OPINION OF THE SCIENTIFIC COMMITTEE ON FOOD ON THE TOLERABLE UPPER INTAKE LEVEL OF FOLATE

(EXPRESSED ON 19 OCTOBER 2000)

FOREWORD

This opinion is one in the series of opinions of the SCF on the upper levels of vitamins and minerals. The terms of reference given by the European Commission for this task, the related background and the guidelines used by the Committee to develop tolerable upper intake levels for vitamins and minerals used in this opinion, which were expressed by the SCF on 19 October 2000, are available on the Internet at the pages of the SCF, at the address: http://www.europa.eu.int/comm/food/fs/sc/scf/index_en.html.

1. INTRODUCTION

Folate is the generic name for a number of compounds having a similar activity as folic acid (pteroylglutamic acid, PGA), i.e. being involved in single carbon (C_1-) transfer reactions. Folic acid (PGA) is a synthetic folate compound used in food supplements and in food fortification because of its stability, and becomes biologically active after reduction. Natural (dietary) folates are mostly reduced folates, i.e. derivatives of tetrahydrofolate (THF), such as 5-methyl-THF (5-MTHF), 5-formyl-THF and 5,10-methylene-THF, and exist mainly as pteroylpolyglutamates, with up to nine additional glutamate molecules attached to the pteridine ring.

2. NUTRITIONAL BACKGROUND

Folates play an important role in the transfer of C_1-groups (i.e. methyl-, methylene- and formyl-groups), maintaining the methylation balance, such as in the biosynthesis of DNA bases and in amino acid metabolism.

Green vegetables and certain (citrus) fruits are important dietary sources of folates. The population reference intake set by the SCF of the EU is 200 µg/day for adults, and 400 µg/day in pregnancy.

Food folates, mainly present as polyglutamates, have to be hydrolysed by a (brush border associated) deconjugase enzyme in the gut before absorption can occur. Folate absorption from natural food is generally lower than synthetic forms (e.g. folic acid) contained in supplements, due to matrix effects and the presence of inhibitors of the conjugase enzyme in some foods. Folic acid (PGA) enters the folate cycle after reduction by a (dihydro-)folate reductase. This enzyme is present in the intestinal mucosal cell, but also in other tissues, such as liver and kidney. Reduction of PGA may be a slow process in some subjects and at higher intake levels (> ca 260 µg) PGA may appear unchanged in the circulation (i.e. in the postprandial state after supplement use (Kelly *et al*, 1997). Under normal conditions 5-MTHF (as monoglutamate) is the only form present in plasma, mainly protein-bound. Tissue uptake is carrier-mediated and/or through folate binding proteins. In tissues folates are retained as polyglutamates and the folate coenzymes can be interconverted in numerous (de-)methylation reactions, such as in DNA synthesis (formation of thymidilate from deoxyuridine), amino acid interconversions, such as the remethylation of homocysteine to methionine. In this latter methionine synthase (MS) reaction vitamin B_{12} is also involved as a cofactor. About 50% of the folate body store, estimated to be 13-28 mg, is considered to be present in the liver (for review see Report of the Standing Committee on the scientific evaluation of dietary reference intakes (DRIs) and its panel on folate and other B-vitamins and choline. Food and Nutrition Board, Institute of Medicine, 1998).

In European countries the average folate intake in adults was found to be remarkably similar, i.e. around 300 µg/day in adult males, and 250 µg in adult women (De Bree *et al*, 1997). This is about the recommended intake level, but lower than recommended for pregnant women and women with a pregnancy wish. For these groups an intake >400 µg/day is considered protective against neural tube defect (NTD). More than 90% of women in the childbearing age range have dietary folate intakes below this optimal level. In The Netherlands and the UK women with a pregnancy wish are advised to take daily a folic acid supplement of 400 µg between 4 weeks before up to 8 weeks after conception. In some European countries, such as the UK, cereals and breads are fortified with folic acid, contributing 25-100 µg per serving.

Additional data on mean and high (P-97,5) intakes, reported for the various EU member states are summarised below.

However, after reviewing the available literature, Dickinson (1995) concluded that there is no convincing evidence for such an effect. A folic acid-induced aggravation or induction of neurological symptoms would be difficult to demonstrate, as the progression of such symptoms in untreated subjects is already highly variable between patients, e.g. between a few months up to 5 years in development of paraesthesiae. Also studies in fruit bats with nitrous oxide-induced vitamin B_{12} deficiency, showing exacerbation of neurological signs after folic acid administration, are not completely convincing because of methodological flaws (van der Westhuyzen *et al*, 1982; see also comment in Dickinson, 1995).

These fruit bats were given large oral daily doses of folic acid (1.54 mg/kg; equivalent to ca 100 mg/day in humans), or daily intramuscular injections of formyl-THF (1.15 mg/kg). The fruit bats given the oral folic acid reached the same stage of neurological impairment ("flight reduced to hops"), but this occurred slightly, but not significantly, earlier.

In another study with experimentally (diet) induced vitamin B_{12} deficiency in rhesus monkeys, three of the nine monkeys received 5 mg/week of supplemental folic acid intramuscularly, followed by 5 mg in the drinking water (5 days/week) (Agamanolis *et al*, 1976). Five animals developed visual impairment and optic atrophy, including the 3 monkeys that received supplemental folic acid. Apparently, the optical nerve lesions occurred earlier (by 10-11 months) in the folic acid-treated animals. It should be noted that the visual lesions observed in these monkeys are only rarely noted in human disease. Spastic paralysis of hind legs and tail was found in 3 animals, including 2 animals receiving folic acid. Other lesions in cranial and peripheral nerves and in the white matter of the spinal cord were observed in some animals, but were apparently not affected by supplemental folic acid.

Vegetarians are at risk to develop a vitamin B_{12} deficiency, while their folate intake is generally high. It has been reported that in this group neurological symptoms due to B_{12} deficiency, such as myelin damage, occur with only minor haematological damage (Herbert, 1994). It was even reported that vegetarians with greater myelin damage had generally higher red cell folate levels. However, there is generally no clear correlation between haematological and neurological features of vitamin B_{12} deficiency.

3.2. Neurotoxic effects of folic acid

Animal studies have shown that folic acid can be a neurotoxin, and can cause convulsions in laboratory animals (e.g. Hommes and Obbens, 1972; Spector, 1972). This evidence is in part based upon *in vitro* tissue and cell culture studies, and/or using very high dose levels (i.v. dosages 60-90 mg). There is however no clear evidence for a folic acid-induced neurotoxicity in humans. Some cases of neurological deterioration have been reported following ingestion of folic acid tablets (3 mg), or folic acid containing multivitamin supplements, but the presence of an (undiagnosed) vitamin B_{12} deficiency cannot be ruled out in these cases (see Dickinson, 1995). In one study with epileptic patients electroencelographic changes were noted after administration of 7.2 mg of folic acid, and seizures after 14.4 and 19.2 mg. However, in other studies no such changes or effects were observed after i.v. dosage of 75 mg (see Campbell, 1996). These studies are therefore inconclusive.

Hunter *et al* (1970) reported disturbing toxic effects, i.e. sleep disturbances and mental changes, after treatment of healthy volunteers with 15 mg folic acid for 1 month. This study was however not placebo controlled, and the results were not confirmed in another double blind, randomised study (Hellstrom *et al*, 1971).

Concern has been expressed that folic acid might exacerbate seizures in persons with uncontrolled, or drug controlled epilepsy. However, no such effects were found in several controlled studies with dosages between 15-20 mg/day. Supplementation studies (15 mg/day for 45 days) with Parkinson disease patients did not show an effect on the incidence of neurological defects. Also after i.v. dosing with dosages up to 150 mg no adverse effects have been reported (for review see Butterworth & Tamura, 1989; Campbell, 1996). As already mentioned, some anticonvulsant drugs may reduce serum folate levels. However, as far as data are available, there seems apparently no increased risk for patients with epilepsy, or interference with anticonvulsant medication, at higher folate intakes.

3.3. Potential adverse effects on zinc absorption and status

Dietary zinc deficiency and a relative shortage of maternal zinc has been associated with NTD in human (Milunsky *et al*, 1992). It has been suggested (Quinn *et al*, 1990) that in the presence of a zinc deficiency the administration of high-dose folate increases the teratogenicity of such a deficiency. The enzyme gamma-glutamyl hydrolase is zinc-dependent and converts polyglutamates to monoglutamates, which

is an important step in the absorption of folate. Therefore, the availability of folate is dependent on the glutamyl hydrolase activity, which is regulated by the concentration of zinc (Canton *et al*, 1990).

Some earlier studies indicated competitive interactions between folic acid and zinc, however, results are conflicting. In reviews on this item from Butterworth and Tamura (1989) and from Zimmerman and Shane (1993) it is concluded that there is as yet no convincing evidence for negative effects of folate supplements on serum or red cell zinc contents (in a study in which women were dosed with 10 mg/day for 6 months), nor for negative effects of folic acid supplementation on zinc status in pregnant women. Contradictory results most likely result from methodological problems in assessment of zinc status/bioavailability.

3.4. Carcinogenicity

Folic acid has been associated with an increased incidence of oropharynx, hypopharynx and all cancers (Selby *et al*, 1989), but, as indicated by the authors of this epidemiological study, these cancers are largely smoking- and/or alcohol-related and this finding likely related to these confounding factors. In other (observational) studies an inverse relation was found between folate intake and/or status and colorectal cancer (e.g. Giovanucci *et al*, 1993; White *et al*, 1997), and with cervical cancer (Butterworth, 1993). Treatment of smokers with 10 mg folic acid plus 500 µg hydroxocobalamin for 4 months resulted in a reduction in atypical bronchial squamous metaplasia (Heimburger *et al*, 1988).

3.5. Decreased efficacy of folate antagonists

Folate antagonists such as methotrexate are used in the treatment of various cancers, e.g. leukemia, and also in rheumatoid arthritis, bronchial asthma and psoriasis. There are also a number of other drugs that interfere with folate metabolism, such as pyrimethamine, phenytoin, colchicine, etc. The FDA discussed the issue of potential effects of increased folate intake on the efficacy of antifolate therapy and concluded that there are relatively little data (Food Labelling: Health Claims and Label Statements; Folate and Neural Tube Defects, 1993). The American College of Rheumatology has stated that a dose of 1 mg/day of folic acid does not appear to inhibit the efficacy of low-dose methotrexate therapy in rheumatoid arthritis. High dose folic acid is also used to reduce methotrexate toxicity (see Campbell, 1996). So, there is currently little scientific data on potential adverse effects of high folate intakes on antifolate medication.

3.6. Assumed hypersensitivity

A limited number of case reports have been published on hypersensitivity reactions to oral and parenteral folic acid, but it cannot be excluded that these reactions were due to other components in the formulations. So, hypersensitivity may occur, but is most likely very rare (see Campbell, 1996).

4. DOSE-RESPONSE ASSESSMENT

From the available data it can be concluded that (synthetic) folic acid can cause adverse effects, while no adverse effects have been reported with the consumption of excess folate from foods. Animal data and *in vitro* tissue and cell culture studies indicate that neurotoxic and epileptogenic effects of folic acid can occur at high dose levels (60-90 mg i.v.). However, there is no clear evidence for a folic acid-induced neurotoxicity in humans. In one study with vervet monkeys a dose of 25.6 mg folic acid per day was given to 3 males for 99 days without any obvious toxic side effect (Venter *et al*, 1993). However, it was not indicated what was examined in this study. The animal data available cannot be used to derive a LOAEL or NOAEL.

The most serious adverse effect known in humans is modification of vitamin B_{12} neurological sequela in PA (pernicious anemia) patients as a result of folic acid supplementation, such as masking of the haematological signs and the potential of progression of neurological symptoms. Although the evidence for an exacerbation of the neurological symptoms in humans is equivocal because of the variability in severity and appearance of these symptoms in PA patients, there is some evidence for such a progression in monkeys.
Masking of the haematological signs in PA patients occurs with high frequencies and consistently with daily intakes of 5 mg; however, insufficient data are available for evaluation of dose levels between 1-5 mg.

This masking effect was considered the most serious adverse effect of folic acid by the Folic Acid Sub-committee of the FDA (in 1993) and used as the basis to derive a safe upper uptake limit (UL). Because of the uncertainty of potential adverse effects in the dose range between 1-5 mg, and because of the

fact that at higher intake levels of folic acid unmetabolised (oxidised) folic acid appears in the blood, exposing body tissues to a form of the vitamin not encountered before, the UL was set at 1 mg per day total folate (dietary folate plus folic acid). Although it was agreed that the safety data are all based upon trials with folic acid, and thus might warrant an UL for folic acid, rather than total folate, it was stated that in all trials folic acid was given on a variable background (dietary) intake of folate, and therefore, it could not be concluded that folic acid nor folate are responsible for the masking effect. In an update in 1996, based upon comments received on the proposal in 1993 for the UL level of 1 mg total folate, this conclusion remained unchanged (FDA, 1996).

More recently, the US Committee evaluating the new dietary reference intakes and the subcommittee on Upper Reference Levels of Nutrients (FNB DRI Report, 1998) concluded that progression of the neurological symptoms due to folic acid supplementation should be considered as the most serious adverse effect, and not the masking effect. Masking of the haematological signs in PA patients was considered a diagnostic problem that could be circumvented by using more specific tests (i.e. measuring serum MMA and/or Hcys) to identify cases of undiagnosed B_{12} deficiency. This Committee set a lowest-observed-adverse-effect level (LOAEL) of 5 mg folic acid and used an uncertainty factor of 5 because no NOAEL could be derived, resulting in an UL of 1 mg of folic acid.

5. DERIVATION OF A TOLERABLE UPPER INTAKE LEVEL (UL)

Although no systematic toxicological studies of folic acid or other folates are available, an upper safe level can be set for (synthetic) folic acid (PGA) on the basis of findings in PA patients treated with high doses of folic acid. There is no evidence for risk associated with high intakes of natural, reduced folates, and thus no data to set an UL for natural folate. Although there is no conclusive evidence in humans, the Committee concludes that the risk of progression of the neurological symptoms in vitamin B_{12}-deficient patients as a result of folic acid supplementation cannot be excluded and should be considered the most serious adverse effect. In nearly all studies showing neurological relapse, dose levels >5 mg folic acid per day have been applied and data on the effect of dose levels between 1-5 mg is limited to a few cases.

In analogy with the US DRI Committee, it is concluded that the best available estimate for a lowest-observed-adverse-effect level (LOAEL), obtained from a sensitive group, for folic acid is 5 mg, and as dosages up to 1 mg of folic acid are unlikely to cause masking of the haematological signs in PA patients, the UL is set at 1 mg of folic acid. No data are available to suggest that other life-stage groups have increased susceptibility to adverse effects of high folic acid intake. Therefore, the UL is also applicable for pregnant or lactating women. It seems prudent, however, to adjust the ULs for children on the basis of bodyweight.

UL for children and adolescents

Age (year)	UL (µg)
1 - 3	200
4 - 6	300
7 - 10	400
11 -14	600
15 - 17	800

6. CHARACTERISATION OF RISK

Average folate intake in Europe is around 300 µg/day in adult men, and 250 µg in adult women (De Bree *et al*, 1997). Dietary folate consists only of reduced folates, and contains no folic acid, unless added. High intake levels (97.5th percentile) for folate from dietary sources around 500 µg/day have been reported, the higher data reported for Austria (see Section 2), are likely from all sources, including supplements.

Data from the 2nd Dutch National Food Consumption Survey on supplement use indicated that the mean folic acid intake from supplements among users is 100 µg, with a 97.5th percentile and maximum intake of 400 and 800 µg, respectively (Ronda *et al*, 1996).

Data from the Boston Nutritional Status Survey on folic acid supplement use among elderly males and females show that the median (P-50) intake from supplements is 400 µg/day; the P-95 was 2400 (M) and 1000 (F) µg/day, respectively (data taken from FNB DRI Report, IOM, 1998).

Regular supplements available on the market usually contain 400-500 µg folic acid. Women with a pregnancy wish are advised to use a daily supplement containing 400-500 µg folic acid between 4 weeks before and up to 8 weeks after conception to reduce NTD (neural tube defect) risk. In some European countries, such as the UK, cereals and breads are fortified with folic acid, contributing 25-100 µg per serving.

Subjects at risk for too high folic acid supplementation are those with an (undiagnosed) vitamin B_{12} deficiency, such as in pernicious anemia (PA) patients and in other conditions associated with cobalamin malabsorption. Figures on the prevalence of PA in W-Europe vary between 1.2/1000 in the UK and 1.98. Among Caucasians, PA is mostly found in elderly people, but in African-Americans individuals it was also reported under 40 years of age. Other cases of hypocobalaminemia, i.e. the occurrence of a low serum cobalamin level without specific anemic abnormalities, were found to be associated with dementia, AIDS, and with malignant diseases. Recent data show a high prevalence (ca 25%) of marginal cobalamin deficiency in elderly, characterised by low serum cobalamin and increased plasma methylmalonic acid (MMA) levels, but not, or hardly associated with haematological abnormalities (van Asselt, 1998).

Other groups at risk for a marginal intake of vitamin B_{12} are groups avoiding animal products, such as vegans and macrobiotics.

7. RECOMMENDATIONS FOR FURTHER RESEARCH

The extent to which a high dietary folate intake affects the symptomatology of a vitamin B_{12} deficiency (i.e. the occurrence of neurological *vs*. haematological signs) remains to be investigated. As elderly are especially at risk for a marginal vitamin B_{12} status, not necessarily related to PA, the potential risks of folic acid supplementation in this group needs further investigation.

The safety and efficacy of synthetic reduced folates, i.e. 5-MTHF, as an alternative for folic acid (PGA), also needs further study.

8. REFERENCES

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