

RIVM report 3402300002/2005

**Masking of vitamin B₁₂ deficiency
associated neuropathy by folic acid**

J.G.C. van Amsterdam, A. Opperhuizen,
E.H.J.M. Jansen

Contact: J.G.C. van Amsterdam

Laboratory for Toxicology, Pathology and Genetics (TOX)

E-mail: JGC.van.Amsterdam@rivm.nl

This investigation has been performed by order and for the account of Department for Nutrition and Health Protection, Ministry of Welfare, Public Health and Sport within the framework of project 340230 Risk assessment folic acid supplementation.

RIVM, P.O. Box 1, 3720 BA Bilthoven, telephone: 31 - 30 - 274 91 11; telefax: 31 - 30 - 274 29 71

RAPPORT IN HET KORT

Vitamine B₁₂-deficiëntie geassocieerde neuropathie

Het Ministerie van VWS overweegt om bepaalde voedselbestanddelen te verrijken met foliumzuur. Foliumzuurverrijking houdt echter een gezondheidsrisico in, omdat het vitamine B₁₂-deficiëntie maskeert, waardoor het de incidentie van megaloblastische anemie en perifere neuropathie kan verhogen. Dit rapport geeft een overzicht van de literatuur met betrekking tot het potentieel maskerende effect door foliumzuur van pernicieuze anemie -het vroege symptoom- van vitamine B₁₂-deficiëntie.

Het vermoeden, dat foliumzuur neurologische complicaties kan geven en verergeren, komt mogelijke door publicaties uit de veertiger jaren, toen foliumzuur voor het eerst - en onjuist - gebruikt werd om patiënten met pernicieuze anemie te behandelen.

Foliumzuursuppletie (zonder co-suppletie met vitamine B₁₂) induceert of stimuleert niet de met vitamine B₁₂-deficiëntie geassocieerde neuropathie. Een belangrijke aanwijzing hiervoor werd recent verkregen in de V.S., waar na de verrijking van granen met foliumzuur, het percentage anemie onder personen met een laag serum vitamine B₁₂ niet significant steeg ten opzichte van de periode daarvoor. Huisartsen dienen alert te zijn op een juiste diagnose van pernicieuze anemie en de hoge prevalentie van vitamine B₁₂-deficiëntie in bepaalde risicogroepen (ouderen en vegetariërs). Maskering van pernicieuze anemie is dan geen argument om de verrijking van voeding met foliumzuur af te wijzen.

Trefwoorden: vitamine B₁₂, foliumzuur, neuropathie, maskering, voedingssupplement

ABSTRACT

Masking of vitamin B₁₂ deficiency associated neuropathy by folic acid

The Dutch authorities consider fortifying certain foods with folic acid. Folic acid supplementation may, however, mask vitamin B₁₂ deficiency and increase the incidence of peripheral neuropathy. This literature review outlines published studies to the potential masking of vitamin B₁₂ -deficiency- related pernicious anaemia by folic acid.

Probably, the assumption that folic acid may precipitate and aggravate neurological manifestations comes from publications in the mid-1940s, when folic acid was first used to treat patients with pernicious anaemia. This resulted in an alarming incidence of relapse or progression of pre-existing neurological lesions.

Indeed, treatment of vitamin B₁₂ -deficient subjects with folic acid is a false and dangerous therapy, though supplementation with folic acid at daily doses less than 5 mg has rarely been linked with a direct adverse effect on vitamin B₁₂ deficiency- associated neurological damage.

After the fortification of cereals with folic acid in the U.S.A., the proportion of subjects without anaemia but with low vitamin B₁₂ did not increase significantly, indicating that increased folic acid intake caused no major increase in masking vitamin B₁₂ deficiency.

There is no conclusive evidence that folic acid supplementation (in the absence of co-supplementation with vitamin B₁₂) precipitates, or enhances vitamin B₁₂-deficiency-associated neuropathy. If practitioners were to properly diagnose vitamin B₁₂ deficiency and beware of its high prevalence in certain high risk groups (elderly and vegetarians), there would be no argument for renouncing the fortification of food with folic acid by masking vitamin B₁₂ deficiency.

Key words: vitamin B₁₂, folic acid, neuropathy, masking, food supplement

Contents

SAMENVATTING.....	5
SUMMARY	6
1. INTRODUCTION	7
1.1 PROBLEM STATEMENT	7
2. PERNICIOUS ANAEMIA.....	9
3. HISTORY.....	10
4. PREVALENCE OF PERNICIOUS ANAEMIA.....	12
5. DETAILED STUDIES	13
5.1 MECHANISTIC BASIS.....	13
5.2 ANIMAL STUDIES.....	13
5.3 HUMAN STUDIES.....	14
6. FOLIC ACID INDUCED NEUROPATHY.....	18
7. FOLIC ACID FORTIFICATION.....	19
8. VITAMIN B12 INTAKE	21
9. CONCLUSION.....	22
REFERENCES.....	22
ACKNOWLEDGEMENT	28

SAMENVATTING

Het Ministerie van VWS overweegt om bepaalde voedselbestanddelen te verrijken met foliumzuur. Foliumzuurverrijking kan echter vitamine B₁₂-deficiëntie maskeren en zo de incidentie van perifere neuropathie verhogen. Dit rapport geeft een overzicht van de literatuur met betrekking tot het potentieel maskerende effect door foliumzuur van pernicieuze anemie - het vroege symptoom- van vitamine B₁₂-deficiëntie.

Het vermoeden, dat foliumzuur neurologische complicaties kan geven en verergeren, komt mogelijke door publicaties uit de veertiger jaren, toen foliumzuur voor het eerst gebruikt werd om pernicieuze anemie patiënten te behandelen. Dit leidde tot een alarmerende incidentie van neurologische lesies.

Inderdaad is de behandeling van vitamine B₁₂-deficiënte personen met foliumzuur een foute en gevaarlijke therapie. Anderzijds is de suppletie met foliumzuur met dagelijkse doseringen van minder dan 5 mg zelden geassocieerd met directe schadelijke effecten op de vitamine B₁₂ deficiëntie geassocieerde neurologische schade.

Na de verrijking van granen met foliumzuur in de V.S. steeg overigens het percentage anemie onder personen met een laag serum vitamine B₁₂ niet significant ten opzichte van de periode daarvoor. Dit is een aanwijzing dat extra inname van foliumzuur geen toename geeft van maskering van vitamine B₁₂-deficiëntie.

Er is geen duidelijk bewijs dat foliumzuur suppletie (zonder co-suppletie met vitamine B₁₂) vitamine B₁₂-deficiëntie geassocieerde neuropathie induceert of stimuleert.

Huisartsen dienen zich bewust te zijn van de hoge prevalentie van vitamine B₁₂-deficiëntie in bepaalde hoog risicogroepen (ouderen en vegetariërs); de maskering door foliumzuur van pernicieuze anemie is dan geen argument meer om de verrijking van voeding met foliumzuur af te wijzen.

SUMMARY

The Dutch authorities consider to fortify certain foods with folic acid. Folic acid supplementation may, however, mask vitamin B₁₂ deficiency and increase the incidence of peripheral neuropathy. The present paper reviews the literature on the potential masking of vitamin B₁₂ deficiency related pernicious anaemia by folic acid.

Probably, the assumption that folic acid may precipitate and aggravate neurological manifestations comes from publications in the mid-1940s, when folic acid was first used to treat patients with pernicious anaemia. This resulted in an alarming incidence of relapse or progression of pre-existing neurological lesions.

Indeed, treatment of vitamin B₁₂ deficient subjects with folic acid is a false and dangerous therapy, though supplementation with folic acid at daily doses less than 5 mg has been rarely associated with a direct adverse effect on vitamin B₁₂ deficiency associated neurological damage.

After the fortification of cereals with folic acid in the U.S., the proportion of subjects with low vitamin B₁₂ without anaemia did not increase significantly which indicates that increased folic acid intake caused no major increase in masking vitamin B₁₂ deficiency.

There is no conclusive evidence that folic acid supplementation (in the absence of co-supplementation with vitamin B₁₂) precipitates, or enhances vitamin B₁₂-deficiency associated neuropathy. Practitioners should properly diagnose and be aware of the high prevalence of vitamin B₁₂ deficiency in certain high risk groups (elderly and vegetarians). If so, masking by folic acid of pernicious anaemia due to vitamin B₁₂ deficiency is no argument to renounce the fortification of food with folic acid.

1. INTRODUCTION

The Dutch authorities consider the fortification of certain foods with folic acid. In addition to megaloblastic anaemia, vitamin B₁₂ deficiency, may elicit peripheral neuropathy, a dangerous neuronal lesion. As it precedes or at least coincides with peripheral neuropathy, megaloblastic anaemia is an important first clinical symptom. Such anaemia is not seen in vitamin B₁₂ deficient subjects, when folic acid supplements or folic acid fortified foods are consumed. As such, folic acid supplementation may mask vitamin B₁₂ deficiency and increase the incidence of peripheral neuropathy. Note that folate is present in natural foods, whereas folic acid is the synthetic form used in supplementation and fortification.

1.1 Problem statement

Deficiency in either vitamin B₁₂ (cobalamin) or folate induces megaloblastic anaemia (“giant germ cell”) anaemia. Pernicious anaemia is caused by lack of intrinsic factor needed in the absorption of vitamin B₁₂. Therefore pernicious anaemia is a kind of megaloblastic anaemia, only caused by vitamin B₁₂ deficiency due to lack of intrinsic factor, a type of megaloblastic anaemia. Vitamin B₁₂ deficiency, but not folate deficiency, produces, however, in addition peripheral neuropathy. Secondly, folate and vitamin B₁₂ deficiency may be present at the same time. As such, the following situations may arise:

1.1.1. Masking

The megaloblastic anaemia that is caused by a vitamin B₁₂ deficiency does not develop in the presence of excess folate (the anaemia is ‘masked’ by folate). The anaemia of vitamin B₁₂ deficiency is often the first clinical symptom to appear, and this symptom is cured (and therefore masked!) by folate. If so, the vitamin B₁₂ deficiency is not timely recognised, and the associated neurological deterioration will progress, ultimately resulting in permanent damage to the nervous system [1-3]. There are, however, few systematic data regarding the level of folic acid intake required to mask vitamin B₁₂ deficiency [4]. Considering that a large part of the general population is exposed (or will be exposed) to folic acid-enriched foods, the potential masking effect of vitamin B₁₂ deficiency by folic acid is (will be) a serious public health issue.

1.1.2. Wrong treatment

If vitamin B₁₂ deficiency is treated with folic acid by mistake, the symptoms of anaemia (megaloblastic anaemia) may lessen, but the severe and irreversible nerve damage induced by inadequate vitamin B₁₂ may continue as it is not cured by folic acid. No controlled studies are, however, available showing that folic acid treatment adversely affects the course of untreated vitamin B₁₂ deficiency. Similarly, no data are available, indicating that rapid progression is anything other than one of the clinical courses to be expected in untreated vitamin B₁₂ deficiency, whether or not folic acid is consumed [5].

1.1.3. False diagnosis

The precise type of megaloblastic anaemia (vitamin B₁₂ or folate deficiency) should be properly diagnosed. This is not that obvious, because folate deficiency induces roughly the same haematological symptoms as vitamin B₁₂ deficiency, but patients with folate deficiency do not have the serious neurological signs, except for loss of short-term memory (“where did I leave my key?”). In making the diagnosis, one should bear in mind that immediately after a high dietary folate intake, serum folate levels can be normal in the presence of low red cell folate stores. Therefore, red blood cell folate is more sensitive for assessment of chronic folic acid deficiency than serum folate i.e. serum folate represents folate intake in the last 24 hours; red cell folate reflects folate intake over the last 4 months (the red cell life span). In summary, a careful serological (which type of deficiency) and neurological assessment is important.

Note: Hematological and epithelial manifestations occur in all megaloblastic disorders, but only vitamin B₁₂ deficiency causes neurological deficits. *Visa versa*, neurological findings may occur in the absence of anaemia and epithelial manifestations of pernicious anaemia. Secondly, pernicious anaemia can present with anaemia, neuropathy, or both, and the view that the disease progresses from anaemia to neuropathy is incorrect. Dickinson [6] reviewed the medical literature in 1995, and reported that in the first half of the 20th century pernicious anaemia presented with neurological symptoms alone in about 25% of the cases.

2. PERNICIOUS ANAEMIA

Pernicious anaemia is induced by a deficiency of vitamin B₁₂. About two-thirds of patients with vitamin B₁₂ deficiency have pernicious anaemia [7-9]. Pernicious anaemia presented with neurological symptoms alone in about 25% of the cases [6].

Vitamin B₁₂ is required for many cells, including blood and nerve cells. As a result, suboptimal vitamin B₁₂ availability induces anaemia and causes neurological problems. Giant germ cell anaemia is the result of pathologic slowing of the doubling of the nuclear DNA necessary for bone marrow nucleated cell growth with only minor slowing of cytoplasmic RNA-synthesis. The neurological symptoms (peripheral neuropathy, originally called sub-acute combined degeneration of the spinal cord; SADC) develop over time due to the gradual damage of sensory and motor nerves in the spinal cord. The mechanism is thought to occur via the interruption of the methylation cycle, and reduced ability to methylate myelin basic protein.

Early symptoms of severe vitamin B₁₂ deficiency (regardless of the cause) may include burning of the tongue, fatigue, weakness, loss of appetite, intermittent constipation and diarrhoea, abdominal pain, weight loss, menstrual symptoms, psychological symptoms, and nervous system problems, such as numbness and tingling in the feet and hands. Most symptoms can occur before the deficiency is severe enough to cause giant germ cell anaemia.

Prognosis for patients with pernicious anaemia is generally good. Many of the symptoms improve within just a few days of beginning treatment (vitamin B₁₂ treatment), although some of the nervous system symptoms may take up to 18 months to improve. Occasionally, when diagnosis and treatment have been delayed for a long time, some of the nervous system symptoms may be permanent [10].

3. HISTORY

The disease was named pernicious anaemia because it was fatal before proper treatment became available. The term pernicious is no longer appropriate, but it is retained for historical reasons. On appropriate treatment, patients presently have a normal life-span.

At the beginning of the 20th century, Whipple, Minot and Murphy investigated the cause of pernicious anaemia and treated pernicious anaemia patients with a liver diet. They saved the life of many patients and received the Nobel prize for their work in 1934. Following the identification and chemical synthesis of folic acid in 1945, but before the isolation of vitamin B₁₂ in 1948, folic acid (at high doses, generally > 5 mg/day), rather than liver extract, was first used to treat patients with pernicious anaemia in the mid-1940s. In 1946 it was, however, recognised in an Editorial in the scientific journal 'Blood' [11] that a considerable period of study would be necessary to ascertain whether folic acid would be as effective as liver extract in the maintenance of remission of haematological symptoms in pernicious anaemia patients.

Around 1947 practising clinicians noted that patients with vitamin B₁₂ deficiency treated with folic acid were at increased risk of neurological damage, because this therapy corrected the anaemia, but did not prevent the development or progression of the signs and symptoms of peripheral neuropathy from developing or progressing. This was based on the following observations:

- i) within one year of commencing folic acid in untreated vitamin B₁₂ deficiency the incidence of vitamin B₁₂ neuropathy was high (up to 80%);
- ii) there appeared to be a particularly high possibility of severe neurological symptoms developing at about three months after starting folic acid in these patients;
- iii) there was some evidence of a higher incidence of neuropathy as the daily dose of folic acid increased from 1.0 mg to 15 mg or more.

The alarming incidence of relapse or progression of pre-existing neurological lesions in patients with pernicious anaemia treated with folic acid alone (reviewed by Dickinson [12]) was given proper emphasis in another editorial that appeared in 1947 [13]. Because the frequency and the severity of the neurological deterioration seemed in some to suddenly have an explosive onset, the assumption arose that folic acid may even precipitate and aggravate the neurological manifestations [14,15].

The first demonstration of dramatic beneficial effects of vitamin B₁₂ in treating pernicious anaemia occurred in 1948.

Comment by the authors:

In the mid-1940s, when folic acid was first used to treat patients with pernicious anaemia (rather than liver extract), an alarming incidence of relapse or progression of pre-existing neurological lesions was observed. This is probably the origin of the still existing assumption that folic acid may precipitate and aggravate neurological manifestations.

4. PREVALENCE OF PERNICIOUS ANAEMIA

Pernicious anaemia is most prevalent in Northern Europe and among African Americans and less so among people from southern Europe and Asia. In the United States, pernicious anaemia is most prevalent among immigrants of either Celtic (i.e. English, Irish, Scottish) or Scandinavian origin. In these groups, 10-20 cases per 100,000 people occur per year. Meanwhile, it has become apparent that pernicious anaemia is more common than was previously recognised and occurs in all racial and ethnic groups. Data in the United States show that pernicious anaemia occurs in equal numbers in both men and women, whereas a female predominance has been reported in England, Scandinavia, and among persons of African descent (1.5 vs. 1). Among Caucasian's, the mean age of onset is 60 years, whereas it occurs at somewhat younger age in black people (mean age of 50 y).

5. DETAILED STUDIES

Whether folic acid intake i.e. folic acid food fortification precipitates or exacerbates the neurological damage of B₁₂ deficiency has been studied in animals and humans. Case studies reporting slow onset or progression of neurological complications in vitamin B₁₂ deficient subjects treated with folic acid supplements have been summarised in Table 1 (derived from the Food and Nutrition Board; reference 21), and are outlined below.

5.1 Mechanistic basis

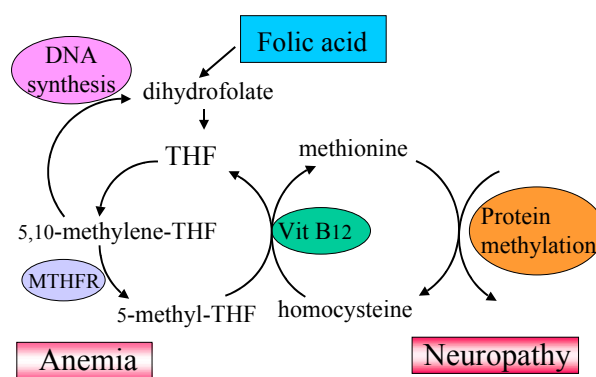


Figure 1. Metabolic routes involving folic acid. Deficiency in folate gives megaloblastic anaemia, whereas vitamin B₁₂ deficiency elicits the same type of anaemia and in some 40% of the cases also symptoms or signs of neuropathy.

Folate is essential for the methylation of various vital compounds in the body. Under sub-optimal level of vitamin B₁₂ this methylation process is, however, disturbed because the available folic acid can not be converted beyond 5-methyl-THF (Fig. 1). Acquiring a methyl group from 5-methyl-THF, homocysteine is remethylated to methionine, and this latter step requires the presence of vitamin B₁₂ as co-factor. Thus 5-methyl-THF is “trapped” or unable to be metabolised to THF (referred as the *methylfolate trap* in Anglo-Saxonian literature) [16].

5.2 Animal studies

Studies in monkeys [17] and fruit bats [18,19] show that vitamin B₁₂-deficient animals receiving supplemental folic acid develop signs of neuropathology earlier than controls do. The studies in fruit bats where vitamin B₁₂ deficiency was induced with nitrous oxide [20], showed exacerbation of neurological signs after folic acid administration. These fruit bats were given

daily large oral doses of folic acid (1.5 mg/kg; equivalent to ca 100 mg/day in humans), or daily intramuscular injections of formyl-THF (1.2 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 the rhesus monkey studies dietary methods were used to induce vitamin B₁₂ deficiency. 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) [17]. 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.

Note: This paragraph was adapted from Food and Nutrition Board, Institute of Medicine [21].

Comment by the authors:

It has been clearly demonstrated that treatment of vitamin B₁₂ deficient animals with folic acid induces severe neurological symptoms.

5.3 Human studies [22]

In contrast with the anaemia in pernicious anaemia patients that is correctable, at least temporarily, by folic acid treatment, neurological damage is not reversed and progresses [23-31]. The majority of available information relates to early case reports of folic acid therapy for the treatment of pernicious anaemia where usually high doses (> 5 mg/day) have been used. Early human case reports (cf. Table 1) show onset or progression of neurological complications in vitamin B₁₂-deficient individuals, receiving supplemental folic acid. A selection of the studies is presented below.

In a report on 10 cases of pernicious anaemia patients treated with 5-25 mg folic acid, one patient experienced neurological symptoms after eight days, and two patients after 4 and 9 months of treatment, respectively [32]. Vilter et al. [33] found that folic acid did not prevent the development of neurological lesions in 4 out of 21 patients receiving 10-50 mg of folic acid

daily. Still larger daily dosages of folic acid from 100 to 500 mg did not halt the progress of degeneration, but liver extract rapidly did.

In another study reported in 1948, neurological symptoms remained stable or improved in 4 out of 70 patients treated daily with 5-20 mg folic acid for a period of 6-12 months, but deteriorated in 3 subjects [34]. One of the six patients without evidence of neuropathy and with normal blood values, developed severe paraplegia while receiving 10 mg of folic acid daily during one year or longer.

In a study from Schwartz et al. [30], 98 pernicious anaemia patients treated with 5 mg of folic acid were followed for a period up to 3½ years, 4 patients relapsed neurologically within 1 year, and 19 in the next year. These studies show that neurological symptoms, especially posterolateral spinal cord disease and peripheral neuritis, occurred in 56 percent of the patients. Although the neurological degeneration was not caused by the folic acid treatment, the data clearly demonstrated the masking potential of folic acid.

Heinle [29] described a case report where the pernicious anaemia patient treated with 10 mg/day of folic acid for 79 days (later increased to 100 mg/day) developed neurological relapse. The relapse was unusually “explosive” and rapidly progressive, and occurred after blood had reached normal levels. Twenty-seven days after the first dose of liver extract (i.m.) the condition of this patient improved. On the other hand from the total of 47 pernicious anaemia patients treated with folic acid only three subjects exhibited neurological relapse (responding readily to therapy with liver extract).

Similar results were reported by Ross et al. [35] who substituted liver extract by folic acid (1-15 mg daily for 8-17 mo.) in 22 cases of pernicious anaemia. Though ten patients showed a significant improvement in blood values, seven patients developed neurological relapse, and four showed progression of neurological lesions. The neurological relapse occurred with considerable suddenness and progressed with great rapidity in several patients. The rapidity was considerably greater than usually observed in untreated pernicious anaemia patients, necessitating in some cases termination of folic acid and reinstatement of liver extract.

Spies et al. [36] similarly reported risk of nervous system degeneration in 28 out of 38 pernicious anaemia patients treated with 10 mg folic acid per day, and neurological degeneration within 2 to 5 months was reported in fourteen patients with vitamin B₁₂ deficiency who were treated with oral doses of 5 to 20 mg folic acid [28]. Studying the long-term (3½ years) therapeutic effectiveness of 5 mg folic acid in 98 pernicious anaemia patients, Schwartz et al. [30] reported that 31 patients relapsed neurologically (9 of them combined with haematological relapse).

Table 1. Dose and duration of oral folic acid administration and the occurrence of neurological manifestations in patients with pernicious anaemia. From: Food and Nutrition Board, Institute of Medicine [21].

No. subjects	Folic acid (mg/d)	Duration	Occurrence ^a	Reference
1	0.35	2 yrs	1 of 1	[38]
1	0.33–1	3 mo	1 of 1	[24]
1	0.5	16 mo	1 of 1	[23]
4	1.25	9–23 mo	1 of 4	[35]
4	1.25 ^b	3.5–26 mo	3 of 4	[39]
2	1.5–2.55	10–39 mo	2 of 2	[40]
1	4.5	3 yr	1 of 1	[41]
98	5	48 mo	32 of 98	[30]
2	5	20–23 mo	1 of 2	[35]
2	5–8	2–2.5 yr	2 of 2	[41]
36	5–10	1–10 yr	16 of 36	[27]
15	5–20	12 mo	4 of 15	[34]
11	5–30	3–25 mo	7 of 11	[39]
20	5–40	35 mo	16 of 20	[42]
10	5–600	12 mo	8 of 10	[32]
1	5.4–6.4	2 yr	1 of 1	[24]
1	6.68	2.5 yr	1 of 1	[40]
12	10	>17 mo	3 of 12	[43]
1	10	26 mo	1 of 1	[44]
1	10	22 d	1 of 1	[45]
6	10–15	± 12 mo	4 of 6	[35]
14	10–15	2–5 mo	3 of 14	[28]
16	10–40	± 12 mo	2 of 16	[29]
1	10–65	5 mo	1 of 1	[46]
1	10–100	4 mo	1 of 1	[29]
38	± 10	24 mo	28 of 38	[36]
7	15	28–43 mo ^c	3 of 7	[35]
1	15	10.5 mo ^c	1 of 1	[39]
2	15–20	4–5 mo	2 of 2	[47]
21	50–500	10–40 ^c	4 of 4	[33]

^a Refers to neurological relapses or progression of pre-existing neurological manifestations while on folic acid therapy; ^b In two patients: minimal or slight neurological progression. Neurological progression was also observed when the dose was increased to 15 mg/day; ^c The initial dosage of 1.25 mg/day was increased to 15 mg/day after variable durations of treatment. Neurological progression occurred only at 15 mg/day in these patients.

In a study [27] on 46 pernicious anaemia patients treated with 30 mg of folic acid three times a week, 36 patients were followed for 1 to 10 years and compared with 40 patients treated parenterally with vitamin B₁₂. Of these 36 patients only three were maintained for 10 years in satisfactory haematological and neurological condition on folic acid therapy alone. The great majority of these patients relapsed in the first four years, whereas the patients given vitamin B₁₂ showed no deleterious effects and maintained patients in good health for 6-7 years.

Of 25 patients treated for 7 to 19 days (< 1 mg/day), none developed nervous system disorder, whereas 6 out of 12 treated with the same dose for 90 to 930 days did so, none of whom had an “explosive” course. Apparently, small doses of folic acid did not induce the striking clinical changes observed in vitamin B₁₂ deficient patients receiving higher (> 5mg/day) amounts of folic acid [37].

In 1951 Conley and Krevans [41] described five patients with pernicious anaemia treated with folic acid (5-8 mg per day; 3 years) in whom neurological signs had developed in the absence of haematological lesions. Similar case reports were described by Fowler and Hendricks [47], Crosby [38], Best [44], Israëls [42], Spiess and Stone [45], Wagley [32], Victor and Lear [40], Berk et al. [43]. Some of them described subjects who took multi-vitamin preparations.

Conversely, some patients with known vitamin B₁₂ deficiency have shown no development of neurological complications when treated with very high (10-100 mg) daily doses of folic acid for a number of years (cf. [3] and references therein). In general, it has been concluded from anecdotal information that supplementation with folic acid at daily doses less than 5 mg is rarely associated with a direct adverse effect on vitamin B₁₂ deficiency associated neurological damage [3,48]. Despite the beneficial anaemic effect of folic acid, pernicious anaemia should be treated with vitamin B₁₂ and not with folic acid. Indeed, Baldwin [23] ends up his paper with the statement that folic acid has no place in multivitamin or “shotgun” anti-anaemic preparations! Victor and Lear [40] state that “prescribing oral multiple vitamin preparations for patients with anaemia, cannot be too strongly condemned.”

Comment by the authors:

The studies cited above demonstrate that treatment of vitamin B₁₂ deficient subjects with folic acid is a false and dangerous therapy as it does not prevent the neurological damage induced by vitamin B₁₂ deficiency. Supplementation with folic acid at daily doses less than 5 mg, however, has been rarely associated with a direct adverse effect on vitamin B₁₂ deficiency associated neurological damage. Anyway, the best treatment for vitamin B₁₂ deficient subjects is supplementation of vitamin B₁₂, and not additional folic acid.

6. FOLIC ACID INDUCED NEUROPATHY

It has been suggested that folic acid supplementation (in the absence of co-supplementation with vitamin B₁₂) may either precipitate, or enhance vitamin B₁₂-deficiency associated neuropathy [29,35]. Such 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 five years in development of paraesthesia. A few uncontrolled trials have further suggested that supplementation with folic acid can reduce serum vitamin B₁₂ levels, but this is very unlikely. Because it would be unethical to withhold vitamin B₁₂ from pernicious anaemia patients in double blind controlled trials, definitive trials examining the effect of folic acid in this setting are not expected [3], and the question as to whether folic acid is harmful to the nervous system will remain unanswered. As such, the claim that folic acid therapy may precipitate or exacerbate neurological dysfunction in vitamin B₁₂-deficient patients remains unproven.

Comment by the authors:

There is no conclusive evidence that folic acid supplementation (in the absence of co-supplementation with vitamin B₁₂) precipitates, or enhances vitamin B₁₂-deficiency associated neuropathy. No data are available or will become available from double blind controlled studies that (will) fully exclude this possibility.

7. FOLIC ACID FORTIFICATION

Folate deficiency (low red cell folate level $<140 \mu\text{g/L}$) is frequently observed. The current averaged daily folate intake in Germany is for example clearly below $300 \mu\text{g}$ (197 to 235 for men and 168 to $214 \mu\text{g}$ for women) so that a large proportion of the general population do not attain the required natural folate intake. Particular risk groups are elderly people, pregnant women, alcohol abuse and patients with renal disease, mal-absorption or cancer [49].

Maintaining a folate intake of 600 - $650 \mu\text{g/day}$, by supplementing $400 \mu\text{g/day}$, may help to lower elevated homocysteine levels. The safe upper limit of folic acid set by the United States Food (FDA) and Nutrition Board (FNB) was 1 mg/day , a round number chosen by the Institute of Medicine [50] and unlikely to produce masking. The increment predicted by the FDA was 70 - $130 \mu\text{g/day}$ [51], and this level of fortification was chosen so that only a very small proportion of the population would receive more than 1 mg .

A recent study [52] on the effect of food fortification on folic acid intake in the U.S. showed, however, that the general population consumed about twice this originally anticipated intake. After the fortification of grain products initiated in 1998 the averaged additional folic acid intake (by subjects not taking additional supplements) is $220 \mu\text{g/day}$. Similar estimates of folic acid intake following food fortification (i.e. $190 \mu\text{g/day}$) in the U.S. have been calculated before by Choumenkovitch et al. [53]. Prevalence of individuals with folic acid intake above the upper tolerable intake level (1 mg/day) increased only among supplement users exposed to fortification (from 1.3 to 11.3%). Interestingly, the number of subjects with a combined low vitamin B₁₂ status ($< 260 \text{ pM}$) and high folic acid intake ($> 1 \text{ mg/day}$) was none (out of 931) respectively three (out of 549) before and following the start of fortification, respectively (all three were supplement users).

In the "Framingham" cohort, the folic acid fortification in non-supplement users has reduced the incidence of abnormal low folate ($<3 \mu\text{g/L}$; 7 nM) from 21% to less than 2% [54]. In the subjects from the same cohort red blood cell folate was measured before folic acid fortification (not exposed) and after implementation of fortification (exposed). Those exposed to folic acid fortification ($N=354$) had a mean red blood cell folate of $450 \mu\text{g/L}$ (1020 nM), a value 38% higher than the mean red blood cell folate of $325 \mu\text{g/L}$ (737 nM) of those who were not exposed ($N=561$) to fortification ($P < 0.001$). The prevalence of individuals with deficient red blood cell folate ($<160 \mu\text{g/L}$, 363 nM) was 4.9% in the non-exposed group, compared with 1.9% in the exposed group ($P < 0.02$). The prevalence of individuals with acceptable red blood

cell folate ($>200 \mu\text{g/L}$, 453 nM) was 87.0% in the non-exposed group compared with 96.1% in the exposed group ($P < 0.001$) [55].

Mills et al. [56] clearly showed in a recent paper from 2003 that fortification of food with folic acid did not increase the proportion of subjects with low vitamin B₁₂ but without anaemia. Of the 1573 subjects with low serum vitamin B₁₂ ($< 258 \text{ pM}$), the proportion without anaemia did not increase significantly from pre-fortification (39.2%) to the post-fortification period to (37.6%). This indicates that increased folic acid intake caused no major increase in masking vitamin B₁₂ deficiency.

Comment by the authors: following the fortification of cereals with folic acid in the U.S., the general population consumed about twice this originally anticipated intake. More importantly, the proportion of subjects with low vitamin B₁₂ without anaemia did not increase significantly which indicates that increased folic acid intake caused no major increase in masking vitamin B₁₂ deficiency.

8. VITAMIN B₁₂ INTAKE

Vitamin B₁₂ is naturally found in foods of animal origin like meat, milk and dairy products, and eggs, but not in plants [57]. Most people need only 2 micrograms of vitamin B₁₂ daily but the average diet provides about 5 to 30 micrograms a day. Obviously, due to their consumption pattern, vegetarians are at increased risk to be vitamin B₁₂-deficient, and strict vegetarians need therefore to be informed about this risk.

Vitamin B₁₂ deficiency (serum level < 260 pM; <400 pg/ml) may affect substantially the older part of the population [58], due to a high prevalence of vitamin B₁₂ mal-absorption. About 14% of elderly Americans and about 24% of elderly Dutch have mild B₁₂ deficiency; the difference probably due to the higher intake of vitamin supplements by the Americans [59,60].

Certain drugs (e.g. nitrous oxide, cholestyramine, p-aminosalicylic acid, neomycin, metformin, colchicine) may also impair vitamin B₁₂ uptake in the ileum. This rarely causes symptomatic vitamin B₁₂ deficiency, because they are not taken long enough to deplete body stores of vitamin B₁₂ (caveat: older subjects). Adults older than 50 years are therefore advised to consult their physician about the advisability of taking folic acid without also taking a vitamin B₁₂ supplement. Finally, the British National Formulary (BNF) notes that folic acid should be prescribed only in the presence of vitamin B₁₂-deficiency.

Comment by the authors: Vitamin B₁₂ deficiency (serum level < 260 pM; <400 pg/ml) may affect substantially the older part of the population and vegetarians. It therefore advised that adults older than 50 years and vegetarians consult with their physician about taking a vitamin B₁₂ supplements.

9. CONCLUSION

The safety issue of masking is still to the argument that folic acid consumption should not be increased. However, nowadays the origin of pernicious anaemia can be and should be properly diagnosed. In addition, practitioners should be aware of the high prevalence of vitamin B₁₂ deficiency in certain high risk groups (elderly and vegetarians). If so, masking by folic acid of pernicious anaemia due to vitamin B₁₂ deficiency is no argument to renounce the fortification of food with folic acid. Data claiming that folic acid may precipitate neurological damage is of limited scientific value, and remains unproven.

REFERENCES

1. Bower C and Wald NJ. Vitamin B12 deficiency and the fortification of food with folic acid. *Eur J Clin Nutr* 1995; 49, 787-793.
2. Weir DG and Scott JM. Brain function in the elderly: role of vitamin B12 and folate. *Br Med Bull* 1999; 55, 669-682.
3. Campbell NR. How safe are folic acid supplements? *Arch Intern Med* 1996; 156, 1638-1644.
4. Koehler KM, Pareo-Tubbeh SL, Romero LJ, Baumgartner RN, and Garry PJ. Folate nutrition and older adults: challenges and opportunities. *J Am Diet Assoc* 1997; 97, 167-173.
5. Oakley G-PJ. Inertia on folic acid fortification: public health malpractice. *Teratology* 2002; 66, 44-54.
6. Dickinson CJ. Does folic acid harm people with vitamin B12 deficiency? *QJM* 1995; 88, 357-364.
7. Healton EB, Savage DG, Brust JC, Garrett TJ, and Lindenbaum J. Neurologic aspects of cobalamin deficiency. *Medicine (Baltimore)* 1991; 70, 229-245.
8. Borch K and Liedberg G. Prevalence and incidence of pernicious anemia. An evaluation for gastric screening. *Scand J Gastroenterol* 1984; 19, 154-160.
9. Shorvon SD, Carney MW, Chanarin I, and Reynolds EH. The neuropsychiatry of megaloblastic anaemia. *Br Med J* 1980; 281, 1036-1038.
10. Toh BH, van Driel IR, and Gleeson PA. Pernicious anemia. *N Engl J Med* 1997; 337, 1441-1448.
11. Editorial. Folic acid. *Blood* 1946; 1, 357.
12. Dickinson CJ. No reliable evidence that folate is harmful in B-12 deficiency. *BMJ* 1995; 311, 949.
13. Editorial. A warning regarding the use of folic acid. *New England J Med* 1947; 237, 713-714.
14. Meyer LM. Folic acid in the treatment of pernicious anamia. *Blood* 1947; 2, 50.
15. Vilter CF, Vilter RW, and Spies TD. The occurrence of combined system disease in persons with pernicious anamia during treatment with L. casei factor (folic acid). *Proc Central Soc Clin Research* 1946; 19, 26.
16. Shane B and Stokstad EL. Vitamin B12-folate interrelationships. *Annu Rev Nutr* 1985; 5,

- 115-141.
17. Agamanolis DP, Chester EM, Victor M, Kark JA, Hines JD, and Harris JW. Neuropathology of experimental vitamin B12 deficiency in monkeys. *Neurology* 1976; 26, 905-914.
 18. Van der Westhuyzen J and Metz J. Tissue S-adenosylmethionine levels in fruit bats (*Rousettus aegyptiacus*) with nitrous oxide-induced neuropathy. *Br J Nutr* 1983; 50, 325-330.
 19. Van der Westhuyzen J, Fernandes-Costa F, and Metz J. Cobalamin inactivation by nitrous oxide produces severe neurological impairment in fruit bats : protection by methionine and aggravation by folates. *Life Sci* 1982; 31, 2001-2010.
 20. Metz J and van der Westhuyzen J. The fruit bat as an experimental model of the neuropathy of cobalamin deficiency. *Comp Biochem Physiol A* 1987; 88, 171-177.
 21. Food and Nutrition Board IOM. Dietary Reference Intakes: A Risk Assessment Model for Establishing Upper Intake Levels for Nutrients. Washington DC: National Acad Press 1998.
 22. Expert group on vitamins and minerals (EVM). Safe upper levels for vitamins and minerals. Food Standard Agency, U K , ISBN 1-904026-11-7 20031-360.
 23. Baldwin JN and Dalessio DJ. Folic acid therapy and spinal-cord degeneration in pernicious anemia. *N Engl J Med* 1961; 264, 1339-1342.
 24. Ellison ABC. Pernicious anemia masked by multivitamins containing folic acid. *JAMA* 1960; 173, 240-243.
 25. Marshall RA and Jandl JH. Responses to 'physiologic' doses of folic acid in the megaloblastic anemias. *Arch Intern Med* 1960; 105, 352-360.
 26. Vilter CF, Will JJ, Wright T, and Rullman D. Interrelationships of vitamin b12, folic acid and ascorbic acid in the megaloblastic anaemias. *Am J Clin Nutr* 1963; 12, 130-144.
 27. Will JJ, Mueller JF, Brodine C et al. Folic acid and vitamin B12 in pernicious anemia. Studies on patients treated with these substances over a ten-year period. *J Lab Clin Med* 1959; 53, 22-38.
 28. Hall BE and Watkins CH. Experience with pteroylglutamic (synthetic folic acid) in the treatment of pernicious anemia. *J Lab Clin Med* 1947; 32, 622-634.
 29. Heinle RW and Welch AD. Folic acid in pernicious anemia: Failure to prevent neurologic relapse. *JAMA* 1947; 133, 739-741.
 30. Schwartz SO, Kaplan SR, and Armstrong BE. The long-term evaluation of folic acid in the treatment of pernicious anemia. *J Lab Clin Med* 1950; 35, 894-898.

31. Katz M. Letter: Potential danger of self-medication with folic acid. *N Engl J Med* 1973; 289, 1095.
32. Wagley PF. Neurologic disturbances with folic acid therapy. *N Engl J Med* 1948; 238, 11-15.
33. Vilter CF, Vilter RW, and Spies TD. The treatment of pernicious and related anemias with synthetic folic acid. 1. Observations on the maintenance of a normal hematologic status and on the occurrence of combined system disease at the end of one year. *J Lab Clin Med* 1947; 32, 262-273.
34. Bethel FH. The relations of therapy in pernicious anemia to changes in the nervous system. Early and late results in a series of cases observed for periods of not less than ten years, and early results of treatment with folic acid. *Blood* 1948; 3, 57-67.
35. Ross JF, Belding H, and Paegel BL. The development and progression of subacute combined degeneration of the spinal cord in patients with pernicious anemia treated with synthetic pteroylglutamic (folic) acid. *Blood* 1948; 3, 68-90.
36. Spies TD, Stone RE, Lopez GG et al. The association between gastric achlorhydria and subacute combined degeneration of the spinal cord. *Postgrad Med* 1948; 4, 89-95.
37. Savage DG and Lindenbaum J. Neurological complications of acquired cobalamin deficiency: clinical aspects. *Baillieres Clin Haematol* 1995; 8, 657-678.
38. Crosby WH. The danger of folic acid in multivitamin preparations. *Milit Med* 1960; 125, 233-235.
39. Chodos RB and Ross JF. The effects of combined folic acid and liver extract therapy. *Blood* 1951; 3, 1213-1233.
40. Victor M and Lear AA. Subacute combined degeneration of the spinal cord. Current concepts of the disease process. Value of serum vitamin B12 determinations in clarifying some of the common clinical problems. *Am J Med* 1956; 20, 896-911.
41. Conley CL and Krevans JR. Development of neurologic manifestations of pernicious anemia during multivitamin therapy. *N Engl J Med* 1951; 245, 529-531.
42. Israels MC and Wilkinson JF. Risk of neurological complications in pernicious anemia treated with folic acid. *BMJ* 1949; 2, 1072-1075.
43. Berk L, Bauer JL, and Castle WB. A report of 12 patients treated with synthetic pteroylglutamic acid with comments on the pertinent literature. *S Afr Med J* 1948; 22, 604-611.
44. Best CN. Subacute combined degeneration of spinal cord after extensive resection of ileum in Crohn's disease: Report of a case. *BMJ* 1959; 2, 862-864.

45. Spies TD and Stone RE. Liver extract, folic acid, and thymine in pernicious anemia and subacute combined degeneration. *Lancet* 1947; 1, 174-176.
46. Jacobson SD, Berman L, Axelrod AR, and Vonder Heide EC. Folic acid therapy: Its effect as observed in two patients with pernicious anemia and neurologic symptoms. *JAMA* 1948; 137, 825-827.
47. Fowler WM. Folic acid and the neurologic manifestations of pernicious anemia. *Am Practitioner* 1949; 3, 609-613.
48. Butterworth C-EJ and Tamura T. Folic acid safety and toxicity: a brief review. *Am J Clin Nutr* 1989; 50, 353-358.
49. Herbert V. The elderly need oral vitamin B-12. *Am J Clin Nutr* 1998; 67, 739-740.
50. Institute of Medicine PoFoBvaC. Dietary reference intakes: thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington DC: National Acad Press 1999.
51. FDA. Food labelling: health claims and label statements; folate and neural tube defects. *Federal Register* 1993; 58, no 197, 53254-53295.
52. Quinlivan EP and Gregory JF. Effect of food fortification on folic acid intake in the United States. *Am J Clin Nutr* 2003; 77, 221-225.
53. Choumenkovitch SF, Selhub J, Wilson PW, Rader JI, Rosenberg IH, and Jacques PF. Folic acid intake from fortification in United States exceeds predictions. *J Nutr* 2002; 132, 2792-2798.
54. Jacques PF, Selhub J, Bostom AG, Wilson PW, and Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *N Engl J Med* 1999; 340, 1449-1454.
55. Choumenkovitch SF, Jacques PF, Nadeau MR, Wilson PW, Rosenberg IH, and Selhub J. Folic acid fortification increases red blood cell folate concentrations in the Framingham study. *J Nutr* 2001; 131, 3277-3280.
56. Mills JL, Von K, I, Conley MR, Zeller JA, Cox C, Williamson RE, and Dufour DR. Low vitamin B-12 concentrations in patients without anemia: the effect of folic acid fortification of grain. *Am J Clin Nutr* 2003; 77, 1474-1477.
57. Herbert V. Vitamin B-12: plant sources, requirements, and assay. *Am J Clin Nutr* 1988; 48, 852-858.
58. Clarke R, Refsum H, Birks J, Evans JG, Johnston C, Sherliker P, Ueland PM, Schneede J, McPartlin J, Nexo E, and Scott JM. Screening for vitamin B-12 and folate deficiency in older persons. *Am J Clin Nutr* 2003; 77, 1241-1247.

-
59. Russell RM. Mild cobalamin deficiency in older Dutch subjects. *Am J Clin Nutr* 1998; 68, 222-223.
 60. Van Asselt DZ, de Groot LC, van Staveren WA, Blom HJ, Wevers RA, Biemond I, and Hoefnagels WH. Role of cobalamin intake and atrophic gastritis in mild cobalamin deficiency in older Dutch subjects. *Am J Clin Nutr* 1998; 68, 328-334.

Acknowledgement

The authors thank Ir. Janneke Kloosterman for her comments and critical reading of the manuscript.