evidence on folate intakes/folate status and health outcomes. In studies of folate status, serum or plasma folate concentrations may not be comparable across different studies because of significant analytical variation between the different assay methods and between different laboratories using similar methods (see SACN 2006 report). Another important consideration is that serum or plasma folate concentrations reflect recent dietary intake rather than long term folate status which is better reflected by red cell folate concentration. Serum or plasma folate concentration is also influenced by genetic variation

(see below). In studies of dietary folate intake, estimated intakes based on different dietary assessment methods and different nutrient databases may not be comparable.

Genetic association studies

32. Meta-analyses of genetic association studies were also considered. In recent years there has been an increase in the use of genetic association to infer nutritional effects on health. There was some consideration of this type of genetic evidence in SACN's 2006 assessment, however very few large studies were available at that time. The value of these studies is that they are not subject to reverse causality and are less prone to potential confounding, both of which limit the causal interpretation of traditional observational studies of nutrient intake and status. They have been likened to '*natural*' RCTs (with genotype corresponding to the dietary intervention) though with the advantage that the numbers of individuals studied are typically much larger and the exposure time is much longer (lifelong) than can be achieved in an RCT. However, there are issues that may affect the interpretation of genetic association studies and these are considered below (see paragraphs 35-37).

33. The basis for genetic association studies is that an observed association between a polymorphism and a disease state or other trait is assumed to arise because the polymorphism has a causal role in determining the trait. This logic may be extended to the assumption that the pathway or process in which the gene is involved is causal and that the nutritional and environmental factors which influence the pathway or process may influence the disease state or trait. This approach has been termed '*mendelian randomisation*' (Davey Smith et al., 2005). The principle of mendelian randomisation is that "*if genetic variants either alter the level of or mirror the biological effects of a modifiable environmental exposure that itself alters disease risk, then these genetic variants should be related to disease risk to the extent predicted by their effect on exposure to the risk factor*" (Davey Smith et al., 2005).

34. The enzyme methylenetetrahydrofolate reductase (MTHFR) converts 5,10-methylene-THF to 5-MTHF (see paragraph 26). A relatively common polymorphism (677C→T; rs1801133) of the gene encoding MTHFR, involving a C to T substitution at base 677 of the gene, produces an enzyme with lower folate-processing capacity. Individuals with the TT genotype have significantly lower blood folate concentrations for the same dietary intake of folate. A meta-analysis (Tsang et al., 2015) estimated that, compared to CC, the TT genotype confers a 16% lower red blood cell folate and a 13% lower serum (or plasma) folate measured using a microbiological assay and a serum (or plasma) difference of 20% when assessed using protein-binding assays. These are substantial effects and this genotype has been exploited to infer the effect of folate on a wide range of health outcomes. The validity of this genetic approach is supported by health outcomes such as NTD where it is known that the condition is influenced by low dietary folate intake and that mothers or infants with the TT genotype have significantly greater risk of an NTD–affected pregnancy than those with the CC genotype (Yan et al., 2012; Zhang et al., 2013).

35. An advantage of genetic studies is that they are not affected by some of the biases that affect other observational studies of folic acid intake and status (e.g., confounding and reverse causation) but, in common with other types of studies, they may be subject to publication bias. Genetic studies also have their own specific problems of interpretation. They may be subject to

bias dues to population stratification and the inference of a nutritional effect may not be valid in all circumstances.

36. Gene products can perform more than one role and it is possible that an association between the MTHFR 677 TT genotype and health may operate through a mechanism that is not currently understood and is not related to the effect of the TT genotype on folate status. Also, the genetic material is inherited in blocks and genetic variants are often linked to others in the same gene and even in other nearby genes. It is therefore possible that another variant within the MTHFR gene, or within another gene close by, may be the factor influencing the health outcome and that the statistical association with the MTHFR C677T variant is not causal.

37. In cases where the MTHFR 677 TT genotype does accurately identify an effect of folate on health, the timing of that effect is generally not known. In the case of NTD the developmental window within which the condition develops is relatively narrow (periconceptional to 12 weeks of gestation) therefore an association with the MTHFR C677T variant points to an effect of folate at a specific developmental stage. For diseases such as cancer, where the timing is not so clear, it may not be possible to specify the critical period of exposure as genotypes do not change across the life-course. Mendelian randomisation may be useful in identifying the role of a nutrient in the development of disease but the practical value of this information is limited if the aim is to use it to make nutritional recommendations for sectors of the population.

Review of the evidence

Cognitive health: Masking or exacerbation of low vitamin B12 status; cognitive decline in older individuals

38. Clinical symptoms associated with vitamin B12 deficiency include anaemia (identical to that of folate deficiency) and neurological impairment. Folic acid treatment can alleviate the anaemia and, as a consequence, 'mask' the vitamin B12 deficiency. This can result in a delay in the diagnosis of vitamin B12 deficiency and lead to irreversible neurological damage.

39. In 2006, SACN considered whether high doses of folic acid could delay the diagnosis of vitamin B12 deficiency and allow the neuropathy to progress. It concluded that intakes of up to 1 mg/d are not associated with adverse effects on neurological function in older people with low B12 status.

Cognitive related outcomes

40. Since 2005, several systematic reviews have evaluated the association of folate status with risk of dementia or changes in cognitive capabilities, as well as the effect of folic acid administration (alone or with other nutrients) on these outcomes. The results of these are summarised in Annex 2 (Tables 1-2).

41. The primary evidence in the reviews focuses on mature and older adults (aged ≥ 55y; mostly aged ≥ 65y). Intervention studies with folic acid alone or in combination with (mainly) other B-vitamins report mixed results but overall no consistent significant effects on any general measures or specific domains of cognition. Cohort studies consistently report either no significant relationships or that relatively higher folate status is associated with lower risks of cognitive decline and dementia.

Masking or exacerbation of low vitamin B12 status

42. No systematic reviews or meta-analyses, evaluating the risk of folic acid masking or exacerbating adverse effects of vitamin B12 deficiency, were identified.

43. Two narrative reviews (Reynolds, 2016; Selhub & Rosenberg, 2016) consider potential risks of folic acid fortification in relation to these outcomes. Masking by folic acid of the anaemia associated with B12 deficiency is well-described in the clinical nutrition literature, but it is further suggested that folic acid may also cause or exacerbate long-term neurological degeneration associated with marginal vitamin B12 status.

44. A key issue is not whether these outcomes could occur, but the susceptible populations and oral doses of folic acid where there might be a significant risk of these occurring. A study which examined nationally representative samples of adults aged ≥ 50y in the USA (Qi et al., 2014) reported that the prevalence of low vitamin B12 status (defined as serum B12 concentration < 148 pmol/L) in the absence of megaloblastic anaemia or macrocytosis did not increase after fortification. However the biochemical measures used to assess vitamin B12 deficiency are of limited reliability and there are no agreed criteria to define deficiency.

45. The bulk of evidence reporting these adverse outcomes comes from interventions with folic acid doses in excess of 1 mg/d, initiated as part of the clinical management of small populations of older patients presenting with pernicious anaemia. At lower doses of folic acid there is very limited and mixed evidence, largely presented as case studies in which causality cannot be confirmed (see Savage & Lindenbaum, 1995). There are also observational data (reviewed by Selhub & Rosenberg, 2016) indicating that higher folate status (plasma or red blood cell concentrations above the normal range) is associated with cognitive impairments in older individuals with low vitamin B12 status. However, these same data indicate beneficial associations of high folate status with cognitive function in individuals with normal vitamin B12 status. The high levels of systemic folate at which the adverse associations are reported most likely occur from intake of folic acid supplements exceeding 1 mg/d.

46. A single nucleotide polymorphism (776C→G) in the gene for transcobalamin (TCN2; rs1801198), which transports vitamin B12 around the body, affects the binding of transcobalamin to vitamin B12 and is associated with a lower concentration of holotranscobalamin (vitamin B12 attached to transcobalamin) (Miller et al., 2002; Afman et al., 2002). Therefore vitamin B12 availability to tissues may be lower in 776G carriers even when vitamin B12 intake is adequate. A study which examined the association of the TCN2 776C→G polymorphism and folate intake with peripheral neuropathy in individuals (n=171; age, ≥ 60y) with normal vitamin B12 status (plasma vitamin B12 concentration >148 pmol/L) reported a significantly higher risk of neuropathy in GG compared to CC genotypes (OR, 3.33; 95% CI, 1.15-9.64) at folate intakes ≥ 800 µg/d (Sawaengsri et al., 2016). However, as with the MTHFR 677 TT genotype, see paragraphs 35-37 regarding the validity of inferring nutritional effects from genetic evidence.

47. Intervention studies with folic acid supplementation (with or without vitamin B12), have generally found no significant effects on cognitive performance and risk of dementia. However, almost all intervention trials were less than 1 year in duration and neither intervention nor observational analyses have been focused on or powered for assessment of effects in individuals with low B12 status specifically. As noted by other authorities, data are

lacking with which to directly assess the risk of adverse neurological effects of higher folic acid intakes in individuals with low vitamin B12 status, particularly for folic acid intakes < 1 mg/d. Longitudinal studies have generally found no association or a significant beneficial association of higher folate status with lower risk of dementia or cognitive decline.

Cognitive health related outcomes in children

48. Two systematic reviews were identified which examined the effects of folic acid supplementation on paediatric populations (Lohner et al., 2012; Skorka et al., 2012).

49. Lohner et al. (2012) reviewed RCTs of folic acid supplementation in infants, children or adolescents. The majority of studies were of premature infants, malnourished populations, or individuals with specific medical conditions; only 6 of the 21 included studies described results for healthy populations. In general, no adverse effects were reported for any outcomes including vitamin B12 status; however, the studies measuring vitamin B12 were of relatively short duration. Cognition was only reported as an outcome in two studies of children with Down syndrome.

50. Skorka et al. (2012) focused on RCTs of folic acid supplementation in mothers and in children aged up to 3y with child mental and psychomotor development/disorders as outcomes. However only 2 studies met the inclusion criteria, both of which involved periconceptual supplementation of mothers with multi-nutrient mixes including folic acid and both found no significant effects on subsequent child development.

51. Another systematic review that examined the links between vitamin B12 and cognition in children (Venkatramanan et al., 2016) cited one RCT from India (Kvestad et al., 2015) in which children (aged 6 to 30 months), from low to middle socioeconomic class families, were supplemented with combinations of folic acid and/or vitamin B12. Six months of supplementation with folic acid alone had no significant effects on measures of gross motor and problem-solving skills, regardless of initial vitamin B12 status.

Summary and conclusions

52. Evidence from intervention studies is inconsistent but, overall, folic acid had no significant effects on cognitive decline. Observational studies have reported no significant relationship, or a lower risk, associated with higher folate status. Concern has been expressed regarding possible adverse effects of high folate status in individuals with low vitamin B12 status.

53. No systematic reviews have evaluated the risk of folic acid masking or exacerbating vitamin B12 deficiency but evidence for this outcome is largely from folic acid interventions in excess of 1 mg/d. Evidence from the US indicates that the prevalence of vitamin B12 deficiency with or without anaemia did not increase after mandatory fortification.

54. Evidence from intervention studies suggests no effect of folic acid on cognitive function or development in children.

Cancer

55. In 2006, SACN concluded that there was insufficient evidence at that time for an adequate risk assessment of folic acid and cancer risk or of the intake levels that might be associated with risk. In 2009, the Committee agreed that there were still uncertainties regarding folic acid and cancer risk.

56. A limitation of RCTs that have assessed the relationship between folic acid and cancer risk is that most were not designed to investigate the effect of folic acid on cancer risk and may not have been adequately powered to detect an association. In addition, cancer usually develops over several years and most trials were of relatively short duration with limited follow-up time.

Prostate cancer risk

RCTs of folic acid supplementation

57. Two meta-analyses of published results (Wien et al., 2012; Qin et al., 2013b) and one pooled analysis of individual participant data (Vollset et al., 2013) examined the effect of folic acid supplementation on prostate cancer risk (Table 3, Annex 2). The pooled analysis of Vollset et al. (2013), which included the largest number of trials (n=12) and cases (n=656), reported no significant difference in prostate cancer risk between participants randomised to receive folic acid (dose range, 0.5-40 mg/d; median, 2.0 mg/d) compared to placebo (RR, 1.15; 95% CI, 0.99-1.34).

Observational studies of folate intake

58. Two meta-analyses of dietary folate and prostate cancer risk were identified (Tio et al., 2014a; Wang et al., 2014). Both included the same individual studies (n=5; 12,898 cases) and reported no association of dietary folate (177-444 µg/d) with prostate cancer risk (Table 4, Annex 2).

59. One meta-analysis of total folate intake (diet plus supplements) and prostate cancer risk was identified (Tio et al., 2014a). The meta-analysis, which included two individual studies, found no association of total folate intake (223-640 µg/d) with risk for prostate cancer (Table 5, Annex 2).

Observational studies of serum or plasma folate concentration

60. Three meta-analyses of published results (Collin et al., 2010; Tio et al., 2014a; Wang et al., 2014) and one pooled analysis of individual participant data (Price et al., 2016) investigated the prospective relationship between serum or plasma folate and prostate cancer risk (Table 6, Annex 2). The pooled analysis (Price et al., 2016) included the largest number of cases (n=6875). Study specific quintile cut-points (not reported) were used to categorise participants into fifths of serum/plasma folate concentration (mean concentrations ranged from 5.7 to 16.9 nmol/L). A significantly higher risk for prostate cancer in men with relatively high compared to relatively low serum or plasma folate was reported (RR, 1.13; 95% CI, 1.02-1.24). Price et al. (2016) also conducted subgroup analyses according to tumour characteristics and reported significant heterogeneity by the histological grade of the tumour, with a high risk for high grade disease associated with high circulating folate concentrations.

61. In the next largest meta-analysis (Tio et al, 2014) (n=5904 cases), which also reported a significantly higher risk of prostate cancer in men with relatively high compared to relatively low folate concentration, the median folate concentration was 17.1 (range 10.3-58.2) nmol/L in the high category and 6.5 (range 4.8-10.9) in the low category.

MTHFR genotype

62. Five meta-analyses investigated the association between MTHFR genotype and prostate cancer risk (Table 7, Annex 2). Four are based only on published results (Zhang et al., 2014; Abedinzadeh et al., 2015; Chen et al., 2015; Guo et al., 2015), while one includes unpublished data (Collin et al., 2009). The studies and numbers of cases included varied, with the largest

number of studies (n=23) and cases (n=11,348) in the meta-analysis by Guo et al. (2015) which reported a significantly lower risk in men with TT genotype (associated with lower serum folate) than in those with CC genotype (RR, 0.90; 95% CI, 0.82-1.00; p=0.04).

Summary and conclusions

63. The data on folic acid/folate and prostate cancer risk, overall, are inconclusive.

64. Meta-analyses of RCTs of folic acid supplementation (median dose, 2.0 mg/d) show no effect of folic acid on prostate cancer risk but the number of cases is small suggesting low statistical power.

65. Observational studies of dietary (median intake in high category, 378 µg/d) or total (median intake in high category, 554 µg/d) folate intake do not suggest an association with prostate cancer risk while observational studies of serum or plasma folate concentration suggest potential adverse effects on prostate cancer risk of relatively high (10.3-58.2 nmol/L; median, 17.1 nmol/L) serum or plasma folate concentration.

66. The genetic studies show a significantly lower prostate cancer risk in men with MTHFR TT genotype, which results in a lower blood folate concentration. This suggests that lower folate concentration may protect against prostate cancer risk.

Breast cancer risk

RCTs of folic acid supplementation

67. Two meta-analyses examined the effect of folic acid supplementation (usually combined with vitamin B12 and sometimes other vitamins) on breast cancer risk (Vollset et al., 2013; Qin et al., 2013b) (Table 8, Annex 2). The largest number of cases (n=297) and trials were included in the pooled analysis by Vollset et al. (2013) which reported no significant difference in breast cancer risk between participants randomised to folic acid supplements (0.5-40; median 2.0 µg/d) compared to placebo (RR, 0.89; 95% CI, 0.66-1.20).

Observational studies of folate intake

68. Three meta-analyses of dietary folate intake and breast cancer risk were identified (Larsson et al., 2007; Chen et al., 2014; Liu et al., 2014) (Table 9, Annex 2). The two most recent (Chen et al., 2014; Liu et al., 2014), which include the same individual studies and almost the same number of cases, found no evidence of an association of dietary folate (126-551 µg/d) with breast cancer risk. Four meta-analyses of total folate intake (diet plus supplements) were identified (Lewis et al., 2006; Larsson et al., 2007; Chen et al., 2014; Zhang et al., 2014) (Table 10, Annex 2). The meta-analysis by Zhang et al. (2014), which included the largest number of studies and cases (n=18,572), found no evidence of an association of total folate intake (150-919 µg/d) with breast cancer risk (RR, 0.97; 95% CI, 0.90-1.05).

Observational studies of serum/plasma folate

69. One meta-analysis (Larsson et al., 2007) found no evidence of an association of circulating folate concentration (6.8-13.6 nmol/L) with breast cancer risk (Table 11, Annex 2).

MTHFR genotype

70. Four meta-analyses investigated the association between MTHFR genotype and breast cancer risk (Table 12, Annex 2). The most recent (Kumar et al., 2015) with the largest number of studies (n=75) and cases (31,315), observed a small but significantly higher risk in women with

TT genotype (associated with relatively lower serum folate) than in those with CC genotype (RR, 1.10; 95% CI, 1.04-1.16).

Summary and conclusions

71. The data overall do not suggest that folic acid increases the risk for breast cancer.

72. Meta-analyses of RCTs of folic acid supplementation (median dose, 2.0 mg/d) show no effect of folic acid on breast cancer risk but they may not have had sufficient power to detect an effect.

73. Observational studies of dietary folate intake (median, >374 µg/d), total folate (median, 489 µg/d) intake and serum or plasma folate concentration (6.8-31.7 nmol/L) show no association with breast cancer risk.

74. Findings from the MTHFR genetic studies suggest lower folate concentrations are associated with higher breast cancer risk.

Colorectal cancer risk

RCTs of folic acid supplementation

75. Two meta-analyses (Cooper et al., 2010; Qin et al., 2015) and one pooled analysis (Vollset et al., 2013) assessed the relationship between folic acid supplementation and colorectal cancer risk (Table 13, Annex 2). The pooled analysis (Vollset et al, 2013), which included the largest number of cases (n=429), reported no effect of folic acid supplementation (0.5-40 mg/d; median dose, 2.0 mg/d) on colorectal cancer risk but the confidence intervals are large and, even when combined, the numbers studied are relatively small.

76. Five meta-analyses (Cooper et al., 2010; Ibrahim & Zekri, 2010; Fife et al., 2011; Figueiredo et al., 2011; van Dijk & Pot, 2016) examined adenoma recurrence in individuals with a history of adenomas (Table 14, Annex 2); one of these (Fife et al., 2011) reported that folic acid supplementation (0.5-2.5 mg/d; median, 1 mg/d) for over 3 years significantly increased the risk of an adenomatous lesion (OR, 1.35; 95% CI, 1.06-1.70). The most recent meta-analysis (van Dijk & Pot, 2016) reported folic acid supplementation (0.5-5 mg/d; median dose 1 mg/d) had no significant effect overall on colorectal adenoma recurrence but the confidence intervals are large and the findings heterogeneous.

Observational studies of folate intake

77. One meta-analysis (Kennedy et al, 2011) and one pooled analysis (Kim et al, 2010) evaluated the association between dietary folate intake and colorectal cancer risk (Table 15, Annex 2). Both found no association with colorectal cancer risk. One meta-analysis (Heine-Broring et al., 2015) evaluated the association between supplemental folic acid intake and colorectal cancer risk and found inconsistent associations, though a comparison of highest (>143 to ≥ 560 µg/d; median, ≥400 µg/d) and lowest (<50 to <101 µg/d; median, <83 µg/d) categories suggests a significant protective effect of higher intakes (Table 16, Annex 2).

78. One meta-analysis (Liu et al., 2015) and one pooled analysis (Kim et al., 2010) investigated total folate intake and colorectal cancer risk (Table 17, Annex 2). The pooled analysis (Kim et al., 2010) (5,720 cases) found no significant association of folate intake with colorectal cancer risk in the continuous regression model but a comparison of highest and lowest quintiles of intake suggested a significant protective effect of higher intakes (RR, 0.85; 95% CI, 0.77-0.95).

Folate intakes in the highest vs lowest quintiles were not specified but the median intake range across the included studies was 259-501 µg/d. The most recent meta-analysis (Liu et al, 2015) with the largest number of cases (n=24,816) reported a reduced colorectal cancer risk when comparing highest (median, > 441 µg/d) with lowest (median, 212 µg/d) folate intake.

Observational studies of serum or plasma folate

79. One meta-analysis (Chuang et al., 2013) assessed the association between serum or plasma folate concentration and colorectal cancer risk (Table 18, Annex 2). Overall no association was found although there was heterogeneity when the studies were considered by assay method. In meta-analysis of studies which measured serum or plasma folate concentration using radioimmunoassay, a higher (median, 31.5 nmol/L) compared to lower (median, 6.1 nmol/L) serum or plasma folate concentration was significantly associated with a lower risk of colorectal cancer, while studies using microbiological assays reported no association.

MTHFR genotype

80. Six meta-analyses (Kennedy et al., 2012; Rai, 2015; Sheng et al., 2012; Taioli et al., 2009; Yang et al., 2012; Zhou et al., 2012) (based on 94 primary studies) investigated the association between the MTHFR genotype and colorectal cancer risk (Table 19, Annex 2). The meta-analysis with the largest number of cases (n=26,731) reported a significant association between the MTHFR 677TT genotype (which results in lower blood folate concentration) and lower colorectal cancer risk (Kennedy et al., 2011). A similar result was reported in the other five meta-analyses though there was overlap in the primary studies included in these.

81. The numbers required to detect an effect depend on the frequency of the MTHFR C677T genotype in the population studied; greater numbers are required when the TT genotype frequency is lower. In European white populations the frequency of the TT genotype is around 10% but has been reported as 2% in West African and African American populations and less than 1% in the northern Chinese and individuals of Mexican descent (Tsang et al., 2015). Therefore in non-European white populations, larger numbers are required to detect effects of the same magnitude.

82. A subgroup analysis by Sheng et al. (2012) also indicated a lower colorectal cancer risk in Asians with the TT genotype. Other studies have shown the same significant association in Asian populations (Yang et al., 2012; Zhou et al., 2012) though Rai et al. (2015) reported a similar odds ratio but the association was not statistically significant. Taioli et al. (2009) conducted a meta-analysis of 29 studies (11,936 cases, 18,714 controls) and a pooled analysis of 14 studies (5068 cases, 7876 controls), with stratification by racial/ethnic group. The TT genotype was significantly associated with a reduced colorectal cancer risk in the whole population and in whites and Asians alone.

Summary and conclusions

83. The data on folic acid/folate colorectal cancer risk, overall, are heterogeneous within most of the study designs and the findings are inconsistent between study designs.

84. The meta-analyses of RCTs of folic acid supplementation (median dose, 2.0 mg/d) on colorectal cancer risk show no significant effect but the numbers studied are relatively small.

85. The observational studies also have a high degree of heterogeneity though the largest suggests a protective association of folate intakes above about 400 µg/d. There is also evidence of a

protective association of high intakes when comparing the highest and lowest categories of intake for total folate and folic acid as a supplement. Observational studies of serum or plasma folate concentration overall suggest no association.

86. The evidence from the MTHFR genetic studies indicates a consistently lower risk of colorectal cancer in individuals with the TT genotype compared to those with the CC genotype in both white and Asian populations. This is indicative of a decreased risk of colorectal cancer in individuals with lower blood folate concentrations associated with the TT genotype.

Overall cancer risk

87. Overall cancer risk represents an average of the different types of cancers and will depend on population and the prevalence of many cancer risk factors.

RCTs of folic acid supplementation

88. Three meta-analyses of published results (Wien et al., 2012; Baggott et al., 2012; Qin et al., 2013b) and one pooled analysis of individual participant data (Vollset et al., 2013) examined the effect of folic acid supplementation on overall cancer risk (Table 20, Annex 2). The meta-analysis with the largest number of trials (n=13) and cases (n=3741) (Qin et al., 2013a) reported no significant difference in cancer risk between participants randomised to receive folic acid supplements (range, 0.5-40 mg/d; median dose, 2 mg/d) compared to placebo (RR, 1.05; 95% CI, 0.99-1.11). There was also no association between intervention duration and cancer incidence risk. The pooled analysis of individual participant data (Vollset et al., 2013), which included the same number of trials and almost as many cases (n=3713), also reported no significant effect of folic acid on cancer risk. Daily dose of folic acid or treatment duration had no effect.

MTHFR genotype

89. One meta-analysis of studies (n=134) investigating the association between the MTHFR polymorphism and overall cancer risk (Tang et al., 2014) (Table 21, Annex 2) reported a significantly higher risk of all cancers in people with TT genotype (lower serum folate concentration) than in those with CC genotype (RR, 1.08; 95% CI, 1.01-1.17).

Summary and conclusions

90. The data on folic acid/folate and overall cancer risk are inconsistent.

91. Evidence from RCTs shows no effect of folic acid supplementation (at doses ranging from 0.5-40 mg/d; median, 2.0 mg/d) on overall cancer risk.

92. Findings from the MTHFR genetic studies suggest lower folate concentrations are associated with higher overall cancer risk.

Unmetabolised folic acid

93. The appearance of unmetabolised folic acid (UMFA) in the systemic circulation has raised concerns regarding potential long term effects of high folic acid intakes.

94. In 2006, SACN noted that "*unmetabolised folic acid has been detected in the systemic circulation following oral doses of folic acid above 260 µg*" but that there were "*insufficient data in humans to assess the long-term effects of exposure to unmetabolised folic acid in the systemic circulation*". Since then, a number of new studies on UMFA have been published and improved detection methods (LC-MS/MS) have been applied in national surveys such as the

National Diet and Nutrition Survey (NDNS) in the UK and National Health and Nutrition Examination Survey (NHANES) in the USA.

95. Studies on UMFA are summarised in Tables 22-25 (Annex 2).

Measurement of serum/plasma UMFA in the systemic circulation

Intervention studies following folic acid supplementation

96. Seven intervention studies (from USA, Ireland and Germany) measured serum UMFA following folic acid supplementation (Table 22, Annex 2). Sample sizes were very small in all studies (n=20-74) and duration ranged from 3-30 weeks (except one study of 1 year duration). The intervention dose ranged from 100 µg to 5 mg/d but was 400 µg/d in most studies. Fasted blood samples were used to measure UMFA in 5 studies; 1 study used non-fasted blood samples; 1 study did not report fasting status.

97. The proportion of individuals with detectable UMFA ranged between 0 and 68% at baseline and 33-97% post intervention. UMFA as a proportion of total serum folate was 1-3% with folic acid doses of 400 µg/d. One study reported that UMFA was 15% of total plasma folate following a supplementation dose of 5 mg/d (Obeid et al., 2011).

98. In one study (Houghton et al., 2009), which measured the effect of three interventions (folic acid, 400 µg/d; 5-MTHF, 416 µg/d; or placebo) on milk folate concentrations of lactating women, UMFA comprised 8% of total breast milk folate concentration regardless of treatment group. Another study that compared the effect of folic acid supplementation (400 µg/d) or placebo during pregnancy on cord blood UMFA concentration reported that UMFA was detected in 15% of the cord samples but there was no difference between the two groups in the proportion of cord samples with detectable UMFA (Pentieva et al., 2016).

99. One small study (n=20) which examined 3 folic acid doses (100, 200 or 400 µg/d) found only the highest (400 µg/d) led to the appearance of UMFA in serum (Sweeney et al., 2007). A study (Tam et al., 2012) which compared the effect of 2 higher doses of folic acid (1.1 vs 5 mg/d for 30 weeks) on plasma UMFA concentration of women of reproductive age (n=40), found no significant differences between the two doses.

Observational studies

100. Seven observational studies (1 prospective; 6 cross-sectional) examined associations between folic acid intake and UMFA (Table 23, Annex 2). Sample sizes ranged from n=11 to 368 and the majority of studies measured UMFA in fasted blood samples.

101. Four studies reported associations between UMFA during pregnancy/in newborns. In three studies (Obeid et al., 2010; Sweeney et al., 2005; Sweeney et al., 2009) from Germany and Ireland (where there is voluntary but not mandatory fortification) the proportion of mothers with detectable UMFA during pregnancy ranged from 44 to 90% (UMFA as % of total folate ranged from 0.43 to 1.3%); the proportion of umbilical cord samples with detectable UMFA ranged from 53 to 100%. In one study from Canada (Plumptre et al., 2015) (where there is mandatory fortification) UMFA was detected in 97% of women (median UMFA as % of total folate = 3.1%) and 93% of cord samples (median UMFA as % of total folate = 1.3%). There was no significant difference in the proportion of maternal or umbilical cord samples with detectable UMFA according to maternal supplement use during pregnancy. UMFA as proportion of total folate was significantly lower in cord than in maternal serum.

102. Out of three population based studies (2 cross-sectional; 1 prospective cohort) in Ireland, two analysed fasted samples (Vaish et al., 2016; Boilson et al., 2012) and reported detectable UMFA in 10% of children ≤ 16y (1.9% of total folate) and in 94% of adults aged 67y (1.3% of total folate). In the study which used non-fasting samples (Sweeney et al., 2009), UMFA was detected in 98% of adults aged 26-39y (2.3% of total folate).

103. One cross-sectional study examined the effects of a polymorphism in the DHFR[5] gene (19 bp deletion in intron-1; rs70991108) on UMFA concentration (Kalmbach et al., 2008). This polymorphism is common in the US population with 23% homozygosity (Philip et al., 2015). Kalmbach et al (2008) reported that the polymorphism was associated with higher plasma UMFA concentration and lower red blood cell folate concentration; the proportion with serum UMFA concentration >1.35 nmol/L was higher in individuals homozygous for the polymorphism (47%), who were consuming >500 µg/d of folic acid, compared with those with one copy of the deletion (21%) or none (24%).

National monitoring programmes

104. Three studies in the USA reported findings from NHANES (2000-2001; 2007-2008; 2011-12) (Table 24, Annex 2). In NHANES 2000-2001 (Bailey et al., 2010), which used fasted samples, UMFA was detected in 38% of the population and accounted for 2.3% of total serum folate. In NHANES 2007-08 (Pfeiffer et al., 2015b) & 2011-12 (Pfeiffer et al., 2015a), which included fasted & non-fasted samples, UMFA was detected in over 95% of the population and accounted for 4% of total serum folate.

105. Data on UMFA concentrations of individuals from the NDNS in the UK (Bates et al., 2014) are shown in Annex 5. The data indicate that UMFA comprises a relatively constant proportion (2-3%) of total blood folate. The median value is slightly lower in children aged below 11y but, overall, the distribution of the proportion of UMFA appears to be relatively constant across the age groups. The medians by age group are: 1.9%, 1.3-3y; 2.1%, 4-10y; 2.9%, 11-18y; 2.8%, 19-64y & 65+y. For the group as a whole, 82% of individuals had a UMFA concentration < 5% of total blood folate and 98% had UMFA < 10% of total blood folate. The percentage of individuals with UMFA concentrations below the limit for detection was: 7.5%, 1.5-3y; 19.2%, 4-10y; 30.1%, 11-18y; 32.6%, 19-64y; and 28.5%, 65+y.

106. It has been suggested that UMFA, rather than reflecting an intake of folic acid which has exceeded the body's capacity to metabolise it, may be produced endogenously from natural folates (Bailey et al., 2010). This could explain the observation that UMFA is routinely detected in samples collected from fasting subjects and that the concentration of UMFA comprises a relatively constant proportion of total blood folate regardless of age and intake.

107. There appear to be two processes that result in UMFA appearing in the blood. Ingestion of a bolus dose of folic acid can exceed the capacity of the intestinal mucosa to metabolise it to circulating plasma folate (5-MTHF) before entering the liver and the wider systemic blood supply and this results in the transient appearance of UMFA in the circulation (Patanwala et al., 2014). It is possible that this could explain some of the UMFA detected in fasting NDNS samples. However, the proportions of UMFA in fasting samples are of the same order as those in the UK, although folic acid intakes are considerably higher in the USA, and it is a relatively

[5] DHFR catalyses the reduction of folic acid to THF (see paragraph 27).

constant proportion regardless of age. It is likely that there is a natural equilibrium point between total folate and UMFA (possibly involving endogenous UMFA synthesis). This complicates the interpretation of UMFA concentrations but it also suggests that the generation of UMFA is a natural process and that concentrations of UMFA are determined by the total intake of folates (natural and synthetic) rather than the intake of folic acid *per se.*

Health outcomes

108. Three observational studies examined associations between potential adverse effects of UMFA on health outcomes (cancer & cognitive test performance) (Table 25, Annex 2).

109. One prospective cohort study (Cho et al., 2015) reported that, overall, prediagnostic UMFA was not associated with colorectal cancer risk; however blood samples were collected before mandatory folic acid fortification so UMFA concentrations were not reflective of those after fortification. One prospective cohort study (Hu et al., 2016) reported no associations between UMFA & overall cancer risk. One cross-sectional study (Morris et al., 2010) reported that in a subgroup analysis of participants with low vitamin B12 status (serum B12 < 148 nmol/L or methylmalonic acid ≥ 2 10 nmol/L) the presence compared with the absence of detectable UMFA was associated with lower cognitive test score.

Summary and conclusions

110. In intervention studies, the proportion of individuals with detectable UMFA ranged between 33 and 97% post intervention. UMFA as a proportion of total folate was 1-3% at folic acid doses over the normal range of consumption (400 µg/d). The presence of UMFA in umbilical cord blood and breast milk was unrelated to maternal FA supplement intake.

111. There is no clear dose-response relationship between folic acid intake and the appearance of UMFA in the systemic circulation.

112. UMFA comprises a relatively constant proportion of total folate in the population regardless of age and is widely detected in samples collected from fasting individuals. It is unlikely that the fasting UMFA concentration reflects an intake of folic acid which has exceeded the body's capacity for metabolic conversion. This is supported by findings from national monitoring programmes in the USA and UK which show similar proportions of UMFA in fasting samples from both countries although folic acid intakes are considerably higher in the USA.

113. The concentration of UMFA in individuals carrying a relatively common polymorphism within the DHFR gene is likely to be higher, for at least part of the day, than in those without the polymorphism or with fewer copies.

114. There are insufficient data to assess whether the presence of UMFA in the systemic circulation is related to any adverse health outcomes.

5) Overall summary and conclusions

Background

115. Conclusive evidence from RCTs has shown that folic acid supplementation during the early stages of pregnancy can reduce the risk of the fetus developing NTDs. All women planning a pregnancy are therefore advised to take a daily supplement of folic acid (400 µg) prior to conception and until the 12th week of pregnancy. Women with a previous pregnancy affected by NTDs or women with spina bifida themselves are advised to take folic acid supplements of 5 mg/d[6].

116. Evidence suggests that this advice has not been followed. It has been estimated that the proportion of women who reported taking folic acid supplements prior to pregnancy has declined from 35% in 1999-2001 to 31% in 2011-12y. Another limitation to the value of recommending folic acid supplementation prior to conception is that about half of all pregnancies in Britain are unplanned. In contrast, significant reductions in NTD prevalence have been reported in countries where mandatory folic acid fortification has been introduced. For example, in the USA, where mandatory fortification was introduced in 1998, there was an immediate 28% reduction in prevalence of anencephaly and spina bifida post-fortification (1999-2011).

117. In 2006, SACN recommended mandatory fortification of flour with folic acid to improve the folate status of women most at risk of NTD-affected pregnancies in the UK (SACN, 2006). It stipulated, however, that it should only be introduced alongside restrictions on voluntary fortification of foods with folic acid. This was to ensure no increase in the numbers of people with intakes above the GL/UL and no substantial increase in mean intakes or folate status of the UK population.

118. In 2007, following publication of two studies suggesting potential adverse effects of folic acid on colorectal cancer risk, SACN was requested by the Chief Medical Officer to conduct a detailed review of these data. In 2009, the Committee concluded that there were uncertainties regarding folic acid and cancer risk but reiterated its previous recommendation for mandatory folic acid fortification together with controls on voluntary fortification, guidance on supplement use and appropriate population monitoring procedures. In addition, people over 50y of age and those with a previous history of colorectal adenomas were advised not to consume supplements containing folic acid above the recommended nutrient intake for folate (200 µg/d).

119. In February 2016, Food Standards Scotland (FSS) informed SACN that in the absence of progress regarding mandatory folic acid fortification in the UK, Scottish Ministers were considering whether to proceed unilaterally with mandatory folic acid fortification of flour in Scotland. FSS was, therefore, seeking advice on whether SACN's 2006 and 2009 recommendations on mandatory folic acid fortification remained applicable. In this context, SACN agreed to conduct a review of evidence published since its 2006/2009 risk assessments.

[6] Diabetes UK recommends that all women with diabetes planning a pregnancy should take a folic acid daily dose of 5 mg.

Terms of reference

120. The terms of reference were to:

- Conduct a review of major new evidence (with an emphasis on systematic reviews and meta-analyses) published since SACN's 2006 and 2009 reports on folate and folic acid to assess whether its previous advice stands.
- Provide comments in relation to the modelling exercise carried out by the FSA for SACN's 2006 report and any updated modelling exercise undertaken by FSS.

Review of the evidence

121. SACN's review of the evidence published since its previous risk assessments in 2006 and 2009 was restricted to potential adverse effects of folic acid. These were: masking or exacerbation of low vitamin B12 status; cognitive decline in older individuals, cancer (prostate, breast, colorectal and overall). The implications for health, of the presence of unmetabolised folic acid (UMFA) in the systemic circulation, was also considered.

Cognitive decline related outcomes

122. The SACN 2006 report highlighted uncertainties regarding evidence relating to potential cognitive and vitamin B12-related adverse outcomes of folic acid (masking of vitamin B12 deficiency & acceleration/aggravation of cognitive decline). It concluded that folic acid intakes up 1 mg/day are not associated with masking anaemia associated with vitamin B12 deficiency. It noted a lack of data on trends in clinical incidence of vitamin B12 deficiency or any related neurological damage but limited data (from national hospital discharge surveys) from countries with mandatory fortification had not suggested an increase post-fortification.

123. Evidence published since the 2006 SACN report reiterates some of these uncertainties but does not provide a substantive basis to change the conclusions of the previous risk assessment with regard to these outcomes, nor to propose changes to the UL for folic acid of 1 mg/d.

Cancer

124. *Prostate cancer:* RCTs of folic acid supplementation have shown no effect on prostate cancer risk and observational studies on dietary folate intake show no association with prostate cancer risk. Observational studies of serum or plasma folate and genetic studies of the MTHFR polymorphism suggest that higher concentrations of folate may be associated with increased risk. The results from the different types of study are inconsistent and overall the evidence is inconclusive.

125. *Breast cancer:* RCTS of folic acid supplementation have shown no effect on breast cancer risk and observational studies on dietary folate intake and observational studies of serum or plasma folate concentration show no association with breast cancer risk. The genetic studies of the MTHFR polymorphism suggest that lower serum or folate concentrations are associated with a higher risk of breast cancer. The results from the different types of study are inconsistent and overall do not suggest an association between folic acid and breast cancer risk.

126. *Colorectal cancer:* RCTs of folic acid supplementation have shown no effect on colorectal cancer risk and observational studies of serum or plasma folate concentration suggest no

effect on colorectal cancer risk. The observational studies of folate intake are heterogeneous and suggest a protective association at intakes above 400 μg/d. The MTHFR genetic studies suggest a decreased risk of colorectal cancer in individuals with lower blood folate concentrations. The results from the different types of study are inconsistent and overall show no clear beneficial or detrimental effect of folate on colorectal cancer risk.

127. *Overall cancer risk:* RCTs of folic acid supplementation show no significant effect on overall cancer risk while the genetic studies suggest lower blood folate concentrations are associated with increased cancer risk. The evidence overall is inconsistent.

128. The evidence base for each outcome is characterised by inconsistencies in the findings of the different study types considered (RCTs, observational studies of folate and folic acid intake, observational studies of folate status, and the indirect inference of folate effects based on genetic MTHFR polymorphism studies).

129. It is difficult to draw firm conclusions on folate and cancer risk on the basis of evidence from nutritional studies (RCTs and observational). Overall the evidence from nutritional studies is no more certain, in either direction, than it was when SACN previously considered the issue (2006 and 2009). Even within a study type the data are heterogeneous and overall do not suggest a detrimental effect of folate on cancer risk. However statistical power is generally low, particularly for the RCTs. In addition, cancer usually develops over a number of years but most trials were of short duration with limited follow-up time. In the observational studies of folate intake there was large variation in the range of folate intake, including in the lowest and highest folate intake categories and some overlap between the two categories.

130. Evidence from genetic studies is relatively consistent within a cancer type but the apparent effects go in different directions for different cancers. The inference from genetic studies is that high blood folate concentrations are associated with increased risk of prostate and colorectal cancer but reduced risk of breast and overall cancer. The usual interpretation of such genetic evidence is that the effect on cancer operates via folate status, but it is possible that the MTHFR 677 TT genotype may have another parallel mode of action that is not currently understood. In addition, although the genetic studies do not suffer from some of the biases that affect other observational studies, they are potentially subject to other biases and uncertainties in interpretation (see paragraphs 35-37).

131. Although there are still uncertainties relating to folate and cancer risk, it is important to note that the folic acid doses (0.5-40 mg/d; median, 2.0 mg/d) used in the trials examining folic acid intake and cancer risk were much larger than the amounts that would be considered for fortification. In the observational nutritional studies where there were apparent associations it is not possible to identify any threshold effect. Genetic studies are not designed to identify threshold effects.

Unmetabolised folic acid

132. SACN's earlier risk assessment (2006) concluded that, overall, there were insufficient data to assess the long-term effects of exposure to UMFA in the systemic circulation. Although more data are now available, most studies relate to measurement of UMFA in the systemic circulation. The widespread use of fasting samples for these measurements, and uncertainties

relating to the nature of the dose-response and the origin and metabolic fate of UMFA in the systemic circulation, make it difficult to interpret the findings.

133. The evidence is still insufficient to assess whether the presence of UMFA in the systemic circulation has any long-term adverse consequences on health.

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ANNEX 1

Summary of previous reports on folic acid

Since SACN's full risk assessment in 2006, five reports on folic acid have been published by the following organisations: European Food Safety Authority (EFSA) (2014), the Food and Drug Administration (FDA) (1998), Food Safety Authority of Ireland (FSAI) (2016), National Toxicology Program (NTP) (2015); and the Norwegian Scientific Committee for Food Safety (VKM) (2015). The overall findings and conclusions of these reports are tabulated below. The conclusions relating to potential adverse effects are summarised in the text below.

POTENTIAL ADVERSE EFFECTS OF FOLIC ACID

Cognitive health related outcomes: masking of vitamin B12 deficiency in older adults; aggravation or acceleration of cognitive decline in older individuals with low vitamin B12 status

The NTP (2015) concluded that evidence suggesting high folic acid intake in the presence of low B12 exacerbates neuropathy is based on early case reports and only supported by limited data from observational studies. In relation to risks for other neurological outcomes (e.g., dementia, depression) it observed that the large volume of literature is indicative of no adverse effects. The FDA (2016) concluded that a folic acid intake up 1 mg/d is unlikely to mask vitamin B12 deficiency or have adverse effects on cognitive decline in older people. The Food Safety Authority of Ireland (FSAI) (2016) cited the NTP report which concluded that high folic acid intake did not increase risk of cognitive impairment. The VKM (2015) and EFSA did not reconsider the evidence on whether folic acid has adverse effects in relation to cognition.

Cancer

The NTP (2015) concluded that there was a consistent enough suggestion in human studies of an adverse effect on cancer growth from supplemental folic acid to justify further research.

The VKM (2015) did not find new evidence for increased risk of cancer related to folic acid in the reviewed literature and concluded that studies examining cancer published after 2009 until 15 October 2014 did not provide support to alter the existing UL for folic acid.

EFSA noted that clarification of the relationship between folic acid and cancer required studies designed with sufficiently long follow-up addressing biological hypotheses for the dual effect of folic acid on cancer development. It concluded that the possible adverse effect of folic acid related to intakes at or above the UL of 1 mg/d.

The FSAI (2016) cited the conclusions of the NTP (2015). It noted that since the introduction of mandatory fortification in the US, rates of all cancers, colorectal cancer and prostate cancer had not significantly increased (National Cancer Institute, 2015).

The FDA concluded that there was insufficient evidence to support an association between high folic acid intake & increased risk of colorectal cancer. In relation to breast cancer it recommended further study regarding potential tumour growth-promoting effects in susceptible populations (breast cancer patients and survivors). For prostate cancer, it noted that increased risk was reported in 2 RCTs with folic acid doses of 1 mg/d (Cole et al, 2007; Figueiredo et al, 2009) but not in a trial with 0.8 mg/d (Ebbing et al, 2009). It concluded that it was not possible to rule out the possibility that folic acid may be associated with increased risk of certain cancer subtypes.

Unmetabolised folic acid

The VKM (2015) concluded that the impact of UMFA in the circulation & extent to which it contributes to development of cancer or other undesirable health effects is still of concern. The FSAI (2015) concluded that at low concentrations the balance of evidence shows it is unlikely that any adverse health effects are associated with the presence of UMFA in the circulation. EFSA (2015) concluded that the metabolic and biological consequences of UMFA in the circulation are, as yet, uncertain. The NTP and FDA did not consider UMFA.

OVERALL SUMMARY

National Toxicity Programme Monograph (US Department of Health and Human Services): *Identifying research needs for assessing safe use of high intakes of folic acid* (August 2015).	Purpose – To inform development of a research agenda for evaluating safe use of high intakes of folic acid. Outcomes considered - 4 high priority outcomes identified based on adverse reports in studies of intakes > 400 µg/d, serum folate > 10 nmol/L or RBC folate > 340 nmol/L: • Cancer – 43 pooled & meta-analyses of 12 cancer subtypes (~600 primary studies identified) • Cognition & vitamin B12 – 28 primary studies & 2 meta-analyses • Hypersensitivity-related outcomes – 43 studies • Thyroid & diabetes-related disorders – 72 studies Conclusions (cancer and cognition & B12 only) *Cancer* • Although inadequate dietary folate intake increases colorectal cancer risk in humans, no benefit for cancer reduction from supplements among people whose baseline folate status is adequate. • Consistent enough suggestion in human studies of an adverse effect on cancer growth from supplemental FA to justify further research. • Need to clarify whether existing evidence from trials suggesting increased risk is driven by effects in specific subgroups (e.g. age, pre-existing neoplasia, genetics, other factors) & to assess long-term outcomes from prior folic acid trials. *Cognition & vitamin B12* • Hypothesis that high folic acid/folate in presence of low B12 exacerbates neurological problems based on early case reports is supported by evidence from observational studies but data are limited. • Data from epidemiological studies hard to interpret because of heterogeneity in vitamin status cut points, cognitive outcomes & omission of other neurological outcomes. Existing intervention studies not designed to answer this question.
Food Safety Authority of Ireland: *Update report on folic acid and the prevention of birth defects in Ireland* (2016).	Purpose • Update on data relevant to prevention of pregnancies affected by NTDs in Ireland; • Recommendations on best practice monitoring systems necessary to generate these data into the future; • Update on latest scientific developments relevant to safe & effective dietary intakes of folic acid; • Recommendations on suitable options for prevention of NTDs in Ireland. Outcomes considered: • Cognitive performance • Unmetabolised folic acid • Growth of colorectal adenomas • Diabetes Conclusions (cognitive performance, unmetabolised folic acid & colorectal adenomas only) *Cognitive performance* • Cite NTP report which concluded available data are limited and no RCTs designed to address this question. *Unmetabolised folic acid* • No definitive studies that have found adverse health effects from exposure to UMFA (Crider *et al*, 2011). • Expert international panel concluded 'it was not aware of any toxic or abnormal effects of circulating folic acid' even from much higher

	exposures than those typically obtained through food fortification (Bailey *et a*l, 2015). • At generally low concentrations arising through food fortification, unlikely that any adverse health effects associated with presence of unmetabolised folic acid in circulation. *Growth of undiagnosed colorectal adenomas* • Cite SACN review (2009) – concluded insufficient data to support concerns that mandatory fortification would promote cancer. • Cite NTP – concluded that although numerous studies of high folic acid intake across multiple cancer types in various populations, results inconsistent in relation to folic acid exposure & cancer risk. However consistent enough suggestion in human studies of an adverse effect on cancer growth from FA supplements to justify further research. • Cite Vollset *et al (*2013) meta-analysis of RCTs - concluded FA supplements neither increased/decreased site-specific cancer within first 5 years of treatment & noted that this finding supported by 2 other meta-analyses (Qin *et al*, 2013; Mackerras *et al*, 2014). • Since introduction of mandatory fortification in the US (1996), rates of all cancer, colorectal cancer and prostate cancer have not significantly increased (National Cancer Institute, 2015)
FDA Memorandum for food additive petition: *Folic acid in corn masa flour* (March 2016).	Purpose - Perform safety evaluation in response to Food Additive Petition (FAP 2A4796) by 6 organisations to amend the food additive regulation by allowing addition of folic acid to corn masa flour (CMF) Outcomes considered • Masking effect of folic acid on vitamin B12 deficiency • Direct effects of FA on B12 deficiency related neurological complications • Effects of prenatal FA on childhood health outcomes • Potential dual actions of FA on carcinogenesis • Asthma & other respiratory problems • Reproduction • Folic acid drug interaction Conclusions *Masking effect of folic acid on vitamin B12 deficiency* • No info found on lowest level of FA associated with masking effect. • Study (Qi *et a*l, 2014) which compared data from NHANES 1991-94 (prefortification) and 2001-2006 (postfortification), prevalence of low B12 status in absence of megaloblastic anaemia or macrocytosis in adults >50 y did not increase after fortification. • Agreed with conclusions SACN, FSANZ, FSAI, Health Council Netherlands -FA up to 1 mg/d not likely to mask B12 deficiency. *Direct effects of FA on B12 deficiency related neurological complications* • Neurological complications – rate of neuropathy progression varied significantly among B12 deficient patients regardless of folic acid treatment. Could not reach definitive conclusion on whether FA enhances or worsens B12 related neuropathy. • Cognitive decline – several cross-sectional studies but cognitive tests used not consistent & different cut-offs used to define *high* & *low* B12 status. Concluded FA < 1 mg/d will not pose immediate health risk for cognitive decline in older people. *Colorectal adenoma (CRA) & colorectal cancer (CRC)* • 6 RCTs in patients with history of CRA. 5 showed no or protective effect on CRA or CRC; 1 suggested positive relationship between FA & recurrence of CRA (Cole *et al,* 2007). • Observational evidence – temporal studies do not provide strong evidence for causative relationship between FA & increased CRC risk. Cohort & case-control studies investigated combined effect of natural folate & synthetic FA, used different cut-offs, generating inconsistent results. • Overall, scientific evidence not sufficient to support association between high folic acid intake & increased risk of CRA or CRC.

	• Could not confirm if FA associated with increased risk of CRC progression in those with cancerous or pre-cancerous lesions. *Breast cancer* • No RCTs on effects of FA alone on breast cancer risk. • 2 RCTs of mixed B vitamins (which incl 2.5 mg FA) did not have higher risk of breast cancer. • Potential tumour growth-promoting effects in susceptible populations (breast cancer patients & survivors) warrant further study. *Prostate cancer* • Increased risk reported in 2 RCTs using 1 mg/d FA (Cole et al, 2007; Figueiredo et al, 2009) but not in trial using 0.8 mg/d (Ebbing et al, 2009). • Cohort/case-control studies suggest no or protective effect of folate/folic acid & many did not differentiate between natural folate & synthetic FA exposure. *Other types of cancer* • Insufficient evidence to support or refute hypothesis that high folic acid intake promotes other types of cancers. • Not possible to rule out possibility that FA may be associated with increased risk of certain cancer subtypes. Overall conclusions: • Evidence linking 2 potential adverse health outcomes with high folic acid intake in adults: 1. Masking vitamin B12 deficiency 2. Accelerating or exacerbating neurological complications & cognitive decline among those who are vitamin B 12 deficient • For other potential adverse outcomes (such as promoting progression of established neoplasms, childhood hypersensitivity & reproductive outcomes evidence not clear. No safety/toxicological concerns regarding request to amend food additive regulation to allow addition of folic acid to CMF.
Norwegian Scientific Committee for Food Safety (VKM): *Risk assessment of folic acid in food supplements* (2015).	Purpose – To assess whether the UL of folic acid should be amended in light of new scientific evidence suggesting possible link between high folic acid intakes and cancer risk. (Norwegian Food Safety Authority currently evaluating national maximum limit for folic acid.) Considered articles published from 2009-15/10/2014. Literature search for studies addressing high intakes of folic acid in food supplements. Publication selection: • Systematic reviews & meta-analyses of human studies; RCTs & prospective studies with data on FA supplementation in at least 1 gp. • Inclusion criteria - results for FA supplementation could be separated from other interventions & could be compared to placebo gp. Outcomes considered • Cancer development (identified 8 meta-analyse, 3 RCTs, 2 case-control studies) • Unmetabolised folic acid (identified 6 studies) Conclusions *Cancer* • Most studies investigated adenomas & colorectal cancer but also breast cancer & total cancer. • None of the studies regarding cancer published after 2009 provide significant support to alter the existing UL for folic acid supplementation. *Unmetabolised folic acid* • Impact of UMFA in circulation & extent to which it contributes to development of cancer or other undesirable health effects, still of concern. • Reviewed studies do not contribute with evidence useful in terms of potential modification of current UL for folic acid. *Overall conclusion* • At this time, no new arguments for increasing or decreasing UL for folic acid in relation to cancer.

EFSA: *Scientific opinion on dietary reference values for folate* (February 2015).	Purpose – To deliver a scientific opinion on Dietary Reference Values for the European population, including folate. Outcomes considered - series of health outcomes to use as a basis for deriving the requirement for folate. Under health consequences of excess, considered: • Masking of B12 deficiency • Cancer • Unmetabolised folic acid. *Masking of B12 deficiency – allowing neurological dysfunction to progress to irreversible subacute combined degeneration of the spinal; cord.* • Accepted UL of 1mg/day set by SCF (2000) (200µg/d for 1-3y; 800µg/day 15-17 y). *Cancer* • Refer to studies by Cole *et al* (2007), Figueiredo *et al* (2009) and meta-analyses by Vollset *et al* (2013) & Mackerras *et al*, 2014. • Noted that follow-up period of trials included in meta-analyses rather short considering development of cancer • Whether or not there is a relationship between folic acid and cancer needs to be clarified by studies designed with sufficiently long follow-up addressing biological hypothesis for the dual effect of folic acid on cancer development. • Noted that possible adverse effect of folic acid related to intakes at or above currently accepted UL. *Unmetabolised folic acid* • Metabolic and biological consequences of unmetabolised folic acid in the circulation are, as yet, uncertain.

ANNEX 2

Studies considered in relation to folic acid and potential adverse health outcomes

Summary of systematic reviews & meta-analyses on folic acid and risk of adverse cognition-related outcome

Table 1. Randomised controlled trials of folic acid supplementation and risk of adverse cognition-related outcomes

First author/date	Design/model	Studies (n)	Participants	Comparison	Results/RR	Comments
Balk et al. (2007)[7]	SR of RCTs of B6 and/or B12 and/or folic acid supplementation Model: SR; no MA	3 RCTs FA alone 6 RCTs FA; 7 other B-vits	n=75 Mean age: 74-80y n=862 Mean age: 73-76y	Placebo Placebo	19 outcome measures. Significant benefit in 4 outcomes (all in 1 trial). All others, NS Total – 37 tests. Placebo better for some tests (in 2 studies). All other NS.	2 out of 3 trials - participants with dementia or cognitive impairment. Limited evidence of benefit or no effect. Doses highly variable.
Dangour et al. (2010)	SR of RCTs of dietary factors in prevention & treatment of AD % dementia Model: SR; no MA	4 RCTs FA alone 6 RCTs FA + other B vits	n=1066 Age: 20-92y n=958 Age: 65+y	Placebo	3 out of 4 studies found FA significantly improved memory & cognitive function for some outcomes. No trials reported increased cognitive performance with FA + other B vits. 3 trials reported trend for increased performance/slower decline in placebo compared to vitamin groups.	Limited or no effect of benefit. Participants included people with cognitive impairment/decline
Ford & Almeida (2012)	SR & MA RCTs of homocysteine lowering treatment on cognitive function Model - random effects	19 RCTs (4 FA alone) 6 RCTs - included participants with cognitive impairment. 13 RCTs – participants without cognitive impairment.	n=5398 Mean age: >70y (except 1 study, mean age, 60y)	Placebo	Summary effect (all cognitive scores combined) - B vits vs placebo: Trials of people with cognitive impairment: SMD = 0.10 (95% CI, -0.08 to 0.28) Trials of people without cognitive impairment: SMD= −0.03 (95% CI, −0.1 to 0.04)	Conclude FA alone or in combination does not improve cognitive function in those with or without cognitive function.
Malouf & Grimley Evans (2008)	SR + MA TCTs FA +/- B12 Model: Fixed-effects unless evidence of heterogeneity	8 trials: 4 in older healthy people 4 in cognitive impaired or demented people	n=1523 Age: 65+y in 6 studies and 56-89y and 50-70y in 2 others.	Placebo (or FA vs FA + B-12)	Did not pool data because of heterogeneity in sample selections, outcomes, trial duration, and dosage. Some evidence of benefit in individual measures and studies but overall no consistent evidence of effect FA w/ or w/o B12 on cognitive function.	

FA, folic acid; MA, meta-analysis; SMD, standardised mean difference; SR, systematic review

[7] Balk et al mark results of p<0.10 as statistically significant. For consistency across papers in this assessment, a p<0.05 criterion has been applied to the results they report.

Table 2. Prospective studies of serum or plasma folate and risk of adverse cognition-related outcomes

First author/date	Design/model	Studies (n)	Cases (n)	Comparison	Results/RR	Comments
Cooper et al. (2015)	SR & MA of longitudinal studies of incident dementia in people with mild cognitive impairment. Model: no MA	3	301	n/a	Higher serum folate predicts less risk of conversion from mild cognitive impairment to dementia in 2 studies, NS in 1 study (smallest, shortest).	
Dangour et al. (2010)	SR prospective cohort studies on association of dietary factors with dementia. Model: no MA	11 (10 with other B vits; 1 of folate alone)	410 (dietary intake) 902 (blood folate)	3 studies reported dietary intake. 6 studies examined folate blood concentrations	2 studies reported significantly decreased risk of developing AD with increased folate intake. Serum folate: low folate increased risk of developing dementia & AD in 1 study & increased risk of conversion from MCI to dementia in 1 study. 4 studies reported no association between folate status & dementia or AD risk.	Higher folate intakes or status either NS or associated with lower risk of dementia.
Raman et al. (2007)	SR of longitudinal and case-control studies on association between folate, B6, B-12 and cognitive function and risk of AD in the elderly. Model: no MA.	10 – serum folate & CTP (8 PCS; 1 RC; 1 case-control) 9 cohorts for AD risk (11 studies)	2910 for folate conc & cognitive function 3886 total, 935 cases	High vs low 4 studies Quantiles – 5 studies Qualitative data – 1 study High vs low - 3 studies Quantiles – 5 studies Qualitative data – 2 studies	Low blood folate concentration associated with poorer outcome Association with high FA intake 'equivocal' (2 studies) Low folate increased risk in 3 cohorts with 384 cases, NS in 6 studies	Threshold for defining low blood folate concentration ranged from 3.6 to 27 nmol/L.
Vogel et al. (2009)	SR of longitudinal & cross-sectional studies on serum folate concentration & cognitive impairment. Model: no MA.	Exact numbers of studies & participants not stated.	Not provided	Not provided.	Narrative and subjective description of literature precludes objective characterisation	Authors conclude majority of data associates low folate with increased risk of cognitive decline & dementia, but direction of cause-effect is not clear.

AD, Alzheimer's disease; CTP, cognitive test performance, MA, meta-analysis; MCI, mild cognitive impairment; NS, not significant; PCS, prospective cohort study; RC, retrospective cohort; SR, systematic review

Summary of systematic reviews and meta-analyses on folic acid and risk of cancer

Prostate cancer

Table 3. RCTs of folic acid supplementation and prostate cancer risk.

Author/Date	Design	Model	Trials (n)/ Dose (mg/d)	Cases (n)	Comparison	RR (95% CI)	Comments
Wien et al. (2012)	Meta-analysis	Random effects	6 Dose Range: 0.8-2.5 Median: 1	632	FA ≥0.4 g/day vs placebo	1.24 (1.03-1.49)	
Qin et al. (2013a)	Meta-analysis	Random effects	5 Dose Range: 1-2.5 Median: 2	508	Treatment vs placebo	1.17 (0.84-1.62)	
Vollset et al. (2013)	Pooled analysis	Fixed effects	13 Dose Range: 0.5-40 Median: 2.0	656	Treatment vs placebo	1.15 (0.99-1.34)	

Table 4. Prospective studies of dietary folate intake and prostate cancer risk

Author/Date	Design	Model	Studies (n)/ Folate intake (µg/d)	Cases (n)	Comparison	RR (95% CI)	Comments
Tio et al. (2014a)	Meta-analysis	Random effects	5 Intake High: 259-444; median: 378 Low: 177-283; median: 215	12,898	High vs low	1.00 (0.96-1.05)	
Wang et al. (2014)	Meta-analysis	Random effects	5 Intake High: >259-444; median: 378 Low: <177-283; median: 215	12,898	High vs low	1.02 (0.95-1.09)	RR, 1.01 (0.99-1.02) per 100 µg/d

Table 5. Prospective studies of total folate intake (diet plus supplements) & prostate cancer risk

Author/date	Design	Model	Studies (n)/ Folate intake (µg/d)	Cases (n)	Comparison	RR (95% CI)	Comments
(Tio et al., 2014a)	Meta-analysis	Random effects	2 High: 468; 640 µg/d Low: 223; 300 µg/d	6,729	High vs low	1.00 (0.79-1.27)	Typo in upper CI, assumed 0.1.27 should be 1.27

Table 6. Prospective studies of serum or plasma folate and prostate cancer risk

First author	Design	Model	Studies (n) Folate conc. (nmol/L)	Cases (n)	Comparison	RR (95% CI)	Comments
Collin et al. (2010)	Meta-analysis	Fixed effects	6	1,807	Various[1]	1.19 (1.03-1.37)	
Tio et al. (2014a)	Meta-analysis	Random effects	6 High: 10.3-58.2; median, 17.1 Low: 4.8-10.9; median. 6.5	5,904	High vs low	1.14 (1.02-1.28)	
Wang et al. (2014)	Meta-analysis	Random effects	5 High: 10.3-58.2; median, 16.6 Low: <4.8-10.9; median, 6	4,451	High vs low	1.21 (1.05-1.39)	Dose response – RR=1.04 (1.00-1.07) per 5 nmol/L increment serum folate
Price et al. (2016)	Pooled analysis	Fixed effects	6 Geometric mean range 5.7-16.9	6,875	High vs low (top v bottom 5th)	1.13 (1.02-1.24)	Significant heterogeneity by grade, RR for high grade=2.30 (1.28-4.12)

[1] Quartiles, quintiles, per 4.5 nmol/l, per 18 nmol/l

Table 7. Genetic studies of *MTHFR* C677T polymorphism and prostate cancer risk

Author/date	Design	Model	Studies (n)	Cases (n)	RR (95% CI) for TT*	Comments
Collin et al. (2009)	Meta-analysis	Random effects	12	10,745	1.05 (0.89-1.23)	Included unpublished data
Zhang et al. (2012)	Meta-analysis	Fixed effects	15	7,306	0.96 (0.85-1.08)	
Abedinzadeh et al. (2015)	Meta-analysis	Fixed effects	22	10,832	0.91 (0.83-1.01)	
Chen et al. (2015)	Meta-analysis	Fixed effects	21	10,529	0.82 (0.69-0.97)	
Guo et al. (2015)	Meta-analysis	Fixed effects	23	11,348	0.90 (0.82-1.00)	

**Results are presented in terms of the OR/RR associated with the TT genotype relative to the CC genotype. The MTHFR TT genotype decreases the concentration of blood folate relative to CC genotype. The inference from an OR/RR> 1.0 is that a high level of folate is protective whilst an OR/RR< 1.0 suggests that a high level of folate increases risk.*

Breast cancer

Table 8. RCTs of folic acid supplementation & breast cancer risk.

Author/Date	Design	Model	Trials (n)	Cases (n)	Comparison	RR (95% CI)	Comments
Vollset et al. (2013)	Pooled analysis	Fixed effects	13 Dose Range: 0.5-40 Median: 2.0	297	Treatment vs placebo	0.89 (0.66-1.20)	99% CI, 95% not available
Qin et al. (2013a)	Meta-analysis	Random effects	4 Range: 1-2.5 Median: 2.25	213	Treatment vs placebo	0.82 (0.63-1.07)	Fixed effects only available for all cancers combined

Table 9. Prospective studies of dietary folate intake & breast cancer risk

Author/Date	Design	Model	Studies (n)	Cases (n)	Comparison/ Folate intake (µg/d)	RR (95% CI)	Comments
Larsson et al. (2007)	Meta-analysis	Random effects	8	8367	High vs low High: >294-522* *Median: >400* Low: <179-296 *Median: <225*	0.96 (0.87-1.05)	200 µg/d increments RR, 0.97 (95% CI, 0.88-1.07)
Chen et al. (2014)	Meta-analysis	Fixed effects	15	24,422	High vs low High: 277-522 *Median: >388* Low: 153-296 *Median: 217*	0.99 (0.94-1.04)	100 µg/d increments RR, 1.0 (95% CI, 0.98-1.01). U-shaped dose-effect relationship: Significant decreased breast cancer risk with intakes 153-400 vs <153µg/d >400 vs <153 µg/d – NS
Liu et al. (2014)	Meta-analysis	Random effects	15	24,083	High vs low High: 249-551 µg/d *Median: >374* Low: 126-296 µg/d *Median: <200*	0.98 (0.90-1.05)	Increments of 220 µg/d not associated with risk of breast cancer. RR, 0.96 (95% CI, 0.95-1.05)

Tio et al. (2014b): Excluded because unable to extract full numbers for the prospective studies; Li et al (2015) excluded because endpoint breast cancer mortality. Did not include 1 study because per 100 µg/d

Table 10. Prospective studies of total folate intake (diet + supplements) & breast cancer risk

Author/Date	Design	Model	Studies (n)	Cases (n)	Comparison	RR (95% CI)	Comments
Lewis et al. (2006)	Meta-analysis	Random effects	9	11,227	100 µg/d increment	0.99 (0.98-1.01)	Most but not all studies reported on diet & supplements
Larsson et al. (2007)	Meta-analysis	Random effects	6	8,165	High vs low High: >351 to >853 *Median:602* Low: 150 to <336 *Median: 219*	1.00 (0.87-1.14)	Increments of 200 µg/d RR, 1.01 (95% CI, 0.97-1.05)
Chen et al. (2014)	Meta-analysis	Fixed effects	11	16,717	High vs low High: >351-3663 *Median: 642* Low: 150-<472 *Median: 237*	0.99 (0.94-1.04)	Per 100 µg/day increment: RR, 1.00 (0.995-1.013)
Zhang et al. (2014)	Meta-analysis	Random effects	14	18,572	High vs low High: 277-918.9 *Median: 489* Low: 150-333.5 *Median: 205*	0.97 (0.90-1.05)	Per 100 µg/day increment: RR, 0.99 (0.98-1.01) Intake of 200-320µg/d associated with reduced risk Intakes >400µg/d associated with increased risk.

Table 11. Prospective studies of serum or plasma folate & breast cancer risk

Author/Date	Design	Model	Studies (n)	Cases (n)	Comparison	RR (95% CI)	Comments
Larsson et al. (2007)	Meta-analysis	Random effects	3	970	High vs low High: 13.6-31.7 nmol/l Low: 6.8-10.4 nmol/L	0.81 (0.59-1.10)	

Table 12. Genetic studies of *MTHFR* C677T polymorphism and breast cancer risk[8] *(OR/RR presented in terms of TT genotype relative to the CC genotype)*

First author/date	Design	Model	Studies (n)	Cases (n)	RR (95% CI) for TT	Comments
Lewis et al. (2006)	Meta-analysis	Random effects	17	6373	1.04 (0.94-1.16)	
Tang et al. (2014)	Meta-analysis	Fixed effects	25	13,874	1.09 (0.93-1.28)	
(Rai, 2014b)	Meta-analysis	Fixed effects	36	8040	1.24 (1.13-1.36)	Restricted to Asian populations
(Kumar et al., 2015)	Meta-analysis	Fixed effects	75	31315	1.10 (1.04-1.16)	

[8] (Macis et al., 2007) excluded because did not report TT relative to CC; (Rai, 2014a) excluded because MTHFR A1298C polymorphism; (Lissowska et al., 2007) excluded because MTHFR A222V and E429A

Colorectal cancer

Table 13. RCTs of folic acid supplementation and colorectal cancer risk

Reference/date	Design / Model/dose (mg/d)	Trials (n)	Cases (n)	Comparison	OR/RR (95% CI)
Cooper et al. (2010)	Systematic review Dose: 2.5-20; median, 2.5 Duration of treatment: 3y	3	128	FA + B vits vs placebo	1.13 (0.77-1.64)
Vollset et al. (2013)	Meta-analysis of individual data Fixed effects Dose:0.5-40; Median: 2.0	13	429	Folic acid vs placebo	1.07 (0.83-1.37)
Qin et al. (2015)	Meta-analysis Model: Fixed effects Dose: 0.5-2.5; Median: 1.5	8	381	FA vs placebo	1.00 (0.82-1.22)

Table 14. RCTs of folic acid supplementation and colorectal adenoma recurrence

Reference/date	Design / Model	Trials (n)	Cases (n)	Comparison	OR/RR (95% CI)
Cooper et al. (2010)	Systematic review Doses: 0.5 & 1.0; median, 0.75 Treatment duration: 3y	2	278	FA vs placebo	1.16 (0.97-1.39)
Ibrahim & Zekri (2010)	Meta-analysis Random & fixed effects Dose: 0.5-5 Median: 1	5	564	FA vs placebo Recurrence (n=4); fixed effects model By dose; random-effects model	 1.08 (0.87-1.33) 0.78 (0.49-1.24)
Fife et al. (2011)	Systematic review Dose: 0.5-2.5 Median: 1	3	850 383	FA supplementation up to 3 y FA supplementation over 3 y	1.09 (0.93-1.28) 1.35 (1.06-1.70)
Figueiredo et al. (2011)	Meta-analysis Model: Random-effects Dose: 0.5-1 Median: 1	3	 682 202	FA vs placebo All adenomas Advanced lesions	 0.98 (0.82–1.17) 1.06 (0.81–1.39)
van Dijk & Pot (2016)	Systematic review & meta-analysis. Model: Random-effects Dose: 0.5-5 Median: 1	4	444	FA vs placebos.	0.93 (0.69-1.25)

Table 15. Prospective cohort studies of dietary folate intake and colorectal cancer risk

Reference/date	Design / Model	Studies (n)	Cases (n)	Comparison	OR/RR (95% CI)/comment
Kennedy et al. (2011)	Meta-analysis Dietary folate intake Model: random effects	9	Not stated.	High vs low High: >212 to >634; median,335.5 Low: 103.3 to-<244; median, 184.4	0.92 (0.81-1.05) No dose effect relationship detected.
Kim et al. (2010)	Pooled analysis Dietary Model: random-effects	13	5720	Dietary folate: highest v. lowest quintile Intake by highest/lowest categories not stated; median intake range: 184-409 100 µg/d increment	0.92 (0.84-1.00) 0.98 (0.95-1.01)
Hou et al. (2013)	Excluded because reports results of other meta analyses already included here				

Table 16. Prospective cohort studies of supplemental folate intake and colorectal cancer risk

Reference/date	Design / Model	Studies (n)	Cases (n)	Comparison	OR/RR (95% CI)/comment
Heine-Broring et al. (2015)	Meta-analysis Model: Random-effect	3	4066	Highest vs lowest Dose-response (increase of 100 ug/d) High: >143 to ≥560; median, ≥400 Low: <50-<101; median, <83	0.88 (0.78-0.98) 0.98 (0.97-1.00) Difficult to interpret (participants may have been consuming other supplements)

Table 17. Prospective cohort studies of total folate intake and colorectal cancer risk

Reference/date	Design / Model	Studies (n)	Cases (n)	Comparison	OR/RR (95% CI)/comment
Kim et al. (2010)	Pooled analysis Model: random-effects	10	4015	Highest vs lowest quintile median intake range: 259-501 100 µg/d increment	0.85 (0.77-0.95) 0.99 (95% CI, 0.96-1.02)
Liu et al. (2015)	Meta-analysis Model: random-effects	19	24816	Highest vs lowest High: ≥212-≥1224; median, >441 Low: <103-422; median, 212	0.88 (0.81-0.95) Objective of study was to consider association with FA supplement intake, authors stated that if results reported both dietary & total vitamins, they used result for 'total' in main analysis.

Table 18. Prospective studies of plasma/serum folate concentration and colorectal cancer risk

Reference/Date	Design / Model	Studies (n)	Cases (n)	Comparison	OR/RR (95% CI)
Chuang et al. (2013)	Meta-analysis Model: fixed-effect flexible meta-regression Folate concentration Upper: -10.8-709.3; median, 31.5 Lower: 4.2-23.8; median, 6.1	8	3477	Serum/plasma folate concentration Regression analysis (linear; risk per 10 nmol/L) Regression analysis (non-linear; risk per 10 nmol/L) Highest vs lowest (radioimmunoassay) Highest vs lowest (microbiological assay)	 0.96 (0.91-1.02) 0.99 (0.96-1.02) 0.80 (0.61-0.99) 1.03 (0.83-1.22)

Table 19. Genetic studies of MTHFR C677T polymorphism & colorectal cancer risk

Reference and search date	Design / Model	Studies (n)	Cases (n)	Comparison	OR (95% CI)[1]
Kennedy et al. (2012)	Meta-analysis Random effects	59	26,731	All gender and ethnicity	0.88 (0.80-0.96)
Rai (2015)	Meta-analysis Random effects	34	9143		0.88 (0.74-1.04)
Sheng et al. (2012)	Meta-analysis Random effects	61	16111	All ethnicities and gender Asian only Males only	0.89 (0.82-0.97) 0.82 (0.69-0.97) 0.82 (0.71-0.93)
Taioli et al. (2009)	Meta-analysis & pooled analysis Fixed effects	Meta-analysis: 29 Pooled analysis: 14	Meta-analysis: 11,936 Pooled analysis: 5,068	*Meta-analysis* All White Asian *Pooled analysis* All White Asian	 0.83 (0.77-0.90) 0.83 (0.74-0.94) 0.80 (0.67-0.96) 0.85 (0.75-0.96) 0.91 (0.77-1.07) 0.68 (0.55-0.85)
Yang et al. (2012)	Meta-analysis Random effects	21	6692	All	0.84 (0.70-1.00)
Zhou et al. (2012)	Meta-analysis Random & fixed effects	n=41	17552	All groups (fixed effects) Asians (fixed effects) White (random effects)	0.83 (0.77-0.88). 0.76 (0.68-0.86) 0.85 (0.72-1.01)

[1]*Results are presented in terms of the odds ratio/relative risk associated with the TT genotype relative to the CC genotype. The MTHFR TT genotype decreases the concentration of blood folate relative to the CC genotype. The inference from an odds ratio greater than 1.0 is that a high level of folate is protective whilst an odds ratio less than 1.0 suggests that a high level of folate increases the risk of CRC.*

Overall cancer

Table 20. RCTs of folic acid supplementation and overall cancer risk

Author/Date	Design/duration	Model	Trials (n)/ Dose (mg/d)	Cases (n)	Comparison	RR (95% CI)	Comments
Wien et al. (2012)	Meta-analysis Included studies FA ≥0.4 mg/d FA +/- other B vitamins Median duration: 38 m	Random effects	10 Dose Range: 0.5-40 Median: 2.25	3515	FA ≥0.4 mg/d vs placebo	1.07 (1.00-1.14)	Increased risk in 4 studies with dose of 0.4-1 mg/d (RR, 1.21; 95% CI, 1.06-1.38) but not in those with doses > 1mg/d Follow-up time>60m – RR, 1.09 (1.1-1.18)
Baggott et al. (2012)	Meta-analysis Intervention duration ≥1y Median duration - 4.2 y	Fixed effects	6 Dose Range: 0.5-2.5 Median: 0.9	2416	0.5-2.5 mg/day vs placebo	1.21 (1.05-1.39)	Did not perform dose-dependent analysis because of narrow dose range
Vollset et al. (2013)	Pooled analysis Mean duration – 5 y	Fixed effects	13 Dose Range: 0.5-40 Median: 2.0	3713	Treatment vs placebo	1.06 (0.99-1.13)	Risk did not increase with treatment duration or daily dose.
Qin et al. (2013b)	Meta-analysis	Fixed effects	13 Dose Range: 0.5-40 Median: 2.0	3741	Treatment vs placebo	1.05 (0.99-1.11)	FA dose ≤ 1mg/d: RR=1.23 (1.06-1.43) No effect of FA dose or treatment duration on risk.

Mackerras et al. (2014): Excluded because unable to verify result in Figure 3 for Smith 2010

Table 21. Genetic studies of *MTHFR* C677T polymorphism and overall cancer risk

Author/Date	Design	Model	Studies (n)	Cases (n)	Reference genotype	RR (95% CI) for TT	Comments
Tang et al. (2014)	Meta-analysis	Fixed effects	134	46207	677 CC	1.08 (1.01-1.17)	

Unmetabolised folic acid

Measurement of UMFA in plasma/serum

Table 22. Intervention studies

Study	Population	Objective/Intervention/duration	Results	Conclusion/comments
Pentieva et al. (2016) Northern Ireland	Pregnant women at 14 wks gestation, who had taken FA supplements (400 µg/d) as recommended during 1st trimester (n=67).	Objective: to investigate effect of FA during pregnancy on UMFA in maternal plasma & newborn cord plasma. Intervention: Start of 2nd trimester 1. FA (400µg/d) 2. Placebo Duration: 22 weeks (until end of pregnancy). Blood collection: *Non-fasting* blood samples collected at weeks 14 & 36. Cord blood samples obtained at delivery. Folate measurement: LC-tandem MS. LOD[9] for UMFA = 0.27 nmol/L	Baseline (14 wks gestation): Proportion with detectable UMFA = 33%. Post intervention (wk 14 to 36): Significant increase in plasma total folate & 5MTHF but not in UMFA conc. or in % of UMFA relative to total plasma folate Proportion with detectable UMFA significantly greater in FA group (p=0.002) 1. 42% 2. 16% Mean plasma UMFA (nmol/L) 1. 0.44 (± 0.80) *(0.51 ± 1.41, pre-intervention)* 2. 0.13 (± 0.49) *(0.46 ± 1.36, pre-intervention)* Plasma UMFA as % of total folate 1. 1.16 (± 2.07) *(1.42 ± 3.7 pre-intervention)* 2. 0.38 (± 1.2) *(1.13 ± 2.7 pre-intervention)* Cord blood: plasma folate & 5-MTHF significantly higher in infants of FA supplemented mothers but no diff between groups in % of cord samples with detectable UMFA (20%, FA gp & 11%, placebo gp; p=0.34).	FA dose of 400 µg/d throughout 2nd & 3rd trimester increased maternal & neonate folate status but did not increase circulating UMFA concentrations. Although significantly higher proportion in FA group had detectable FA in plasma (42% v 16% in placebo gp; p=0.002), this did not correspond to a significant increase in UMFA concentration in maternal circulation. Findings suggest FA metabolised to reduced folates in mother before transfer to placenta.

[9] Limit of detection.

Study	Population	Objective/Intervention/duration	Results	Conclusion/comments
Houghton et al. (2009) Canada	Lactating women (36 wk gestation) intending to exclusively breast feed for >4m (n=69) < Mean age: 32 ± 4y	Objective: compare effects of folic acid & 5-MTHF on total milk folate & determine if UMFA present in breast milk. Intervention: (within 1 wk after birth of infants) 1. Folic acid (400µg/d) 2. 5-MTHF (416 µg/d; equimolar dose) 3. Placebo All received daily supplement of B6 (1 mg), B12 (3 µg), ferrous fumarate (4 mg) Duration: 16 weeks Blood/milk collection: Blood collected 16 wks postpartum (did not specify fasting status); milk samples collected between 1300 & 1450 because of wide variation in milk folate conc. over 24 hrs. Folate measurement – Plasma & RBC folate & total milk folate measured by microbiological assay; milk FA measured using HPLC. LOD for UMFA not specified.	All reported consumption of FA containing supplements during pregnancy (mean FA intake, 911 ± 251 µg/d). After 16 wks: Mean plasma folate con significantly greater in supplemented participants compared to placebo group ($p<0.0001$) Milk folate concentration similar between all groups & remained stable from 4-16 weeks postpartum. No significant correlation between maternal blood folate concentrations & total milk folate concentrations. UMFA detected in 96% of milk samples & represented ~ 8% of total milk folate concentration; no group differences in UMFA concentrations at 16 wk postpartum.	Did not measure UMFA in lactating mothers. Milk folate concentrations similar to those reported before fortification.
Tam et al. (2012) Canada	Women (n=40) Age: 18-45 y	Objective: (1) Examine relationship between plasma UMFA concentration & dietary FA & total folate intake (2) examine effect of FA supplementation on fasting plasma concentrations of UMFA. Intervention: Randomised open label trial 1. FA (1.1 mg/d) 2. FA (5 mg/d) Duration: 30 weeks Blood collection: *Fasting* sample (min 6h): baseline, 2, 4, 6, 12 & 30 wks. Folate measurement: total folate, microbiological assay; FA, affinity-HPLC. LOD for UMFA = 0.18 nmol/L	*Baseline % with detectable UMFA in plasma* 1. 63% 2. 68% Significant change in % with detectable UMFA over time in both grps but no diff between grps. 1. Out of 7 with undetectable UMFA at baseline, all had undetectable UMFA at 30 wks; out of 12 with detectable UMFA at baseline, 11 (58%) had detectable UMFA at 30wks. 2. Out of 6 with undetectable UMFA at baseline, 5 had undetectable UMFA at 30 wks; out of 13 with detectable UMFA at baseline, 10 (53%) had detectable UMFA at 30wks. *Median plasma UMFA concentration* (nmol/L) *Baseline* *30 weeks* 1. 4.8 3.1 2. 3.7 4.4 No significant change over 30 wks in either grp. When data pooled from both grps, change in UMFA significant ($p=0.019$).	Significant decline in UMFA between weeks 12 & 30 despite ongoing supplementation & sustained total folate concentrations. Plasma UMFA at wk 30 not significantly different from baseline. Effect of supplementation small relative to natural variation in circulating UMFA. Data suggest homeostatic mechanisms that limit systemic exposure to UMFA, such as downregulation of carrier-mediated transport systems & upregulation of FA metabolism.

Study	Population	Objective/Intervention/duration	Results	Conclusion/comments
Sweeney et al. (2007) Ireland	Adults (n=20) Age: 20-40y	Objective: appearance of UMFA after consumption of FA for prolonged period. Intervention: FA fortified bread (400, 200, 100 µg/d) Phase 1 – all, 400 µg/d FA for 14 wks. Phase 2 – 2 slices bread supplying total: Wk 1 - 400 µg/d FA. Wk 2 – 200 µg/d FA. Wk 3 - 100 µg/d FA. Between each wk consumed 400 µg/d FA supps for 7d to re-saturate total serum folate levels. Blood collection - *fasted* sample Phase 1: collected wks 10 & 14, 6h post supplementation. Phase 2: Day 7 of each regimen; sample taken pre-prandially then after 1st slice, collected every hr for 4 hrs. 2nd slice then administered & sample collected every hr for 3h. Folate measurement: Microbiological assay used to measure total serum folate & red cell folate; HPLC for UMFA. LOD for UMFA >0.31 nmol/L	Baseline - UMFA not detected in any samples. Intervention phase 1 Serum & red cell folate increased significantly (p<0.001). At wk 14 – UMFA detected in 18/19 (95%) participants but only slightly elevated in 7 (37%) & below level for accurate quantification. Mean UMFA = 0.048 nmol/L; as % of total serum folate = 1.4% Intervention phase 2 UMFA present in 17/19 (89.5%) participants on day 7 of consuming 400 µg/d pre-prandially. UMFA increased post-prandially in all participants after consumption of slice 1 (200 µg) and slice 2 (200 µg) (p<0.001). UMFA not detected in any participants on day 7 of regimen consuming 200 or 100 µg/d pre- or post-prandially. Total folate concentration – no significant change over 8 hrs after consuming bread containing 400, 200 or 100 µg/d.	Out of the 3 fortified bread regimens, only the highest (400 µg/d) led to appearance of UMFA.
Kirsch et al. (2015) Germany	Individuals invited to participate via local newspapers or during a stay in geriatric centre (n=59) Mean age: 67y	Objective: measure changes of serum & whole blood folate forms after 1 y supplementation with FA, B12 & B6 in older adults. Intervention: DBCRT; daily 1. FA (500 µg), B12 (500 µg) B6 (50 mg), Ca (456 mg), vitamin D (30 µg) 2. Ca (456 mg), vitamin D (30 µg) Duration: 1 year. Blood collection: Fasting samples (≥8hr) collected at baseline, 6 m & 1y after supplementation. Folate measurement: measured using UPLC-MS/MS LOD for UMFA= 0.20 nmol/L	5-MTHF Baseline: comparable between groups (85.6%). After 6m & 1y, 5-MTHF ~. 2.2-2.6 x higher in gp 1 UMFA: Baseline: 5% with detectable serum UMFA. 6m: 29% gp 1 & 7% gp 2 with detectable UMFA (p=0.031) 1y:81% of gp 1 & 11% in gp 2 had detectable UMFA (p<0.001) Mean UMFA (nmol/L) Baseline: 0.05 (±0.08) 6m: 0.15 (±0.13) 1y: 0.45 ((±0.09) (p<0.05) UMFA concentration showed direct correlation with 5-MTHF at baseline (r=0.25; p=0.057) & after 1y (r=0.35, p=0.089).	Mean UMFA after 1y similar to values reported in study in Ireland where FA fortification not mandatory (Boilson, 2012) suggesting 500 µg/d FA over 1y is effectively metabolised to other forms. UMFA correlated to serum 5-MTHF even in unsupplemented people (at baseline). Therefore, possible that 5-MTHF endogenous source of FA. Co-supplementation with B6 & B12 could have impact on folate form distribution in serum.

Study	Population	Objective/Intervention/duration	Results	Conclusion/comments
Obeid et al. (2016) Germany	Residents in geriatric centre for recovery from bone fractures or other surgery (n=58) Median age: 82y (range 69-96)	Objective: compare effect of short-term supplementation with FA or B-complex on serum UMFA. Intervention (daily) single blind, non-placebo controlled: 1. FA (400 µg) 2. FA (400 µg) + B12 (10 µg) +B6 (8 mg) Duration: median 23 days. Blood collection: Fasting samples (overnight) collected on 2nd day of study and day before discharge. Folate measurement: UPLC-MS/MS LOD for UMFA = 0.20 nmol/L	Serum 5-MTHF[10] & UMFA increased significantly in both grps ($p<0.001$) with no difference between grps in extent of increase. Serum UMFA increased significantly in both grps: 1. From <LOD at baseline to 0.61 nmol/L ($p<0.001$) 2. From <LOD at baseline to 0.17 nmol/L ($p=0.002$) Proportion with detectable UMFA 1. From 20% at baseline to 76% ($p<0.001$) 2. From 7% at baseline to 33% ($p=0.109$) UMFA as % of total 5MTHF (0% at baseline): 1. 3.26 ($p<0.001$) (3.3% total folate) 2. 0.77 ($p=0.039$) (1.1% total folate)	Compared to FA alone group, serum UMFA lower in FA+B12 & B6 group. Consuming FA together with vitamins that enhance its metabolism may lower prevalence of detectable UMFA.
Obeid et al. (2011) Germany	Older adults in geriatric health centre (n=74). Median age = 82y	Objective: examine concentrations of primary folate forms (5-MTHF, THF, FA) before & after high dose FA. Intervention: DBRCT 1. FA (5 mg), B12 (1 mg), B6 (40 mg)/day 2. Placebo Duration: 3 weeks Blood collection: Fasting samples (> 8h) collected on 3rd day of admission & 3 weeks later before discharge. Folate measurement: UPLC-MS/MS LOD for UMFA = 0.20 nmol/L	At baseline: 26% with detectable plasma UMFA After 3 wks 1. 97% with detectable UMFA; median plasma concentration, 15.3 nmol/L (0.07 nmol/L at baseline) Median 5-MTHF & total folate significantly increased ($p<0.001$). 2. 41% with detectable UMFA; median plasma concentration, 0.17 nmol/L (0.08 nmol/L at baseline). Median 5-MTHF & total folate concentrations slightly lower than those at start. UMFA as % of total folate: 2.6% in placebo & 14.9% in FA grp. UMFA concentration positively related to 5-MTHF & THF concentrations ($p<0.05$).	FA grp - substantial increase in UMFA - related to higher conc of THF & 5MTHF. Increase in 5MTHF stronger than that of THF; suggests FA effectively reduced & further converted into active forms. Wide range of interindividual variations in FA (144%), THF (58%), 5-MTHF (52%) in FA gp suggest factors other than folate intake might be responsible. Differences in absorption, storage, or activities of folate cycle enzymes may account for such variations.

[10] 6S)-5-methyltetrahydropteroylglutamate [(6S)-5-CH_3-H_4Pte]

Table 23. Observational studies

Author (year)/Country	Population	Objective/study details	Results	Conclusion/Comments
Obeid et al. (2010) Germany	Pregnant women recruited from consecutive deliveries in hospital (n=87) including 24 mother-infant pairs Newborns (n=29) Non-pregnant women (n=25) not taking FA supplement *Cross-sectional*	Objective: - investigate primary folate forms in pregnant women and umbilical cord blood at delivery. Also tested effect of MTHFR polymorphism. Methods Blood sample (non-fasted) obtained from mothers 1-12h before birth. Folate forms measured using Ultra Performance LC/MS. LOD for UMFA = 0.20 nmol/L	Maternal – n=25 consumed FA (400 µg/d) throughout pregnancy. Higher total serum folate & 5-MTHF conc in supplemented pregnant women not associated with significant difference in UMFA compared with non-supp pregnant women. No significant difference in % pregnant women with detectable UMFA according to FS supplement use (44% in both groups)) Umbilical cord blood No difference in conc of folate forms by maternal FA use. % with detectable UMFA did not significantly differ according to maternal supplement use: 52.6% from non-supplemented & 60% from supplemented. UMFA as % of total folate did not differ by maternal supplement use: 0.54%, supplemented & 0.43%, non-supplemented. Mother cord pairs No significant diff in serum UMFA conc between maternal & cord. UMFA as % of total folate significantly lower in cord than in maternal serum (0.49% vs 2.4%) UMFA positively correlated to 5-MTHF in maternal & cord serum. Genotype – No difference in folate form conc. by MTHFR.	Detectable but small amounts UMFA observed in cord serum at birth. Study might be underpowered to detect significant differences in cord blood UMFA according to maternal vitamin use. Alternatively, speculate that FA might be produced in vivo from internal nonenzymatic oxidation of 5-MTHF or THF. Maternal FA supplement use did not explain detection of UMFA in maternal or cord blood.
Plumptre et al. (2015) Canada	Pregnant women between 12-16 wks gestation (n=368). Mean age: 32.5 (5)y *Prospective*	Objective: assess maternal & cord blood folate & UMFA in cohort of pregnant women & newborns & effect of maternal intakes of folate & FA supp on maternal & cord blood conc of folate & UMFA. Effect of 4 variants in fetal MTHFR & DHFR genes on cord blood folate & UMFA. Methods: Maternal non-fasted blood drawn at wks 12-16 of pregnancy & at delivery. Mean time since food/supps consumed before blood draw = 5h. Umbilical cord blood obtained at delivery. Serum & RBC folate concentrations measured using protein-binding immmunoassays; UMFA measured using LC/MS. Dietary intake - semi-quantitative FFQ. LOD for UMFA = 0.2 nmol/L	Maternal early pregnancy - UMFA detected in 97% of women. Median % of UMFA to total serum folate = 3.09% (IQR, 1.90-8.93%). Maternal UMFA conc did not differ according to FA supplement use. Cord blood - UMFA detectable in 93% samples Median % UMFA to total serum folate = 1.34% (IQR, 0.92-82.12%). Cord plasma UMFA 72% lower than maternal plasma UMFA (p<0.0001) Plasma UMFA not significantly higher in cord blood from mothers who supplemented in late pregnancy (p=0.07). RBC folate & UMFA weakly correlated in maternal blood in early pregnancy (r=0.18; p=0009) but not in cord blood (r=0.08; p=0.23); serum folate & UMFA strongly correlated in maternal blood (r=0.81; p<0.0001) & weakly correlated in cord blood (r=0.24; p=0.0002). Genotype - No significant associations between 3 fetal MTHFR & DHFR variants & serum & RBC folate or UMFA; fetal MTHFR 677TT genotype associated with significantly lower cord serum folate & higher cord RBC folate than wild type.	Results suggest UMFA does not accumulate in the fetus even with a high folate status and detectable UMFA in mothers. Fetus has limited folate storage in liver & must use what is immediately available via the placenta. Therefore the UMFA that reaches the fetus is likely metabolised to active folate forms in a more efficient manner.

Author (year)/Country	Population	Objective/study details	Results	Conclusion/Comments
Sweeney et al. (2005) Ireland	Newborn infants (n=11) *Cross-sectional*	Objective: Examine UMFA in cord blood of newborns of mothers consuming normal diet & serum from 4d old infants post formula feeding. Methods: Blood collected from umbilical cord. Follow-up serum sample (n=9) collected 4d after birth (90 mins after last formula feed). Serum UMFA assay – HPLC/microbiological assay. LOD for UMFA = 0.31 nmol/L	Cord blood - UMFA present in all samples Mean UMFA concentration = 0.42 nmol/L None of the mothers were consuming FA supplements. At 4d (with formula feed) UMFA detected in 7 samples (78%) Mean UMFA concentration = 1.06 nmol/L	Since none of the mothers were consuming FA supplements, FA in cord blood must have arisen from FA intake from fortified foods.
Sweeney et al. (2009) Ireland	Mothers about to undergo caesarean section (n=20) & offspring immediately after delivery. Age: 26-39y *Cross-sectional*	Objective: examine whether infants *in utero* have UMFA in cord blood after period of maternal fasting. Methods: Blood collection: from mothers & umbiglical cords immediately after caesarean section. All had fasted for ≥ 8h prior to surgery. None consuming FA at start of study. Plasma folate & RCF: microbiological assay; UMFA – column switching HPLC. LOD for UMFA – not reported	Maternal - UMFA present in 90% Significant correlation between maternal plasma folate concentration & maternal plasma UMFA (r^2=0.300; p=0.007) Mean total plasma folate mothers: 29.8 (19.6) nmol/L Mean UMFA in mothers: 0.39 (0.325) nmol/L (1.3% of total folate) Significant correlation between maternal FA & cord blood FA concentration (r=0.38; p=0.004) Cord blood – UMFA present in 85% Mean UMFA in cord blood: 0.27 (0.15) nmol/L	Samples from fasting non-pregnant females were not included in study
Vaish et al. (2016) Ireland	Healthy children attending routine minor surgery (n=68) Age: ≤16y 0-5y – 23% 6-10y – 38.5% 11-16y – 38.5% *Cross-sectional*	Objective: Explore concentrations of fasting UMFA in circulation of children exposed to voluntary folic acid fortification in Ireland. Methods: Fasting blood samples (8h) collected from children before minor surgery. Plasma folate & RCF measured using microbiological assay; UMFA - LC-MS. Diet assessed using short dietary questionnaire for parents. LOD for UMFA = 0.53 nmol/L	UMFA detected in 10% of sample (95% CI, 4.2-20.1%) Mean UMFA concentration: 0.07 (±0.23) UMFA contributed 1.94% of plasma total folate. Mean plasma folate: 35 nmol/L (range: 7-104 nmol/L)	Limitation: small convenience sample not representative of the Irish population – difficult to say if findings can be translated to the population of children in Ireland.

Author (year)/Country	Population	Objective/study details	Results	Conclusion/Comments
Sweeney et al. (2009)) Ireland	Blood donors (n=50) Age 27-60y *Cross-sectional*	Objective: Explore if UMFA present in Irish people exposed to current range of vol fortified foods. Methods: Blood collection: unfasted samples collected from blood donors attending routine blood donation. Plasma folate & RCF: microbiological assay; UMFA – column switching HPLC. LOD for UMFA – not reported	UMFA present in 98% of blood donors. Plasma folate correlated with UMFA concentration (r=0.110; p=0.011) Mean plasma folate – 32 (17.3) nmol/L Mean UMFA: 0.72 (0.9) nmol/L (2.25% of total folate)	Majority of population in Ireland now exposed to liberal voluntary fortification, appears to have circulating UMFA. Samples from fasting males were not included.
Boilson et al. (2012) Ireland	Participants ≥60y from the Lifeways Cross-Generation Cohort Study (n=135) Mean age: 67.4y (±5.9) *Cross-sectional*	Objective: (i) Measure persistent UMFA conc. in non-institutionalised older people exposed to liberal voluntary fortification. (ii) Whether genes involved in folate metabolism are associated with plasma UMFA concentration. Methods: Blood collection – early morning fasting sample. 2 dietary intake questionnaires: semi-quantitative FFQ. Plasma folate & RCF – microbiological assay; UMFA – column switching HPLC LOD for UMFA = 0.07 nmol/L	UMFA detected in 94% of participants. Mean UMFA concentration = 0.39 nmol/L (range 0.07-1.59), accounting for 1.3% of total folate 19% consumed FA supplements habitually; 8% had taken recently (within last 24h but not previous 8h). UMFA significantly lower in those with no recent or habitual FA intake (0.11 ±3 compared with 0.43 ± 0.02; p<0.0005). Habitual FA intake significantly related to UMFA (p<0.0005) but effect of recent FA intake not significant (p=0.108). Dose-response between habitual FA intake & UMFA (regression analysis): doubling FA intake = 14.3% (95% CI, 9.7-18.8%) increase in UMFA. Genotyping analysis: No significant correlations with MTHFR 677C→T, DHFR 19-bp intron deletion, or MTHFDIL	UMFA in plasma of most of this older cohort even after overnight fast. No threshold effect for habitual FA intake & UMFA.

Author (year)/Country	Population	Objective/study details	Results	Conclusion/Comments
Kalmbach et al. (2008) USA	Participants of 6th examination of Framingham Offspring Study (Jan 1995-Aug 1998 (therefore includes people unexposed & exposed to mandatory FA fortification) (n=1258) Mean age: 58y *Cross-sectional*	Objective: determine functional impact of DHFR polymorphism on UMFA, total folate, tHcy & RBC folate concentrations & evaluate possible interaction between FA intake & DHFR genotype with any measures of folate status. Hypothesised DHFR polymorphism alters capacity to reduce dietary FA & thereby limits assimilation of FA into endogenous folate forms. Methods: Fasting blood samples. Plasma total folate & RBC measured using microbiological assay. UMFA measured using updated affinity/HPLC method Dietary intake assessed by semi-quantitative self-administered FFQ. LOD for UMFA = not reported.	n=126, homozygous for deletion allele (del/del); n=646, heterozygous for deletion allele (WT/del); n=396, homozygous for wildtype allele (WT/WT). Prevalence of high serum UMFA (defined as >1.35 nmol/L) higher in del/del homozygotes with FA intakes >500 µg/d (47%; 95% CI, 32-63%) compared with WT/del heterozygotes (21%; 95% CI, 14-29%) and WT/WT (24%; 95% CI, 14-35%). Prevalence of high serum UMFA in those with intakes <500 µg/d unrelated to DHFR genotype. Also interaction (p=0.01) between FA intake & DHFR genotype & RBC folate. In those with FA intakes <250 µg/d, mean RBC folate was 732.3 nmol/L (95% CI, 669.1-801.4) for del/del compared with 793 nmol/L (95% CI, 750.3-837.4) WT/del and 844.4 nmol/L (95% CI, 787.8-905.1) WT/WT. RBC folate in those consuming < 250 µg/d did not differ between the 3 genotypes. No significant interaction between DFHR genotype & FA intake with plasma total folate.	Describes an effect of a 19-bp deletion allele in DHFR gene on measures of folate status that depends on FA intake. Homozygotes for 19-bp deletion in DHFR consuming >500 µg/d FA had increased prevalence of high UMFA compared with WT/WT & WT/del genotypes. Homozygotes who consume < 250 µg/d of FA had lower RBC folate compared with WT/WT genotype. Results suggest DHFR polymorphism directly impairs FA metabolism at both low & high FA intakes.

Table 24. National Monitoring Programmes (NHANES)

Author (year)/Country	Population	Objective/study details	Results	Conclusion/Comments
Bailey et al. (2010) USA	NHANES 2000-2001 (surplus sera project analysed UMFA conc of individuals ≥ 60y) (n=1121) Mean age: 70y *Cross-sectional*	Objective – to examine UMFA concentrations in relation to dietary & supplemental folate & status biomarkers in NHANES 2001-2002 Methods: Blood sample – Obtained details on fasting before blood draw. UMFA & 5-MTHF measured using affinity/HPLC method. Dietary data obtained from one 24-hour recall. LOD for UMFA = 0.18 nmol/L	UMFA detected in 38% of population (fasted for mean of 10h). UMFA concentration: mean = 4.4 (0.6) nmol/L; median = 1. 2 (0.2) nmol/L UMFA represented ~2.25% of total serum folate in population. In those with detectable UMFA, it represented ~6% of total serum folate. In those with detectable UMFA, % contribution of UMFA did not vary by 5-MTHF quartile (5.5-7.5%) however mean UMFA concentration significantly higher in those in highest 5-MTHF quartile. 47% of population reported use of FA containing supplements. Significantly higher % of FA dietary supplement users in those with detectable UMFA. Total FA intakes (food & supplements) significantly higher in detectable UMFA grp but relation between UMFA & total FA intake not strong when examined in whole population.	Could not identify specific amount of FA leading to UMFA appearing in serum. Although supp intake higher in detectable UMFA gp (235 µg/d) than undetectable UMFA grp (140 µg/d), 40% of undetectable UMFA gp were supp users and 39% of detectable UMFA grp not supp users. Supp use alone is insufficient factor in determining UMFA. Suggest detectable UMFA gp represents sensitive subpopulation with altered FA metabolism – genetic differences may be contributing determinant in FA variability.

Author (year)/Country	Population	Objective/study details	Results	Conclusion/Comments
Pfeiffer et al. (2015b) USA	NHANES 2007-2008 (population aged ≥1y) (n=2676) 26% children (1-11y) 12% adolescents (12-19y) 62% adults (≥20y) *Cross-sectional*	Objective: describe distributions of serum 5-MTHF & UMFA concentrations in US population & assess how they correlate with dietary folate intake. Also evaluate factors associated with UMFA concentrations > 1 nmol/L. Methods: Blood samples included fasting & non-fasting 5-MTHF & UMFA measured using HPLC-MS/MS. Dietary intake assessed by two 24-hour recalls. LOD for UMFA = 0.3 nmol/L	38% fasted ≥8h; 27% reported using FA supplements in previous 30d. UMFA detected in >95% of population. When samples grouped according to weighted 5-MTHF quartiles, mean UMFA significantly higher in Q4 (5.6 nmol/L) than in Qs 1-3 (1.8, 1.4, 3.4 nmol/L respectively) but relative UMFA contribution to sum of 5-MTHF & UMFA, fairly constant across quartiles (3.1-5%). In fasting samples (≥8h) relative UMFA conc. appeared lower (2.2-3.5%). Mean UMFA concentration significantly higher ($p<0.05$) in supplement users vs non-users (1.5 & 0.79 nmol/L respectively). Fasting status significantly associated with lower UMFA conc in SUP+ & SUP- gps with larger concentration diffs between non-fasting (<3h) & fasting (≥8h) in SUP+ gp (1.17 nmol/L) than SUP- gp (0.269 nmol/L) Prevalence of UMFA >1 nmol/L: 33% overall & 21% in fasting (≥8h) adults. UMFA > 1 nmol/L associated with being older, non-Hispanic black, nonfasting (<8h), smaller body surface area, higher total FA intake (diet & supps) & higher RBC concentrations.	UMFA concentration >1 nmol/L mainly associated with non fasting & higher FA intake from diet & supplements. Findings raise possibility that UMFA concentration may be an indicator of a mismatch between supply (i.e., intakes) and cellular demand (i.e., requirements) but some common genetic polymorphisms may also have an impact.
Pfeiffer et al. (2015a) USA	NHANES 2011-12 (population aged ≥1y) (n=7462) 27% children (1-11y) 14% adolescents (12-19y) 59% adults (≥20y) *Cross-sectional*	Objective: describe serum concentrations of several folate forms in the US population ≥1 y (NHANES 2011-12). Methods: Blood samples included fasting & non-fasting Serum folate forms measured using HPLC-MS/MS; whole blood folate measured by microbiologic assay. LOD for UMFA = 0.14 nmol/L	UMFA detected in 99.9% of samples. Mean UMFA: 1.21 nmol/L (95% CI, 1.15-1.92); range: 0.14-282 nmol/L UMFA contributed 4% to serum total folate. Correlation between UMFA & 5-MTHF (r=0.44) & total serum folate (r=0.5). Mean UMFA (nmol/L) according to fasting time (h): <3 1.52 (95% CI, 1.44-1.61) 3 - <8 1.40 (95% CI, 1.29-1.52) ≥8 0.98 (95% CI, 0.92-1.04) p=0.01 Mean UMFA (nmol/L) according to FA sup use (last 24h) Yes 2.10 (95% CI, 1.92-2.29) No 1.02 (95% CI, 0.96-1.09) p<0.0001	UMFA significantly higher in persons who reported consuming FA containing supplements during previous 24h. Focus seems to be on MeFox (an oxidation product of 5-MTHF). Patterns observed for MeFox suggest altered folate metabolism dependent on biological characteristics.

Table 25. Studies examining the association between UMFA and adverse health outcomes

Author (year)	Health outcome/objective/study type	Population/Methods	Results	Conclusions/comments
Cho et al. (2015)	Colorectal cancer Objective: Evaluate association between prediagnostic plasma UMFA and CRC risk *Prospective cohort*	Population: Nurses Health Study (NHS)/Health Professionals Follow-up Study (HPFS) Methods: Participants grouped into 3 categories: 3. Undetectable 4. < 0.5 nmol/L 5. >0.5 nmol/L Adjusted for multiple risk factors UMFA measurement: LC/MS/MS LOD for UMFA = 0.25 nmol/L	Follow-up time: NHS, 20-21y; HPFS, 15-17y. Cases, 618; controls, 1207 UMFA detected in 21.4% of controls & 22% of cases. Those with detectable UMFA: median conc = 0.61 nmol/L Overall, prediagnostic UMFA not associated with CRC risk Compared to those with undetectable UMFA: RR=1.03 (95% CI = 0.73-1.46) for less than 0.5 nmol/L. RR = 1.12 (95% CI, 0.81-1.55) for ≥0.5 nmol/L.	Blood samples collected prior to mandatory folic acid fortification so concentrations not reflective of those after fortification which occurred during follow-up. Study suggests prediagnostic UMFA plasma concentrations from prefortification period in US not associated with CRC risk.
Hu et al. (2016)	Cancer Objective: Examine associations of folate intake, folate biomarkers, & presence of UMFA with overall cancer incidence among adults ≥57y in NHANES 1999-2002. *Prospective cohort*	Population: Adults ≥57y to 85y (n=3997) Methods: Data from NHANES 1999-2002 participants linked to Medicare data & National Death Index. Folate & FA intake assessed from 24h recall. RBC folate & serum folate measured by radioassay; UMFA - revised affinity/HPLC. LOD for UMFA = 0.18 nmol/L	Median follow-up time 6.3y; 125 cancer cases. No associations observed between presence of UMFA & risk of cancer incidence (data were not shown). Significant inverse association between highest quartile of RBC folate (956 nmol/L) vs reference group (539 to <721 nmol/L) with overall cancer incidence. Adjusted HR = 0.54 (95% CI, 0.31-0.93).	Absence of relationship between UMFA & cancer incidence may be due to insufficient power. Examined association between presence vs absence of UMFA rather than amount of UMFA & risk of cancer incidence.
Morris et al. (2010)	Cognitive test performance Objective: examined associations between 5-MTHF & UMFA in blood samples obtained from NHANES (1999-2002) participants with cognitive test performance. *Cross-sectional*	Population: Adults ≥60y from NHANES (1999-2002); n=1430; mean age, 70y; Methods: combined data from 2 surveys in which cognitive data were collected into single dataset Dietary data assessed from 24h recall. Fasted blood sample collected (6-9h fasting time) Plasma folate measured using radioassay; UMFA measured using affinity/HPLC LOD for UMFA = 0.027 nmol/L	UMFA detected in 32% – on average UMFA 6% of total serum folate (IQR: 1-8%). UMFA concentrations up to 111 nmol/L but 90% <1.78 nmol/L. Presence vs absence of detectable UMFA related to increased odds of anaemia in alcohol users: OR=3.37 (95% CI, 1.42-8.01). In participants with low B12 status (serum B12 <148 pmol/L or MMA ≥210 nmol/L) mean DSST score for those with detectable UMFA almost 5 points lower than that of unexposed participants: -4.86 (-9.09 to -0.63); p=0.03.	UMFA linked to increased odds of haemotologic & cognitive problems in combination with low B12 status.

.

ANNEX 3

Literature search methodology

1. Literature searches were conducted to identify relevant research on folic acid and health outcomes, which had been published since SACN's *folate and disease prevention report* (SACN, 2006) and SACN's *report to the Chief Medical Officer on folic acid and colorectal cancer risk* (SACN, 2009).

Search

Folic acid

2. Structured searches of Embase, MEDLINE, Scopus and the Cochrane Library were undertaken between April and June 2016. Three consecutive searches were conducted as follows: the first search (07/04/16) was limited to meta-analyses and systematic reviews; the second search (03/06/16) was limited to pooled analyses; the third search (28/06/16) combined the previous searches of MEDLINE and Embase and removed a database review filter which had resulted in a number of important studies not being identified. The results of all three searches were then combined.

3. The search was restricted to studies since 2005. Recently published reviews from international organisations (European Food Safety Authority, 2014; Food and Drug Administration, 2016; Food Safety Authority of Ireland, 2016; National Toxicology Program, 2015; Norwegian Scientific Committee for Food Safety, 2015) were also hand searched for relevant citations.

4. The Embase search strategy used on 28/06/16 is provided in the Appendix.

Inclusion and exclusion criteria

5. Meta-analyses, systematic reviews and pooled analyses of randomized controlled trials and prospective cohort studies in humans were included. Meta-analyses of case-control studies were included only for polymorphism publications as case-control studies are most often used to assess the relationship between polymorphisms and disease risk. All other study types were excluded. Only studies in healthy populations were considered; studies that investigated folic acid as a treatment for people with a disease or medical condition were excluded. Foreign language publications were excluded.

6. All health outcomes associated positively or adversely with folic acid supplementation were initially included. Due to the limited time available for completing the review, it was subsequently agreed to focus on the following potential adverse outcomes: i) cancer (prostate, breast, colorectal and total); ii) masking of vitamin B12 deficiency and iii) acceleration of cognitive decline in older individuals with low vitamin B12 status. It was also agreed that methylenetetrahydrofolate reductase polymorphism studies related to these outcomes would be included.

7. Publications which combined folic acid with other vitamins or minerals were initially excluded. As this would result in the exclusion of many important meta-analyses, it was subsequently

agreed to include those that assessed folic acid + B12 or other B vitamins. Publications that assessed the association between folate status and health outcomes were also included.

Screening

8. The search results were screened in duplicate by a member of the SACN secretariat and a colleague in Food Standards Scotland. Publications were screened in two phases: i) the combined first and second search results ii) any new publications identified in the third search. Studies identified from each screening phase were then combined.

9. Publications were initially screened based on title and abstract; the full article was reviewed if there was any uncertainty about whether it should be included. Any differences were resolved by discussion before the list of publications to be considered by the working group was agreed. The working group agreed the final list of publications to be included in the review.

Unmetabolised folic acid

10. A separate search of primary literature was undertaken on 3 May 2016 to identify studies that investigated: i) the effect of folic acid supplementation on plasma or serum unmetabolised folic acid (UMFA); ii) the metabolism of folic acid; iii) the relationship between UMFA and health outcomes. It was agreed to search for primary research studies as there is still limited research in this area. Four databases were searched: Embase, MEDLINE, Scopus and the Cochrane Library. The Embase search strategy is shown in the Appendix.

Search Results

11. The combined literature search of folic acid and health outcomes identified 2644 citations of which 51 were included in the review (Figure 1). Although it did not meet the inclusion criteria for study type, it was agreed that one cross-sectional study by Qi et al (2014) should be included that provided evidence on the masking of B12 deficiency as there is very limited evidence in this area.

12. For the literature search on unmetabolised folic acid, 225 studies were identified, of which 23 were included in the review (Figure 2).

Figure 1: Flow chart of search and selection process of meta-analyses, systematic reviews and pooled analyses included in update

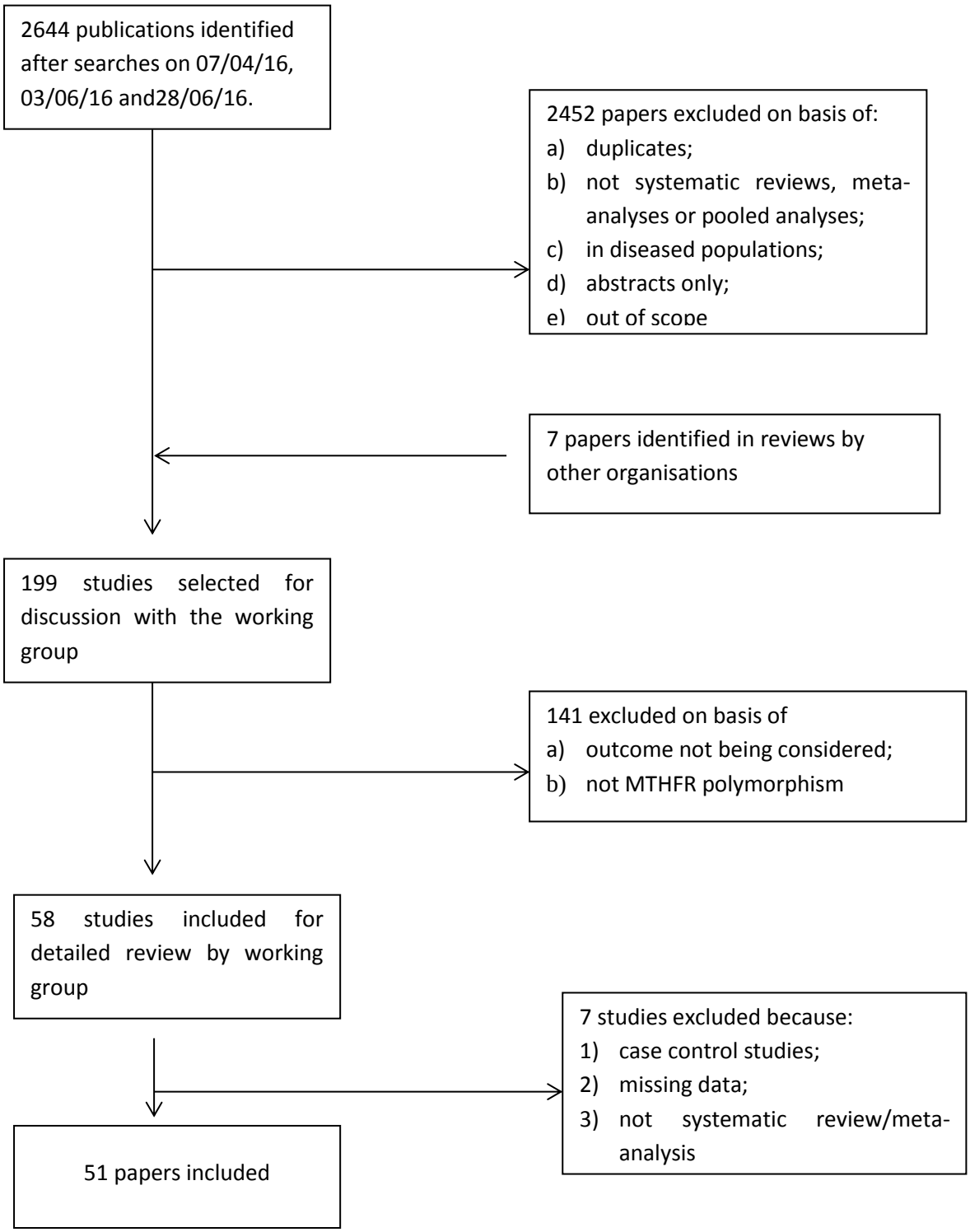
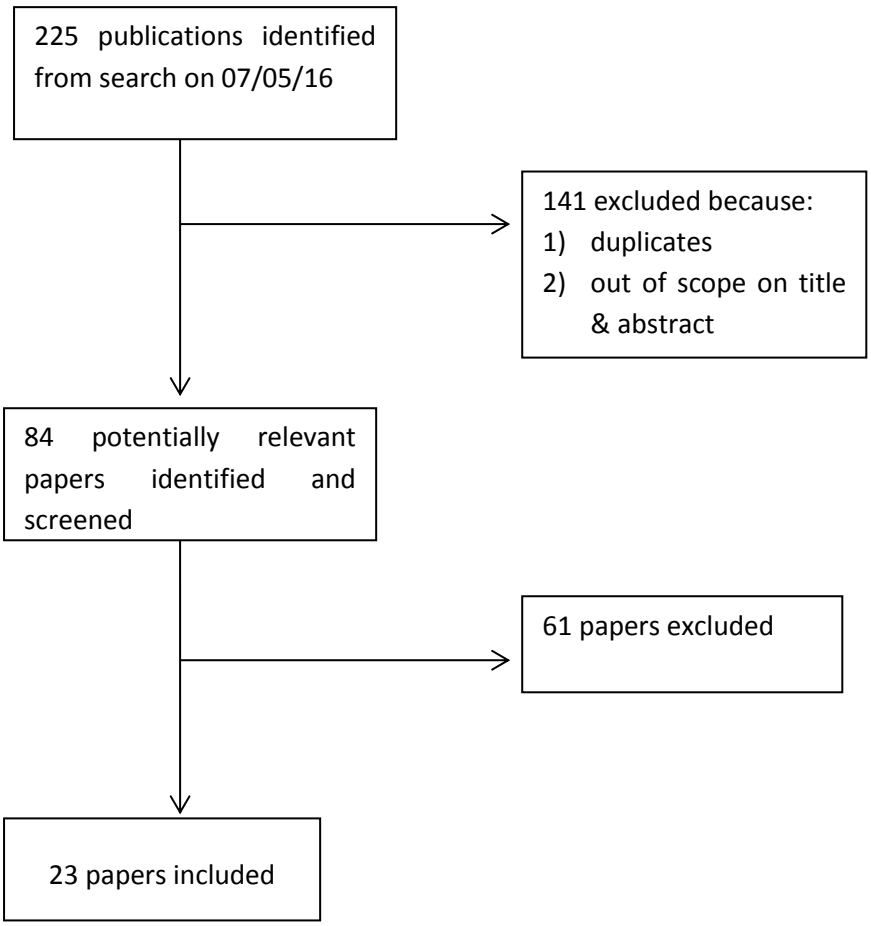


Figure 2: Flowchart of search and selection process for studies on unmetabolised folic acid included in update



Embase search strategy

Folic acid

1. exp FOLIC ACID/
2. "folic acid " OR folate*
3. exp SYSTEMATIC REVIEW/
4. exp META ANALYSIS/
5. ("systematic review*" OR meta-analys* OR "meta analys*").ti,ab
6. (pooled ADJ (analys* OR mean OR estimate*)).ti,ab
7. 1 OR 2
8. 3 OR 4 OR 5
9. 7 AND 8
10. 1 AND 6
11. 9 [Limit to: Publication Year 2000-2016 and (Publication Types Review)]
12. 10 [Limit to: Publication Year 2000-2016 and (Publication Types Review)]

Unmetabolised folic acid

1. exp FOLIC ACID/
2. FOLIC ACID DEFICIENCY/
3. ("folic acid " OR folate*).ti,ab
4. (unmetaboli?ed OR un-metaboli?ed OR metaboli?ation).ti,ab
5. 1 OR 2 OR 3
6. 4 AND 5

ANNEX 4

Individual studies included in each meta-analysis/systematic review

Cognitive decline in older adults with vitamin B12 deficiency

Folate intake – prospective cohort studies			
Individual Studies	**Cooper (2015)**	**Dangour (2010)**	**Vogel (2009)**
Corrada (2005)		x	
Morris (2006)		x	
Luchsinger (2007)		x	
Blasko (2012)	x		

Folate intake – randomized controlled trials						
Individual studies	**Balk (2007)**	**Malouf (2008)**	**Vogel (2009)**	**Dangour (2010)**	**Ward (2010)**	**Ford (2012)**
Rapin (1988)						
Deijen (1992)						x
Fioravanti (1997)	x	x		x	x	
Fioravanti (1998)			x			x
Sommer (1998)		x				
Bryan (2002)	x	x	x	x	x	x
Seal (2002)						x
Clarke (2003)		x				x
Sommer (2003)	x		x	x		
Vital (2003)				x		
Garcia (2004)						x
Hvas (2004)			x			x
Obeid (2005)				x		
Stott (2005)				x	x	x
Lewerin (2005)			x	x	x	x
Eussen (2006)		x		x	x	x
McMahon (2006)			x	x	x	x
Pathansali (2006)		x			x	x
Durga (2007)		x	x	x	x	x
McNeill (2007)					x	
Aisen (2008)			x			x
Connelly (2008)		x				x
Kang (2008)						x
Ford (2010)						x
De Jager (2011)						x
Kwok (2011)						x

Folate status – prospective cohort studies				
Individual studies	**Raman (2007)**	**Dangour (2010)**	**Michelakos (2013)**	**Cooper (2015)**
Ortega (1996)[11]			x	
Clarke (1998)	x			
Ebly (1998)[1]			x	
Lindeman (2000)[1]			x	
Wang (2001)	x	x		
Bowirrat (2002)	x			
Jones (2002)	x			
Maxwell (2002)	x	x		
Sheshadri (2002)	x			
Snowden (2002)[12]				

[11] Cross sectional study

Stewart (2002)[1]			x	
Marengoni (2004)			x	
Quadri (2004)[1]			x	
Elias (2005)	x			
Mooijaart (2005)[1]	x		x	
Kado (2005)	x			
Nurk (2005)	x			
Ramos (2005)			x	
Ravaglia (2005)	x	x		
Tucker (2005)	x			
Velestino study (2005)[1]			x	
Annerbo (2006)		x		
Hin (2006)[1]			x	
Kang (2006)	x			
Ravaglia et al (2006)		x		x
Clarke (2007)			x	
Haan (2007)		x		
Morris (2007)[1]			x	

Total cancer

Folate intake – prospective cohort studies	
Individual Studies	**Wein (2012)**
Lashner (1997)	x
Skinner (2004)	x
Stolzenberg-Solomon (2006) (PLCO)	x
Slatore (2008) (VITAL)	x
Maruti (2009) (VITAL)	x
Oaks (2010) (PLCO)	x

Folate intake – randomized controlled trials					
Individual studies	**Baggot (2012)**	**Wien (2012)**	**Qin (2013)**	**Vollset (2013)**	**Mackerras (2014)**
Paspatis (1994)					x
Kim (2001)					x
Zhu (2003)		x			x
Charles (2004)/(2005)		x			
Liem (2004) (FOLARDA)					X
Toole (2004) (VISP)		x	x	x	X
Liem (2005) (GOES)					x
Sato (2005)					x
Bonaa (2006) (NORVIT)			x	x	x
Flicker (2006)and Ford (2008)					x
Lonn (2006) (Hope 2)	x	X	x	x	x
Righetti (2006) (total cancer mortality)			x		
Cole (2007) (AFPPS)	x	x	x	x	x
den Heijer (2007) (VITRO)				x	
Durga (2007) (FACIT)					x
Fernandez Miranda (2007)					x
Jamison (2007) (HOST)		x	x	x	
Aisen (2008)					X
Ebbing (2008)	x		x		x
Jaszewski (2008)					x
Logan (2008) (UKCAP)	x	x	x	x	x
Albert (2008) (WAFCS)					x
Van Uffelen (2008)					x
Zhang (2008) (WAFCS)	x	x	x	x	x
Ebbing (2009) (WENBIT) and (NORVIT)		x		x (WENBIT only)	x

[12] Retrospective cohort study

Figuerido (2009) (AFPPS)					x
Hodis (2009) (BVAIT)			x		X
Wu (2009) (NHS/HPPS Folic Acid prevention Trial)		x	x	x	x
Armitage (2010) (SEARCH)		X	x	x	X
Clarke (2010) (VISP)					X
Galan (2010) (SU-FOL-OM3)				x	
Heinz (2010) (total cancer mortality)			x		
Smith (2010) (VITACOG)					x
VITATOPS Trial Study Group (2010)				x	x
Walker (2010)					x
Kwok (2011)					x
Andreeva (2012)			x		
Hankey (2012) (VITATOPS)			x		x

Folate status – MTHRF polymorphism case control studies	
Individual studies	**Tang (2014)**
Head & Neck	
Kureshi (2004)	x
Neumann (2005)	x
Capaccio (2005)	x
Reljic (2007) (+breast)	x
Suzuki (2007)	x
Breast	
Campbell (2002)	x
Sharp (2002)	x
Ergul (2003)	x
Langsenlehner (2003)	x
Semenza (2003)	x
Grieu (2004)	x
Lee (2004)	x
Lin (2004)	x
Shrubsole (2004)	x
Chen (2005)	x
Deligezer (2005)	x
Justenhoven (2005)	x
Kalemi (2005)	x
Chou (2006)	x
Hekim (2007)	x
Jakubowska (2007) (+ovarian cancer)	x
Stevens (2007)	x
Vollset (2007)	x
Langsenlehner (2008)	x
Ericson (2009)	x
Tuo (2009)	x
Alshatwi (2010)	x
Naushad (2011)	x
Jakubowska (2012)	x
Lung cancer	
Shen (2001)	x
Jeng (2003)	x
Shi (2005)	x
Suzuki (2007)	x
Cui (2011) (China)	x
Cui (2011) (Korea)	x
Esophageal cancer	
Song (2001)	x
Stolzenberg- Solomon (2003) (+ stomach cancer)	x
Zhang (2004) (Germany)	x

Zhang (2004) (China)	x
Wang (2005) (+ stomach)	x
Sarbia (2005) (+stomach cancer)	x
Yang (2005)	x
Li (2008)	x
Qin (2008)	x
Umar (2010)	x
Stomach cancer	
Miao (2002)	x
Kim (2005)	x
Shen (2005)	x
Granziano (2006)	x
Lacasana-Narvarro (2006)	x
Weng (2006)	x
Boccia (2007)	x
Mu (2007)	x
Zeybek 2007 (+ colorectal)	x
De Re (2010) (2009)	x
Galvan- Portillo (2009)	x
Colon cancer	
Ma (1997) (colorectal)	x
Park (1999) (colorectal)	x
Slattery (1999) (colon)	x
Ryan (2001) (colorectal)	x
Keku (2002) (colon)	x
Plaschke (2003) (colorectal)	x
Toffoli (2003) (colon)	x
Curtin (2004) (colon)	x
Kim (2004) (colon/rectal)	x
Ulvik (2004) (colon/rectal)	x
Yin (2004) (colorectal)	x
Le (2005) (rectal)	x
Van Guelpen (2006) (colorectal)	x
Wang (2006) (colon/rectal)	x
Murtaugh (2007) (rectal)	x
El Awady (2009) (colorectal)	x
Lacopetta (2009) (colorectal)	x
Komlosi (2010) (colon/rectal)	x
Promthet (2010) (colon)	x
Chandy (2010) (colorectal)	x
Kang (2011) (colorectal)	x
Pardini (2011) (colorectal)	x
Sameer (2011) (colorectal)	x
Zhu (2011) (colorectal)	x
Cervical cancer	
Kang (2005)	x
Shekari (2008) (+ AML)	x
Tong (2011)	x
Bladder cancer	
Moore (2004)	x
Lin (2004)	x
Rouissi (2009)	x
Izmirli (2011)	x
Haematological cancers	
Skibola (1999) (ALL)	x
Matsuo (2001) (Malignant lymphoma)	x
Gemmati (2004) (ALL/malignant lymphoma)	x
Habib (2005) (Malignant lymphoma)	x
Lightfoot (2005) (Malignant lymphoma)	x
Da Costa Ramos (2006) (AML)	x

Siraj (2007) (Malignant lymphoma)	x
Kim (2009) (ALL/AML/CML/Myelodyspastic syndrome)	x
Lightfoot (2010) (ALL/AML)	x
Sadananda (2010) (ALL)	x
Damnjanovic (2010) (ALL)	x
Karathanasis (2011) (ALL)	x
Brain cancers	
Kafadar (2006) (High-grade gliomas/Meningiomas)	x
Da Costa (2012) (Astrocytic tumors)	x
Oral cancers	
Weinstein (2002)	x
Vairaktaris (2006)	x
Thyroid cancer	
Ozdemir (2012)	x
Liver cancer	
Mu (2007)	x
Cui (2012)	x
Gallbladder cancer	
Srivastava (2008)	x
Bile duct cancer	
Ko (2006)	x
Prostate cancer	
Singal (2004)	x
Van Guelpen (2006)	x
Ovarian cancer	
Jakubowska (2012)	x
Endometrial cancer	
Esteller (1997)	x
Paynter (2004)	x
Multiple myeloma	
Chiusolo (2006)	x
Kim (2007)	x
Acute leukemia	
Wiemels (2001)	x
Cancer	
Jin (2009)	x

Breast cancer

Individual Studies	Lewis (2006)	Larsson (2007)[13]	Chen (2014)	Liu (2014)	Tio (2014)[3]	Zhang (2014)	Li (2015)
Holmes (1999)							x
Zhang (1999)	x	x	x	X	x	x	
Rohan (2000)	x	x	x[14]	X		x	
Sellers (2001)	x	x	x	X	x	x	
Sellers (2002)			x[4]		x		x
Cho (2003)	x	x	x[4]	X		x	
Feigelson (2003)	x	x	x[4]	X		x	
Le Marchand (2004)	x						
Sellers (2004)			x[4]		x[4]	x	
Baglietto (2005)	x	x	x[4]	x		x	
Tjønneland (2005)	x	x		x			
Zhang (2005)			x		x		
Lajous (2006)		x	x	x	x	x	
McEligot (2006)							x
Stolzenberg-Solomon (2006)	x	x	x	x	x	x	

[13] case-control studies also included in meta-analyses but not listed here

[14] Not included in the meta-analysis

Tjønneland (2006)				x		x	x
Cho (2007)			x	x	x		
Ericson (2007)			x		x	x	
Ischitani (2008)							
Kabat (2008)			x	x	x		
Larsson (2008)			x	x	x	x	
Xu (2008)					x		x
Duffy (2009)			x[4]		x	x	x
Nuehouser (2009)							
Pocobelli (2009)							
Larsson (2010)							
Roswall (2010)			x		x	x	
Stevens (2010)			x	x	x	x	
Shrubsole (2011)			x	x	x	x	
Harris (2012)							x

Folate status – prospective cohort studies	
Individual Studies	**Lewis (2006)**
Wu (1999)	x
Zhang (2003)	x
Rossi (2006)	x

Folate status – MTHRF polymorphism case control studies						
Individual studies	**Lewis (2006)**	**Lissowaska (2007)**	**Macis (2007)**	**Rai (2014) Asia**	**Rai (2014)**	**Kumar (2015)**
Sharp (2002)	x	x	x		x	x
Campbell (2002)	x	x	x			x
Semenza (2003)	x	x	x			x
Langsenlehner (2003)	x	x	x			x
Ergul (2003)	x	x	x	x	x	x
Shrubsole (2004)	x	x	x	x	x	x
Forsti (2004)	x	x	x		x	x
Lee (2004)	x	x	x	x		x
Grieu (2004)	x	x	x			x
Lin (2004)	x	x	x	x		x
Le Marchand (2004)	x	x	x	x	x	x
Qi (2004)	x			x	x	x
Chen (2005)	x	x	x		x	x
Kalemi (2005)		x	x			x
Deligezer (2005)	x	x	x	x		x
Justenhoven (2005)	x	x	x		x	x
Chou (2006)		x	x	x	x	x
Kalyankum& Jamil (2006)				x		x
Xu (2007)					x	x
Hekim (2007)	x	x	x	x		x
Jakubowska (2007)					x	
Kan (2007)				x	x	x
Lissowska (2007)		x			x	x
Macis (2007)						x
Reljic (2007)						x
Stevens (2007)					x	x
Yu (2007)				x		x
Inoue (2008)				x	x	x
Kotsopoulos (2008)					x	x
Suzuki (2008)				x		x
Cheng (2008)				x	x	x
Langsenlehner (2008)						x
Mir (2008)				x	x	x

Ericson (2009)					x	x
Gao (2009)				x	x	x
Ma (2009)				x	x	x
Platek (2009)					x	x
Hennquez-Hernandez (2009)						x
Cam (2009)				x		x
Maruti (2009)						x
Ma (2009)						x
Li (2009)				x		x
Yuan (2009)				x		x
Jin (2009)				x		x
Bentley (2010)						x
Alshatwi (2010)				x		x
Lin (2010)					x	
Sangrajrang (2010)				x		x
Weiner (2010)					x	x
Wu (2010)				x		x
Batschauer (2011)						x
Cerne (2011)						x
Hosseini (2011)				x	x	x
Mohammed (2011)				x		
Hua (2011)				x	x	x
Nausad (2011)				x		x
Prasad (2011)				x		x
Akram (2012)				x	x	x
Barbosa (2012)						x
Diakite (2012)						x
Jakubowska (2012)						x
Lajin (2012)				x		x
Papandreou (2012)					x	
Wu (2012)				x	x	x
Liu (2013)				x	x	x
Ozen (2013)				x	x	x
He (2014)						x
Huang (2014)						x
Jiang-hua (2014)						x
Wang (2014)						x
Weiwei (2014)				x	x	x
Kakkoura (2015)						x
Lopez-Cortes (2015)						x
Lu (2015)						x
Singh (2015)						x
Italian Chemoprevention Trial (IEO Study)			x			

Colorectal cancer

Folate intake – prospective cohort studies				
Individual Studies	**Kim (2010)**	**Kennedy (2011)**	**Heine-Broring (2014)**	**Liu (2015)**
Giovannucci (1995)	x			
Glynn (1996)	x			
Bandera (1997)	x			
Giovannucci (1998)	x			
Kato (1999)	x			
Su (2001)		x		x
Flood (2002)	x	x		x
Fuchs (2002)				x
Harnack (2002)	x	x		x
Konings (2002)	x			
Sieri (2002)	x			

Terry (2002)	x			
Jacobs (2003)	x			x
Larsson (2005)	x	x		
Zhang (2006)	x	x		x
Ishihara (2007)		x		x
Kabat (2008)		x		
Schernhammer (2008)		x		x
de Vogel (2008)		x		x
Shrubsole (2009)				x
Gibson (2011)				x
Lee (2011) (multivitamins and Folic acid)			x	x
Stevens (2011)			x	x
Zschahiz (2012)				x
Bassett (2013)				x
Razzak (2013)				x

Folate intake – randomized controlled trials							
Individual studies	**Carroll (2010)**	**Fife (2011)**	**Figueirerdo (2011)**	**Ibrahim (2010)**	**Qin (2015)**	**van Dijk (2016)**	**Cooper (2010)[15]**
Paspatis (1994)				x			
Kim (2001)				x			
Zhu (2002)	X						
Baron (2003) (AFPPS)	X		x				
Zhu (2003)	X						
Cole (2005) (AFPPS)	X						
Lonn (2006) (HOPE-2)	X				x		
Lonn (2006) (HOPE-2)	X				x		
Cole (2007)(AFPPS)	X	x	x		x	x	
Figueirerdo (2008)				x			
Jaszewski (2008)	X			x		x	
Logan (2008) (ukCAP)	X	x	x	x	x	x	
Nodic Cochrane Centre (2008) (Health outcomes prevention evaluation 2006)		x					
Zhang (2008) (WAFACS)	X				x		
Wu (2009)					x	x	
Armitage (2010)					x		
Fife (2011) (NHS/HPFS)			x				
Hankey (2012)					x		
Gao (2013)					x		

Folate status – nested case control studies	
Individual studies	**Chuang (2013)**
Eussen (2010) (EPIC)	x
Kato (1999) (NYUWHS)	x
Le Marchand (2009) (MEC)	x

[15] No inclusion of supplement or references

Lee (2012) (HPFS)	x
Lee (2012) (NHS)	x
Lee (2012) (PHS)	x
Otani (2008) (JPHC)	x
Shrubsole (2009) (SWHS)	x

Folate status – MTHRF polymorphism case control studies							
Individual studies	**Tailoli (2009)**	**Kennedy (2012)**	**Sheng (2012)**	**Yang (2012)**	**Zhou (2012)**	**Figueirerdo (2013)**	**Rai (2015)**
Chen (1996)	X		x		x		
Ma (1997)	X		x		x		
Park (1999)	X	x	x	x	x		x
Slattery (1999)			x		x		
Slattery (2000)	X		x				
Delgado- Enciso (2001)		x					
Ryan (2001)	X		x		x		
Keku (2002)	X	x	x		x		
Le Marchand (2002)	X	x	x		x		x
Matsuo (2002)	X	x	x	x	x		x
Sarchse (2002)	X		x		x		
Shannon (2002)	X	x	x		x		
Yin (2002)							x
Heijmans (2003)			x		x		
Heijmans (2003)[16]		x					
Huang (2003)				x			x
Plaschke (2003)	X	x	x		x		
Pufulete (2003)	X	x	x		x		
Toffoli (2003)	X	x	x		x		
Curtin (2004)					x		
Kim (2004)	X		x	x	x		x
Jiang (2004)		x		x			
Ulvik (2004)	X	x	x		x		
Yin (2004)	X	x	x	x	x		x
Jiang (2005)	X	x	x		x		x
Kono &Chen (2005)						x	
Landi (2005)	X	x	x				
Matsuo (2005)	X	x	x	x	x		x
Miao (2005)		x		x			x
Otani (2005)	X	x	x	x	x		x
Le Marchand (2005)	X	x	x		x		
Battistelli (2006)		x	x				
Chang (2006)			x				
Koushik (2006)	X		x		x		
Van Guelpen (2006)	X	x	x		x		
Wang (2006)	X	x	x		x		x
Huang (2007)						x	
Hubner & Houlston (2007)		x				x	
Lima (2007)	X	x	x		x		
Chang (2007)	X	x		x	x		x
Curtin (2007)		x					
Jin (2007)		x		x			x
Murtaugh (2007)	X	x	x		x		
Osian (2007)		x	x				

[16] Prospective cohort study

Zeybek (2007)	X	x	x		x		x
Cao (2008)		x	x	x			x
Eklof (2008)			x				
Kury (2008)		x	x				
Lightfoot (2008)		x					
Mohebbi (2008)					x		
Mokarram (2008)		x	x				x
Sharp (2008)	X	x	x		x		
Theodoratou (2008)		x	x		x		
Zhang (2008)		x		x			x
Arreola (2009)			x				
De Vogel (2009)[6]		x	x				
Derwinger (2009)		x	x				x
El Awady (2009)		x					
Gallegos- Arreola (2009)		x	x				x
Haghighi (2009)		x	x				
Iacopetta (2009)			x		x		
Peralta (2009)			x				
Reeves (2009)						x	
Taioli (2009)			x				
Vogel (2009)		x	x				x
Chandy (2010)		x	x	x	x		x
Cui (2010)		x					
Eussen (2010)		x					
Fern´andez- Peralta (2010)		x	x				
Karpinski (2010)			x				
Kim (2010)		x	x				
Komlosi (2010)		x	x				x
Naghib alhossaini (2010)			x				
Pardini (2010)		x	x	x			x
Promthet (2010)		x					
Wettergren (2010)				x			x
Yang (2010)				x			x
Zhu (2010)		x					
Abuli (2011)			x				
Eussen (2011)		x			x		
Guimaraes (2011)		x			x		
Jokie (2011)			x	x			x
Kang (2011)							x
Li (2011)		x			x		
Pardini (2011)		x		x			x
Kim (2011)		x	x		x		x
Sameer (2011)		x	x				x
Prasad and Wilkhoo (2011)			x				
Vossen (2011)		x	x	x			x
Zhu (2011)						x	
Zacho (2011)		x		x			x
Kim (2012)		x					
Lee (2012)							x
Ozen (2014)							x

Prostate cancer

Folate status – prospective cohort studies				
Individual studies	**Colin (2010)**	**Price (2016)**	**Tio (2014)**	**Wang (2014)**
ATBC		x		

CARET		x		
EPIC		x		
Janus		x		
NSDDC		x		
ProtecT		x		
Weinstein (2003)[17]	x		x	x
Hultdin (2005)[3]	x		x	x
Rossi (2006)	x			
Johansson (2008)[3]	x		x	x
Belby (2010)[3]			x	x
Collin (2010)[3]			x	
De Vogel (2013)[3]			x	x

Folate status – randomized controlled trials	
Individual studies	**Colin (2010)**
Figueiredo (2009)	x
Ebbing (2009)	x

Folate status – MTHRF polymorphism case control studies					
Individual studies	**Abedinzadeh (2015)**	**Collin (2009)**	**Guo (2015)**	**Yadak (2016)**	**Zhang (2012)**
Kimura (2000)	x	x	x		x
Heijmans (2003)	x	x	x		x
Cicek (2004)	x	x	x	x	x
Singal (2004)	x	x	x	x	x
Amundadottir (2006) (deCODE)		x			
Van Guelpen (2006)	x	x	x	x	x
Johansson (2007)	x	x	x		x
Reljic (2007)	x	x	x		x
Yeager (2007) (CGEMS)		x			
Eeles (2008) (UKGPCS)		x			
Marchal (2008)	x	x	x	x	x
Stevens (2008)	x	x	x	x	x
Collin (2009)	x		x	x	x
Muslumanoglu (2009)	x		x	x	x
Cai (2010)	x		x	x	x
Safarinejad (2010)	x		x	x	x
Wu (2010)	x		x	x	x
Kucukhuseyin (2011)	x		x		x
Fard-Esfahani (2012)	x		x		
Mandal (2012)			x		
Raju K (2012)	x				
Kobayashi (2012)	x		x		
Vidal (2012)	x		x	x	
Jackson (2013)			x	x	
López-Cortés (2013)	x		x	x	
de Vogel (2013)	x		x		
Ghasemi (2014)	x				

[17] Nested case control study

ANNEX 5

UMFA concentration in the NDNS (years 1-4), UK

Figure 1: UMFA concentration in participants aged 1.5-3y

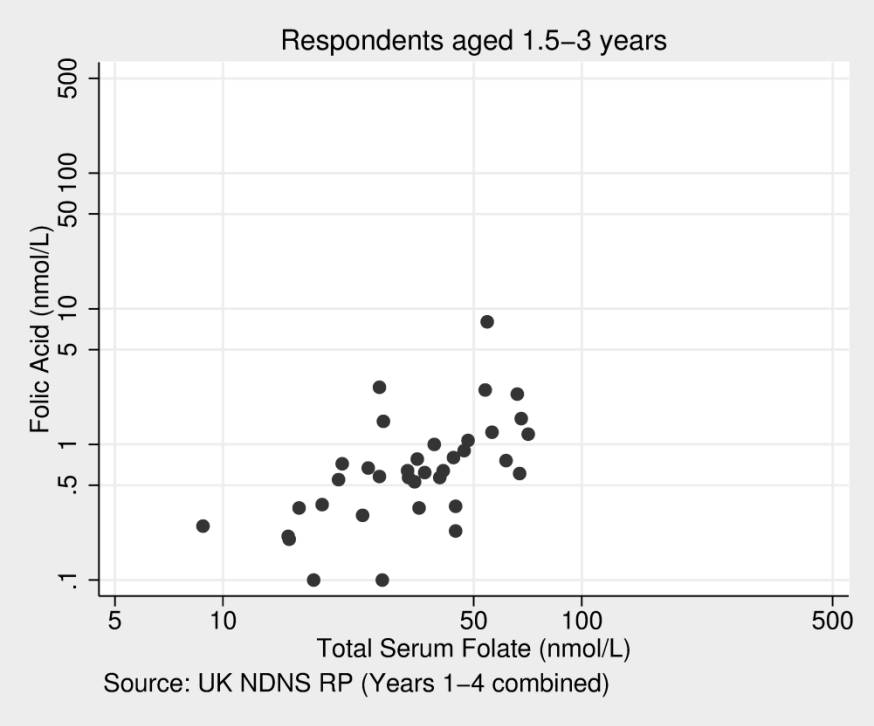


Figure 2: UMFA concentration in participants aged 4-10y

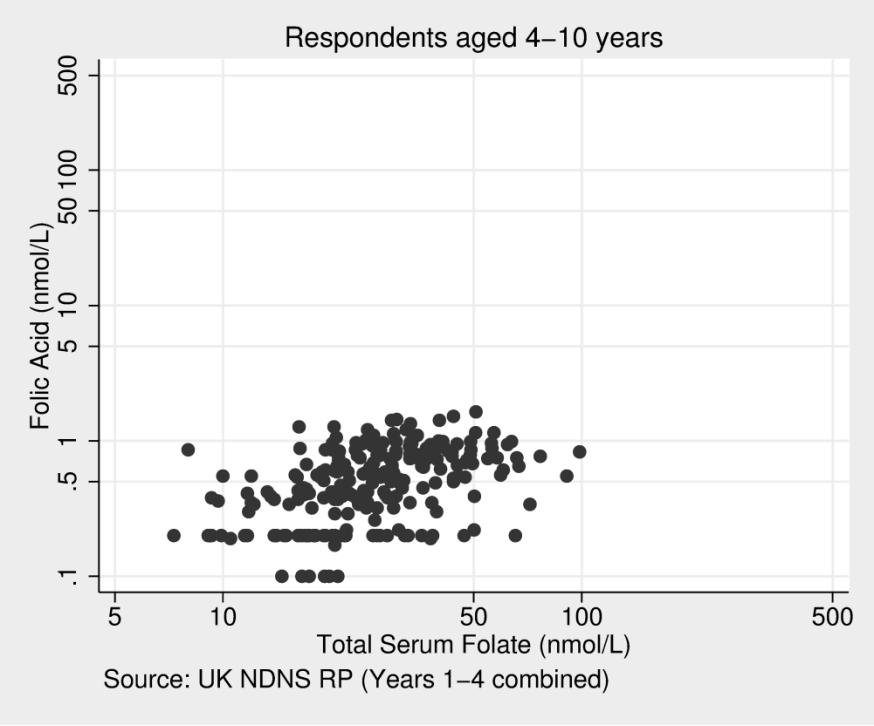


Figure 3: UMFA concentration in participants aged 11-18y

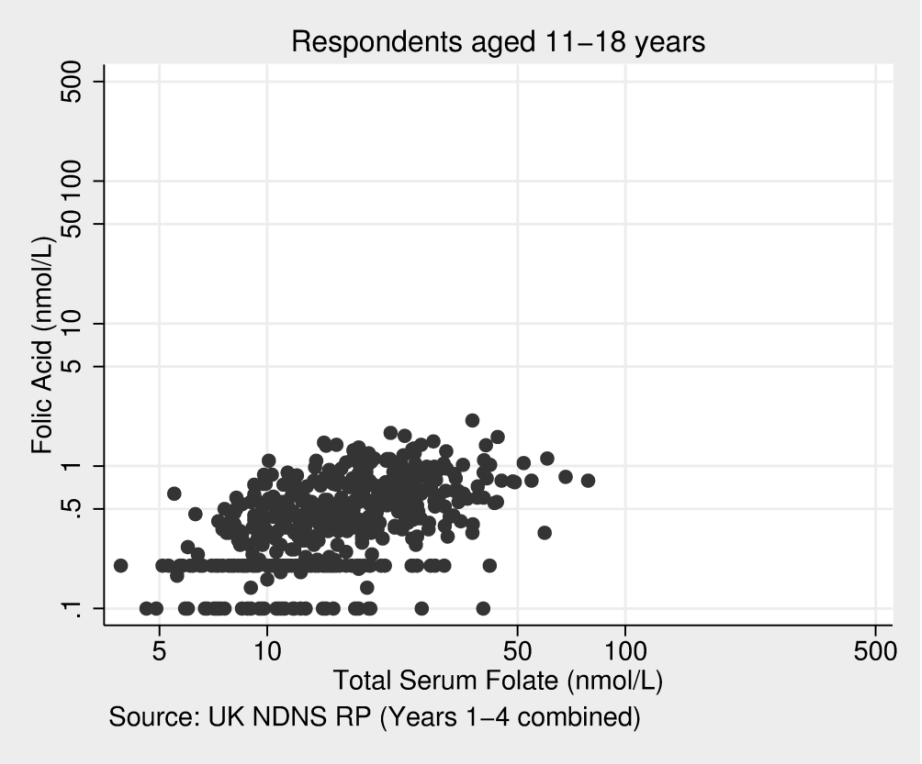


Figure 4: UMFA concentration in participants aged 19-64y

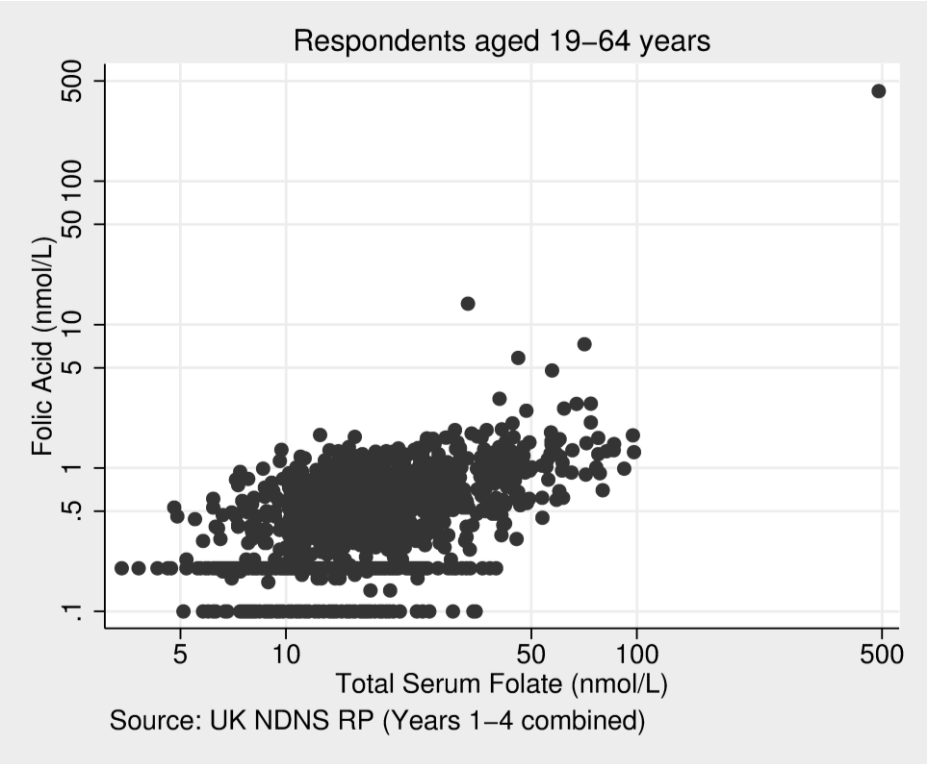


Figure 5: UMFA concentration in participants aged 65+y

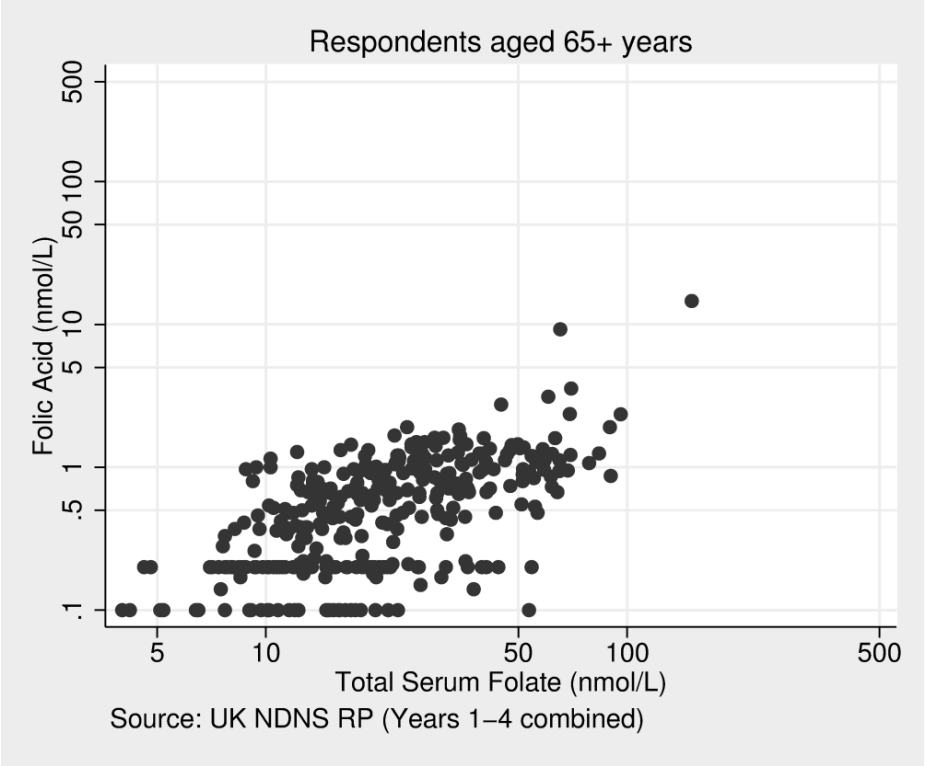
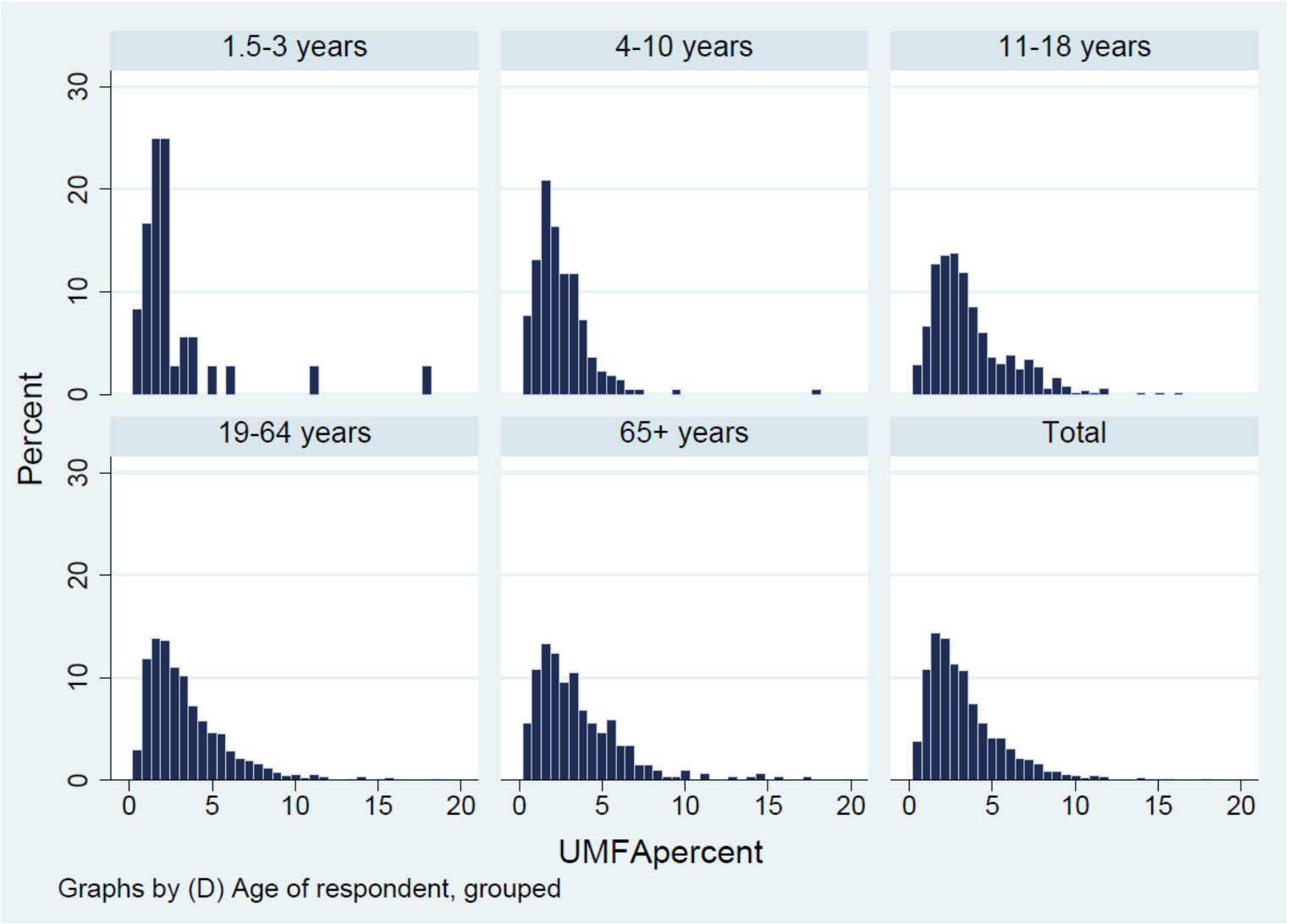


Figure 6: UMFA as a proportion of total folate



ANNEX 6

SACN's recommendations on mandatory fortification of flour with folic acid (2009)

1. As previously recommended by the Committee on Medical Aspects of Food and Nutrition Policy (Department of Health, 2000[18]), all women who could become pregnant should take 400 μg/day folic acid as a medicinal or food supplement prior to conception and until the twelfth week of pregnancy. Women with a previous pregnancy affected by a neural tube defect (NTD) are advised to take 5 mg/day of folic acid prior to conception and until the twelfth week of pregnancy.

2. Individual long-term intakes of folic acid from fortified foods and supplements above the Guidance/Tolerable Upper Level (GL/UL)[19] per day for folic acid (1 mg/day for adults; lower amounts for children[20]) should be avoided. A proportion of the UK population[21] is currently exceeding the GL/UL per day due to consumption of foods fortified with folic acid on a voluntary basis and supplement use. The current risk posed by voluntary fortification of food with folic acid and supplement use in contributing to intakes above the GL/UL per day for folic acid needs to be addressed.

3. Mandatory fortification of flour with folic acid would improve the folate status of women most at risk of pregnancies affected by NTDs. It would also improve the folate status of other population groups in the UK. However, mandatory fortification, combined with the current practice of voluntary fortification of foods with folic acid and inappropriate supplement use, would increase the numbers in the population consuming levels of folic acid above the GL/UL per day. Therefore, mandatory fortification should only be introduced in the UK if it is accompanied by:

    - action to restrict voluntary fortification of foods with folic acid;
    - measures for careful monitoring of emerging evidence on any adverse effects of long-term exposure to intakes of folic acid above the GL/UL per day; and
    - guidance on supplement use for particular population groups.

4. Mandatory fortification of flour[22] alongside restrictions on voluntary fortification will confer a more even distribution of folic acid intakes across the population compared to current voluntary fortification and supplement use. It will not lead to a substantial increase in the average population intake of folic acid but will reduce the risk of intakes exceeding the GL/UL and increase intakes of those currently consuming the lowest total folate intakes (from foods containing naturally occurring folates and foods fortified with folic acid).

5. The introduction of mandatory fortification will require: acquisition of new baseline data on folic acid intakes and blood folate concentration to ensure that mandatory fortification does not lead to an increase in folic acid intakes above the GL/UL and to permit monitoring of trends in future surveillance programmes; adoption of a sufficiently robust common standard analytical method for

[18] Department of Health. *Folic acid and the prevention of disease.* Report on health and social subjects 50. London: TSO, 2000.
[19] In the UK, the Expert Group on Vitamins and Minerals set a GL of 1 mg/day of folic acid for adults. The GL is based on limited data and is an approximate indication of intakes that would not be expected to cause adverse effects. In the USA and Europe, a UL of 1 mg/d of folic acid was set for adults; the UL represents the highest level of daily nutrient that is likely to pose no risk to health.
[20] GLs were not set for children in the UK. ULs were set for children in the USA and Europe based on body weight. ULs for children, Europe: 4-6y, 300 μg/d; 7-10y, 400 μg/d; 11-14y, 600 μg/d; 15-17y, 800 μg/d. ULs for children, USA: 1-3y, 300 μg/d; 4-8y, 400 μg/d; 9-13y, 600 μg/d; 14-18y, 800 μg/d.
[21] Approximately 106,000 people.
[22] Careful consideration would need to be given to the issue of overage.

measurement of folate status at baseline and in all future surveillance studies; and establishment of suitable reference ranges to predict folate adequacy and deficiency.

6. If mandatory fortification were introduced, all women who could become pregnant and those with a history of a previous NTD-affected pregnancy should continue to supplement their diet with 400 μg and 5 mg per day of folic acid respectively prior to conception and until the twelfth week of pregnancy.
7. There are no specific recommendations on folic acid supplementation for other population groups (i.e., children, women above child bearing age, and men) except on medical advice. For people who choose to take supplements, as a precaution, it would be advisable for those aged over 50 years not to consume supplements containing folic acid above the recommended nutrient intake (RNI)[23] for folate of 200 μg/day since the risk of developing colorectal adenomas/colorectal cancer increases after this age (Winawer *et al,* 1997[24]; American Cancer Society, 2008[25]). For people with a previous history of colorectal adenomas, folic acid supplementation should also not exceed 200 μg/day without medical guidance. This recommendation is relevant to current consumption patterns and those which would prevail if mandatory fortification were introduced.
8. Evidence on the benefits and hypothesised risks of folic acid should be reviewed after an appropriate period of time which should be no later than five years.
9. There are a number of uncertainties regarding the GL/UL per day set for folic acid which is based on limited data and relates to concerns regarding vitamin B_{12} deficiency. Further research is required on safe upper levels of folic acid intake in relation to other postulated risks, such as cancer.
10. More reliable diagnostic indices to identify vitamin B_{12} deficiency should be developed. The development of a clinical strategy to manage issues related to vitamin B_{12} is necessary irrespective of a decision on future mandatory fortification of flour with folic acid.
11. The prevalence of poor vitamin B_2 (riboflavin) status in the UK population needs to be addressed.

[23] The RNI represents the amount of a nutrient that that is sufficient to meet the requirements of 97.5% of the population.
[24] Winawer SJ *et al.* Colorectal cancer screening: clinical guidelines and rationale. *Gastroenterology.* 1997; 112:1060 and 1998; 114:625.
[25] American Cancer Society. *Cancer Facts & Figures 2008.* Atlanta: American Cancer Society; 2008.