FOLATE

Disclaimer

This chapter describes the background for setting dietary reference values for Folate in the 6th edition of the Nordic Nutrition Recommendations (NNR2022). Anne-Lise Bjørke-Monsen and Per Magne Ueland has been assigned as authors. The present version of the chapter has been peer reviewed by Cornelia Witthöft and David Smith, and considered by the NNR2022 Committee. The chapter is now open for public consultation. The hearing responses will be publicly available and carefully considered by the NNR2022 Committee. All input considered by NNR2022 Committee as scientifically valid and relevant will be forwarded to the authors for consideration. Please note that sustainability aspects and other issues such as obesity, physical activity, and burden of diseases will be integrated at a later stage, if relevant. The NNR Committee is responsible for setting the dietary reference values. The suggestion for setting of dietary reference values will be open for public consultation at a later stage, before the NNR2022 Committee reach the final conclusion, and are not included in the document now available for public consultation.

1. **Abstract:**

Folate is an essential micronutrient for normal development and metabolic function and folate deficiency is associated with an increased risk of cancer, cardiovascular disease, mental dysfuntion and negative pregnancy outcome.

When estimating folate requirements, one must consider different bioavailability and functionality between synthetic folic acid and dietary folate, a long with increased needs of folate in women of fertile age, pregnant and lactating women, preterm and small for gestational age weight infants and in individuals who are homozygote for the MTHFR gene polymorphism.

In order to give an adequate metabolic status based on the metabolic marker total homocysteine, and not only on the absence of clinical signs of folate deficiency, the recommended intake of folate differs according to age, pregnancy and lactation. According to WHO a decision limit for folate deficiency in adults is serum folate level below 10 nmol/L and in women of fertile age a red cell folat level below 906 nmol/L in order to prevent neural tube defects.

Both de novo and qualified systematic reviews along with identified relevant literature have been used for this chapter.

2. Introduction:

The aim of this chapter is to describe the totality of evidence for the role of folate for health related outcomes as a basis for setting and updating DRVs. Folate is an essential micronutrient in the B-complex vitamins. It is involved in different physiological processes and particularly essential for growth and fetal

development because of its role in the synthesis, repair, and methylation of DNA, contributing to the formation of new cells and tissues.

Folate is present in most foods, and high concentrations are found in liver, green vegetables, and legumes. Folate in food are labile to light and oxidation, and partly destroyed by cooking, compared to the chemically most stable folate form, synthetic folic acid, which is found in supplements (1).

Higher folate requirements are found in infants, children, pregnant and lactating women and also in patients with intestinal disease, severe skin diseases, hemolytic anemia, patients taking antiepileptic medications and in people with certain gene polymorphisms (2, 3).

The significance of folate status remains conflicting for many conditions, including cancer(4), cardiovascular disease(5), asthma(6, 7), mental function(8, 9) and pregnancy outcome(10, 11). More than 30 years ago, the British Medical Research Council showed that maternal intake of folic acid starting before pregnancy prevents most cases of infant spina bifida and anencephaly, two major NTDs(12). Mandatory food fortification with folic acid is considered a safe and cost-effective intervention to prevent NTDs, however, this has not been implemented in many countries, including the Nordic countries (13).

Serum folate is the primary marker of folate status in both children, adults and pregnant women. The metabolic marker plasma total homocysteine (tHcy), increase with decreasing serum folate levels. Plasma tHcy are also affected by vitamin B12 and B6 status, age and renal function (3). In young children serum folate concentrations are commonly high (>20 nmol/L) (14). In adults, the World Health Organization (WHO) recommends a serum folate concentration > 10 nmol/L and a red cell folate of 906 nmol/L in women of fertile age(15).

In a recent study, median folate intake was 246 µg/day in a Swedish adult population (18-80 years, n=1797), 25% had a serum folate concentrtion < 10 nmol/L and none of the women of reproductive age had erythrocyte folate concentrations associated with the lowest risk of neural tube defects (16). Women may reach a preventive RBC folate concentration of more than 906 nmol/L within four weeks of supplementation with daily intake of 800 µg folic acid(17), while a dietary folate intake of at least 350 µg/d has been considered necessary to prevent an increase in plasma homocysteine levels of the adult population (18).

3. **Methods:**

This review follows the protocol developed within the NNR2022 project “The Nordic Nutrition Recommendations 2022 – Instructions to authors of chapter” which can be found on the official NNR2022 website. The sources of evidence used in the chapter follow the eligibility criteria described in the paper “The Nordic Nutrition Recommendations 2022 – Principles and methodologies“(19).

The main literature search for this chapter was performed in MEDLINE March 1st, 2021 with a search string: ((folate[MeSH Terms] AND review[Publication Type] AND ("2011"[Date - Publication] : "3000"[Date - Publication]) AND Humans[Filter])) AND (("Diet" OR "Dietary" OR "Food" OR "Nutrition" OR "Nutritional")). The number of hits was 578. Only one qualified SR was identified for folate(20). We also identified relevant literature for this chapter via "snowballing"/citation chasing that was relevant for the background information.

4. **Physiology:**

Dietary folates mostly occur as polyglutamyl derivatives and undergo hydrolysis in the gut to monoglutamates before intestinal absorption(21). Folates are transported across the jejunum by a carrier-mediated process, as folic acid, 5-methyl-tetrahydrofolate (5-MTHF), and 5-formyl-tetrahydrofolate (22, 23). Folates can also be absorbed by diffusion, a process that is linearly related to lumenal folate concentrations and can account for 20-30% of folate absorption at high folate intakes (21).
Folates taken up by the intestinal mucosal cell are reduced to THF, which can either be transferred to the portal circulation without further metabolism. THF is taken up by the liver, methylated to 5-MTHF and 10-formyl- THF, and transported to the peripheral tissues. Folate is transported to the tissues as monoglutamate derivatives in the plasma(2). Within the cell, THF is methylated to 5-methyl- THF, which is converted to folate polyglutamates containing up to 7 glutamyl residues. Polyglutamation traps folate inside the cell at concentrations greater than extracellular fluids (24).
The chemically most stable folate form is synthetic folic acid. The bioavailability of food folate is commonly estimated at 50% of folic acid bioavailability when establishing food recommendations, but this should be considered a rough estimate, as data on the bioavailability of food folate vary between 30% and 98%(1, 25, 26). When adults receive daily folate doses < 200 µg, little or none is lost in the urine, but at higher doses in the pharmacological range, as used by pregnant women on antiepileptic medication(27), the urinary loss is considerable: 6% of a 1 mg dose, 10% of 2 mg, 50% of 5 mg, and 80% of 15 mg(28).
Folate requirements and recommendations for folate intake are expressed as dietary folate equivalents that adjust for the greater degree of absorption of folic acid compared with folate naturally found in foods (1 µg of folate equals 0.6 µg folic acid added to food or taken with food or 0.5 µg folic acid (as a supplement) taken on an empty stomach(29).
About half of the total body folate pool (5-10 mg) is stored in the liver. Plasma folate consists almost entirely of 5-MTHF (1). A small amount of plasma folate is bound to a folate-binding protein.
Folates acts as coenzymes for enzymes that mediate single-carbon metabolism. The fully reduced form (tetrahydro-) serves as an acceptor or donor of a single-carbon unit in reactions involved in the synthesis of pyrimidines, purines, serine and methionine(2). Thymidine monophosphate is produced by the methylation

of uridine monophosphate. The coenzyme delivering the necessary methyl group in this reaction is 5,10-methylenetetrahydrofolate, which may be reduced to 5-methyltetrahydrofolate for synthesis of methionine (Met) from homocysteine (Hcy) or oxidized to 10-formyltetrahydrofolate for use in purine synthesis (2).

In the Nordic population 5%–8% have a polymorphism in the gene coding for the 5,10-methylene tetrahydrofolate reductase (MTHFR) (C677T, Ala --> Val) (30). This mutation is associated with a decreased activity of the enzyme and results in hyperhomocysteinemia, primarily when folate levels are low. It is recommended that people with MTHFR polymorphism should have a serum folate>15 nmol/L(31).

Pregnancy

In women of reproductive age, WHO recommends a RBC folate threshold of > 400 ng/mL (906 nmol/L) to be used as an indicator of folate insufficiency, as RBC folate concentrations above this limit will achieve the greatest reduction of neural tude defects (NTDs) (15). Pregnancy is associated with higher demands for folate due to growth and fetal drainage, as well as increased folate catabolism and excretion(32, 33). Serum folate levels decrease continuously during pregnancy and folate stores are depleted after 3 months or sooner if dietary supplements are not provided(34).

Lactation

During lactation, folate is preferentially taken up by actively secreting mammary glands. 5-Methyl tetrahydrofolate is the predominant form of folate in human milk(35). While colostrum is relatively low in folate, milk folate increases during the lactation period (36). Despite reduced maternal folate status, average milk folate levels are reported to be maintained at recommended dietary allowances for infants (37). In a 16 weeks intervention study, there were no differences in total milk folate or in unmetabolized folic acid concentration in the breast milk of women provided with either a low dose of folic acid, a [6*S*]-5-methylTHF supplement, or a placebo during lactation(38).

Infants

Maternal folate deficiency is associated with low folate levels in the infants (14, 39).). Significantly lower folate levels at birth have also been observed in low birth weight (<2500 grams)(40), and premature infants (41). These infants also experience a fall in folate concentrations in early life (42).

In exclusively breast-fed infants, plasma folate levels are reported to be elevated after the age of 2 months and are then 2-3-fold higher than maternal levels (14, 43, 44). In formula-fed infants, more than 70 % of plasma folate concentrations are below the lowest concentration for the breast-fed infants(45). The opposite was observed in Korean infants; where the overall folate intakes in formula-fed infants were

significantly higher than those in human milk-fed infants, and this was associated with significantly higher folate and lower tHcy in formula-fed infants than human minl-fed infants at 5 months(46).
Median serum folate was 27.0 (IQR 20.4–36.3) nmol/L in Norwegian newborns (4 days) and median 31.6 (IQR 21.3–43.3) nmol/L in infants from 6 weeks to 6 months. Exclusive breastfeeding decreased from 73% at 6 weeks to 35% at 6 months (14).

Older children

Serum folate remains high up to age 12 months and then decreases to values observed in older children and adults during the first 1 to 3 years of life(14) (Figure 1).

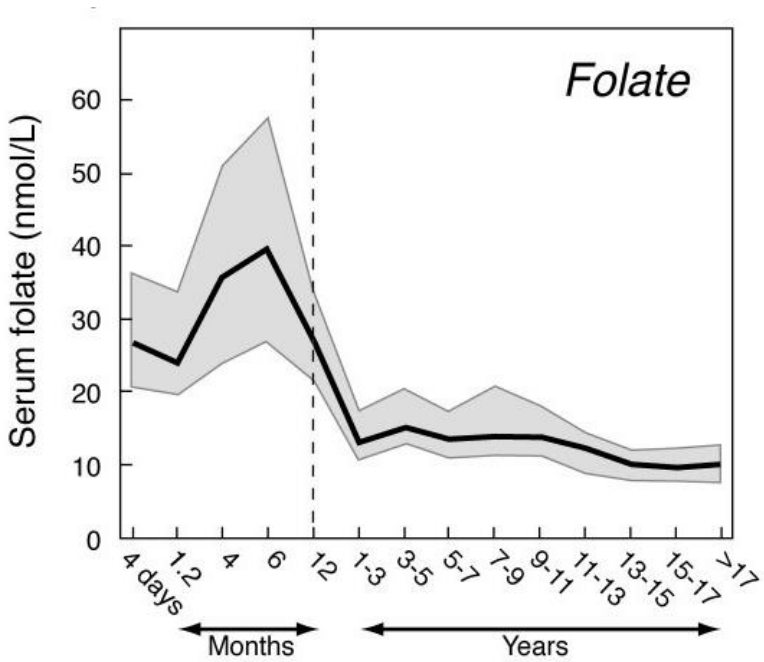

Figure 1. Serum folate concentrations in children aged 4 days to 18 years.

Malabsorption

Malabsorption occurs with intestinal diseases, such as celiac disease, Crohn disease, ulcerative colitis and tropical sprue (3, 47). Folate can bind to food matrices, and many foods (e.g. oranges, lentils, cabbage) contain inhibitors of the intestinal folate conjugase, which reduces folate absorption. Iron and vitamin C deficiency are associated with impaired food folate utilization(48).

5. **Assessment of nutrient status**.

Serum or plasma folate is the primary marker of folate status in both children, adults and pregnant women. Intake of food before blood sampling, may affect serum folate concentrations, however, most

laboratories do not demand a fasting condition. The microbiological assay is considered the gold standard for both serum and RBC folate(49), but more often modern immunoassay are used in clinical laboratories. Although erythrocyte (RBC) folate is considered to be a better indicator of body stores and nutritional status, as there is considerable uncertainty about the reliability of the analytical methods for RBC folate, many laboratories do not longer offer this analysis (50-52).

A higher intake of folate or folic acid is associated with a higher serum and RBC folate. After initiation of mandartory folic acid fortification in 1998, serum folate concentrations more than doubled and RBC folate increased by approximately 50% in the US population(3). Initially in folate deficiency, serum folate decreases, then plasma tHcy increases and a reduction in RBC folate becomes evident. Folate deficiency gives rise to megaloblastic changes in the bone marrow and other rapidly dividing tissues(53), hypersegmentation in neutrophils and generation of micronuclei in lymphocytes, biomarkers of chromosome breakage or loss(54). Plasma tHcy may increase to 40-50 µmol/L in severe folate deficiency. In patients who are homozygous for the *C677T*-polymorphism in the *MTHFR*-gene plasma tHcy may increase to 100 µmol/L. Plasma tHcy also increases with reduced renal function and age, so it is necessary to use age specific decision limits(55).

Many laboratories still use the 2.5 reference limit to define folate deficiency, ranging from 5- 7 nmol/L in the Nordic countries. The reference interval is typically defined as the interval between the two reference limits (2.5th and 97.5th percentiles) derived from the distribution of results from an apparently healthy reference reference population(56). However, a reference interval is merely a description of the folate status in a specific population, and will differ according to the diet in the tested population. The mean folate concentration was 29.5 (95% confidence interval: 27.3–31.7) nmol/l in a population based study including 750 individuals aged⩾12 years in 2017 from Brazil, where folic acid fortification of wheat and maize flours has been mandatory since 2004 (57). In Norway, where folic acid fortification has not been implemented, the mean serum folate was 18.0 (SD 13.8) nmol/L, 60% lower, in 158 Norwegian women of fertile age in 2015 (own unpublished data).

For clinical interpretation of serum folate, one must have clinical decision limits, which defines a value above or below a threshold associated with a significantly higher risk of adverse clinical outcomes or diagnostic for the presence of a specific disease (56). The decision limit may vary according to outcome used. When WHO used megaloblastic anemia as a outcome for folate deficiency, the decision limit was < 6.8 nmol/L (58). When WHO used the metabolic marker homocystein as a marker for folate deficiency, the decision limit was < 10 nmol/L (58). WHO considered that folate status needs to be optimal in women of fertile age to prevent NTDs, and suggested that blood cell folate concentrations below 906 nmol/L (serum folate 25-27 nmol/L) should be a decision limit for deficiency in this age group(15). As Figure 2 shows, plasma tHcy starts to increase already when serum folate falls below ~ 25-27 nmol/L, indicating suboptimal intracellular folate stores, and increases more sharply below ~ 10 nmol/L, indicating

biochemical deficiency(59). A similar relation between serum folate and plasma tHcy is observed in women in pregnancy (60).

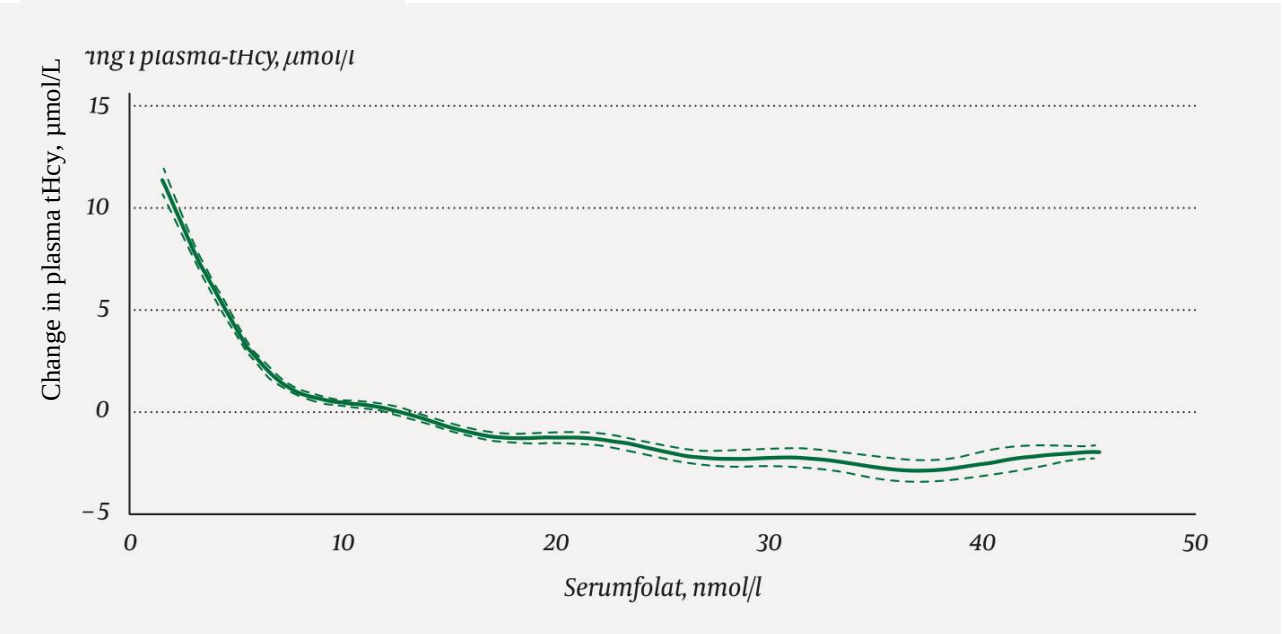

Figure 2. Change in plasma tHcy in relation to serum folate in adults > 16 years with normal renal function and serum cobalamin > 275 pmol/L, (n=12988) by generalized additive models (GAM). The values on the y-axes represents the difference from mean plasma tHcy. Published with permission of Tidsskrift for den norske legeforening(59).

6. **Dietary intake in Nordic and Baltic countries**.

Folate is present in most foods, and high content are found in liver, green vegetables, and legumes. The folates in foods are almost exclusively in reduced form as polyglutamyl derivates of tetrahydrofolate (FH_4)(48), and reduced forms are labile to light and oxidation, and partly destroyed by cooking. The bioavailability of food folate is commonly estimated at 50% of folic acid bioavailability when establishing food recommendations, but this should be considered a rough estimate, as data on the bioavailability of food folate vary between 30%(26) and 98%(25), depending on the methodological approach used. Synthetic folic acid, which is a stable oxidized form of pteroylmonoglutamate, is considered to be more bioavailable than natural folate, with a bioavailability when taken with food of 85% and under fasting conditions close to 100% (24, 61).

The average dietary intake in adults in the Nordic countries is 257 µg per day for women and 293 µg per day for men (see the chapter on dietary intakes in Nordic countries). It is of interest than average intake in Denmark is approxiately 38% higher in both men and women compared to the other Nordic countries.

People with low folate intake, malabsorption or increased folate requirements have a risk of developing folate deficiency. Chronic alcoholism is associated with severe folate deficiency linked to poor dietary intake, intestinal malabsorption, impaired hepatic uptake with reduced storage of endogenous folates, and increased renal excretion(62). Children and pregnant and lactating women have an increased demand for folate. So have patients with haemolytic anemia, malignancy, patients undergoing renal dialysis or patients using anticonvulsant drugs (phenytoin, primidone), sulfasalazine (used in the treatment of inflammatory bowel disease), triamterene (a diuretic); and metformin (used in type 2 diabetes)(3).

7. **Health outcomes relevant for Nordic and Baltic countries.**

Cancer

Research has evaluated the potential impact of folate on cancer risk with conflicting findings. Studies have demonstrated increased risk, no effect, and decreased risk. Evidence from animal and human studies suggests that folic acid supplementation may prevent neoplastic initiation, but may promote the progression of established precancerous lesions (4) and the relation between folic acid and dietary folate intake, folate status and cancer still remains an unresolved issue(63).

Elevated homocysteine levels and folate deficiency, as determined by serum folate level, were associated with increased overall risk of cancer in a meta-analysis of 83 case-control studies (64)[1. Folate level was inversely associated with most cancer types except prostate, bladder, pancreatic, and breast (64). Folic acid supplementation and higher serum levels are associated with increased risk of prostate cancer. Gene polymorphisms may impact risk in certain ethnic groups(65).

The differences in bioavailability and metabolism of synthetic folic acid and natural dietary folate as well as variation in the baseline characteristics of subjects and various methods of folate status assessment in various studies have been suggested as a reasons for the controversies regarding colorectal cancer prevention versus promotion(63). Both Randomized Controlled Trial (RCT) and cohort studies have however shown beneficial effects of both supplementary folic acid and dietary folate on the primary prevention of colorectal adenomas(66-69). A recent systematic review including a total of 24 cohort studies involving 37 280 patients and 6 165 894 individuals showed that high folate intake was associated with a reduced risk of colorectal cancer, particularly in people with middle or high alcohol consumption. However, the authors concluded that this still needs to be further confirmed(70).

Cardiovascular disease

An adequate dietary folate intake (i.e. according to the recommendations) has been shown to be inversely associated with both severe and subclinical cardiovascular disease outcomes (71). A systematic review of RCTs from 2106 indicate a 10% lower risk of stroke and a 4% lower risk of overall cardiovascular disease

with folic acid supplementation. Folic acid supplementation had no significant effect on risk of coronary heart disease(5). A meta-analysis which included search for both folate and vitamin B12, found that homocysteine lowering with B-vitamins among high vascular risk patients who are not taking antiplatelet therapy, was related to a significant reduction (29%) in overall stroke risk(72). In the China Stroke Primary Prevention Trial, daily supplementation with 0.8mg folic acid reduced the incidence of a first stroke by 21% with greater benefit in those with lower folate levels or higher homocysteine levels(73).

Mental health

In 2008 a Cochrane reviewe concluded that there is no consistent evidence either way that folic acid, with or without vitamin B12, has a beneficial effect on cognitive function of unselected healthy or cognitively impaired older people(9). However, in Swedish adolescents, higher folate intake and lower homocysteine status have been associated with improved achievement in school and this effect was consistent after correcting for parental education and other confounders(74). A Norwegian study on 2189 elderly community subjects followed for 6 years showed higher memory scores in those with higher serum folate in the range 4-24 nmol/L(75). A recent meta-analysis found that low folate status was associated with an increased risk of cognitive decine or dementia whereas folate supplementation protected against the development of dementia(76).After controlling for vitamin B-12, creatinine, demographic variables, and depressive symptom score, an American study from 2005 concluded that RBC folate was directly associated with cognitive function scores and inversely associated with dementia in 1789 people aged ≥ 60 years exposed to folic acid fortification(77). A Norwegian study including 2203 people aged 72-74 years, unexposed to mandatory folic acid fortification, showed that plasma folate was associated with cognitive performance. Among the elderly participants with vitamin B12 concentrations in the lower range, the association between plasma folate and cognitive performance was strongest(8).

Obesity

There is an increasing prevalence of obesity in most parts of the world (78), including the Nordic countries(79). BMI has been inversely correlated with concentrations of folate in healthy children aged two moths to 18 years(80), in in women of fertile age (81), in pregnant women (82) and in non-pregnant adults (83).

Pregnancy outcomes

A Cochrane Review found consistent results showing that folic acid, alone or in combination with vitamins and minerals, prevents neural tube defects (NTD), but does not have a clear effect on other birth defects(84) or pregnancy outcomes(10). The US Preventive Services Task Force (85) reviewed systematically results from RCTs on supplementation in pregnancy and neural tube defects confirming

previous conclusions on protective effects. As demands for folate increase during pregnancy, the mother is in risk of developing folate deficiency throughout pregnancy. Folate deficiency has been associated with anemia and peripheral neuropathy in mothers(86). Recent reviews have found limited evidence for an association between folate status or folic acid supplementation in pregnancy and offspring neurodevelopment (87, 88). Continued folic acid supplementation in pregnancy beyond the early period which is currently recommended to prevent NTD, is reported to benefit neurocognitive development of the child (89). Use of folic acid supplements during pregnancy was associated with improved neurodevelopment in 4-year-old Spanish children when adjusting for socio-demographic and behavioural factors(90). The absence of folic acid supplementation in early pregnancy was associated with a higher risk of behavioural problems in the offspring at 18 months of age(91). A detrimental effect of high dosages of folic acid supplements (>5000 versus 400-1000 μg/d) during pregnancy on psychomotor development after the first year of life has also been shown(92). Although a more recent Norwegian study found a 23% increased risk of asthma in children aged 7 whose mothers had a folate intake of >578 μg/day in pregnancy(7). The evidence for an association between folate intake or status in pregnancy and offspring risk of asthma and allergy appears inconclusive (93). There also appears to be inconclusive evidence for a protective association with hypertensive disorders in pregnancy (94). On the otherhand, there is evidence from India of an increased risk of insulin resistance and of obesity in children of women with high serum folate and low serum B12 (95).

Toxicity

The European Upper Tolerable Intake Level of folic acid is set to 200 μg/d for children aged 1-3 years, 300 μg/d for children 4-6 years, 400 μg/d for children 7-10 years, 600 μg/d for children 11-14 years, 800 μg/d for children 15-17 years, 1000 μg/d for adults >17 years , pregnant and lactating women(96).
Observations indicating adverse effects from excess folic acid intake, elevated serum folate and unmetabolized folic acid concentrations remain inconclusive (97). Although harmful effects in elderly with low B12 status have been reported in several countries, as reviewed recently(98), the data do not yet provide the evidence needed to affect public health recommendations(97).
Adverse effects are exclusively reported from use of the synthetic compound folic acid and no adverse effects have been associated with the consumption of excess folate from foods (99). Only intake of folic acid in excess of 5000 μg/day may mask hematological manifestations of cobalamin deficiency, as well as antagonize anticonvulsant therapy and zinc physiology (4).
Unmetabolized folic acid is detected in nearly all serum samples from US children, adolescents, and adults(100). Concerns have been raised about the potentially untoward effects of unmetabolized synthetic folic acid with regard to cancer, depression and cognitive impairment(101). In postmenopausal women unmetabolized folic acid, but not total folate, in plasma has been found to be related to a decrease in NK

cell cytotoxicity(102). The causative role for unmetabolized folic acid in this study has been questioned(3), and there are still gaps in understanding the factors that contribute to unmetabolized synthetic folic acid accumulation in plasma and the metabolic effects(97).

8. **Requirement and recommended intakes**.

EFSA concluded in 2014 for adults on an Average Requirement (AR) of 250 µg dietary folate equivalents (DFE)/day and a Population Reference Intake (PRI) of 330 µg DFE/day. This was based on the folate intake required to maintain folate adequacy characterised by serum folate of ≥ 10 nmol/L and red blood cell folate concentrations of 340 nmol/L. They assumed a coefficient of variation (CV) of 15 % to account for the additional variability associated with the higher requirement for folate in individuals with the MTHFR 677TT genotype(103).

A dietary folate intake of at least 350 µg/d has been considered necessary to prevent an increase in plasma homocysteine levels of the adult population (18). In a study on elderly men and women (66–94 y), a gradual decrease in plasma tHcy from ~ 11.0 to ~ 8.5 µmol/L was evident with increasing folate intake ranging from 160 to ~850 µg/day(104). An intake of ~ 300 µg folate per day was associated with a plasma tHcy of ~10 µmol/L, indicating that a higher folate intake than recommended by NNR 2012 (AR for adults: 200 µg/d and RI to 300 µg/d) may give a better folate status.

Women of reproductive age

To promote optimal NTD risk reduction at the population level, WHO recommend that the population red blood cell (RBC folate) concentrations should be above a threshold of 906 nmol/L (400 ng/mL) in women of reproductive age(15). It is considered that a RBC folate concentration below this level indicates folate insufficiency and suboptimal prevention. A RBC level of 906 nmol/L corresponds to a plasma/serum folate concentration threshold of 25.5 nmol/L for optimal NTD prevention(105). This level coincides with increased geneomic stability and stable plasma tHcy concentrations(106).

As far from all pregnancies are planned, an RI of 400 µg/d for all women of reproductive ages is considered necessary to provide adequate folate supply to women experiencing unplanned pregnancies. It has been shown that women may reach a preventive RBC folate concentration of more than 906 nmol/L within four weeks of supplementation with daily intake of 800 microg folic acid(17). The prevalence of having a RBC folat < 906 nmol/L was 35 % after 40 weeks with a daily folic acid supplement of 140 µg and 18% with 400 µg(107).

Pregnant women

In NNR 2004, the recommended intake during pregnancy was set to 500 µg per day. This was based on previous studies indicating that 400–500 µg/d was considered sufficient to meet the increased requirement from fast growing tissues during pregnancy (67) and the recommendation was kept unchanged in NNR 2012.

The EFSA Panel considers that it is not possible to set an AR for pregnancy and proposed an AI for folate for pregnancy at 600 µg DFE/day based on a study on maintenance of serum and red blood cell folate concentrations in pregnancy(103). This study reported that a total of 450 µg/d of dietary folate in addition to synthetic folic acid was sufficient to maintain folate status in pregnant women. This level of intake was considered eqvivalent to ~ 600 µg/d dietary equivalents, assuming 50 and 75% availability of dietary folate and synthetic folic acid consumed with meals, respectively(108).

In a study published in 1997, mean dietary folate intake in adults ws 291 µg/d (range 197–326) for men and 247 µg/d (range 168–320) for women in Europe(18). Currently, most women in the Nordic countries still do not meet the RI 500 µg folate per day. Median folate intake was 246 µg/day for Swedish adults aged 18-80 years and for women of reproductive age 227 µg/day(16). Finnish studies report mean folate intakes in women of reproductive ages ranging from 215 µg/d to 230 µg/d (65, 66).

In the Norwegian MoBa study, women who were regular folic acid supplements users had a total mean intake of folate of 615 (SD 270) µg per day from supplements, and mean 275 (SD 95) µg per day came from diet alone (109). In pregnancy week 18, women from the MoBa study, with a regular intake of folic acid supplement from 4 week before pregancy to week 17, had median serum folate 15.7 (IQR 9.4-23.1) nmol/L compared to median 10.2 (IQR 7.3-16.6) nmol/L in irregular users and median 5.7 (IQR 4.3-7.7) nmol/L in non-users(110), showing that a regulat intake of folic acid supplements is necessary to achive a better folate status. However, even among regular supplements users, less than 25% had a serum folate in the range of 25.5 nmol/L, considered optimal for NTD protection. Based on this, recommendations on folate intake should be considered adjusted.Additionally, folate supplementation should if necessary be combined with vitamin B12 supplemention in view of the commonly found low B12 status in pregnancy and the risk of harm to the child of high folate and low B12 (111). This recommendation applies especially to women who are vegans and not currently taking a B12 supplement(112).

Lactation

NNR 2012 recommended 500 µg/d to lactating women and this amount was also considered to allow replenishment of stores before a possible new pregnancy. This was based on the following: The concentration of folate in human milk varies throughout the lactation period and is highest between 3 and 6 months(113). Smith and coworkers reported the average concentration of folate in human milk to be 85 µg/L(114). Based on a daily milk production of 0.75 L and a bioavailability of 50%, the diet should contain approximately 100 µg of extra folate.

The EFSA (2014) added an additional intake of 130µg DFE/day considered to cover folate losses via breast milk to the AR for non-lactating women, and a PRI of 500 µg DFE/day was derived for lactating women(103).

Infants and children

The calculated folate intake for infants from birth to 6 months of age was estimated by EFSA to be 64 µg/day, based on a mean folate concentration of mature breast milk ~ 80 µg/L (range 45–99 µg/L)(103), and mean breast milk intake per day the first 6 months ~ 0.8 L/day. In a study based on healthy, well-nourished lactating mothers and infants published in 1980, the mean breast-milk folate level was 141.4 µg/L and total daily folate intake for breast-fed infants was assessed at 14 to 25 µg/kg body weight (115). Approximately the same levels were reported in a recent study from Korea (46). Mean breast milk folate contents ranged from 88.4 to 160.6 µg/L with an overall mean of 128.0 µg/L, and the contents peaked at 2 months postpartum. Folate intake in the infants ranged between 100 – 140 µg per day during the first 12 months. Serum folate in the infants at age five months was mean 72.0 (SD 39) nmol/L and at 12 months mean 76 (SD 40) nmol/L with adequate plasma tHcy levels of mean 4.4 (SD 1.5) and 3.5 (0.8) µmol/L at five and 12 months respectively(46). Mainly breast-fed Norwegian infants have high serum folate levels the first 6 months of life (median 31.6 (IQR 21.3 – 43.3) nmol/L, indicating that folate content in breast milk is adequate(14).

For infants aged 7–11 months, EFSA recommended an Adequate Intake (AI) of 80 µg DFE/day, by extrapolating upwards from the estimated folate intake in exclusively breast-fed infants, including relating this to metabolically active body mass (median weight or infants at 3 months (6.1 kg) and at 9 months (8.6 kg)(103).

For older children, EFSA extrapolated the ARs from the AR for adults using allometric scaling and growth factors and considering differences in reference weights. PRIs ranging from 120 µg DFE/day for age-group 1–3 year-old, to 330 µg DFE/day age-group 15–17 years were derived(103).

In a study from Germany, only children who ate food enriched with folic acid had a folate intake corresponding to recommended EFSA intake. For age-group 6 – 12 months, the folate intake was ~105 µg DFE/day and increased to 323 µg DFE/day in age-group 15-18 years, compared to infants who did not eat folic acid enriched food: ranging from ~63 µg DFE/day to 164 µg DFE/day from 6 – 12 months to 15-18 years(116).

During the first 2 months of life, exclusively breastfed low birth weight (<2500 gram) and /or preterm infants (≤32 gestational weeks) could be at risk for folate deficiency, especially when mothers are smokers and/or do not receive folic acid supplementation during pregnancy(117).

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