

## Age-associated B vitamin deficiency as a determinant of chronic diseases

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The number of elderly individuals is growing rapidly worldwide and degenerative diseases constitute an increasing problem in terms of both public health and cost. Nutrition plays a role in the ageing process and there has been intensive research during the last decade on B vitamin-related risk factors in vascular and neurological diseases and cancers. Data from epidemiological studies indicate that subclinical deficiency in most water-soluble B vitamins may occur gradually during ageing, possibly due to environmental, metabolic, genetic, nutritional and pathological determinants, as well as to lifestyle, gender and drug consumption. Older adults have distinct absorption, cell transport and metabolism characteristics that may alter B vitamin bioavailability. Case-control and longitudinal studies have shown that, concurrent with an insufficient status of certain B vitamins, hyperhomocysteinaemia and impaired methylation reactions may be some of the mechanisms involved before a degenerative pathology becomes evident. The question that arises is whether B vitamin inadequacies contribute to the development of degenerative diseases or result from ageing and disease. The present paper aims to give an overview of these issues at the epidemiological, clinical and molecular levels and to discuss possible strategies to prevent B vitamin deficiency during ageing.

### Ageing: Micronutrients: B vitamin deficiency: Degenerative diseases

Micronutrients such as vitamins and minerals that are essential to biological reactions have been shown to play a role in the ageing process. In developed countries, classical vitamin-deficiency syndromes, such as scurvy, beriberi and pellagra, are now uncommon but specific population subgroups remain at risk for modest vitamin insufficiency. This is notably the case in elderly individuals, who frequently display an inadequate status of vitamin D and water-soluble vitamins including many of the B group. Extensive research in the last decade has suggested that subtle deficiencies in B vitamins are risk factors for degenerative diseases, including vascular diseases, mental health conditions such as dementia and depression, and cancer. The present review aims to report the latest findings on (i) B vitamin bioavailability and status in elderly individuals and (ii) the association of B vitamin insufficiency with chronic diseases. Finally, whether natural foods could deliver enough

B vitamins to achieve the required status and help to prevent or delay the onset of age-associated diseases will be discussed.

### B vitamin intake, bioavailability and status in the elderly

#### *A brief general overview*

Intake of B vitamins is dependent on the food supply. Most B vitamins are widely distributed in foods, but can be at relatively low concentrations (for example, vitamin B<sub>1</sub>), while others are only provided by certain categories of food (for example, dairy products and meat for vitamin B<sub>12</sub>). Synthetic forms of vitamins are also used for food fortification purposes (for example, folic acid), and for supplements (for example, cobalamin for strict vegetarians).

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**Abbreviations:** AD, Alzheimer's disease; CRC, colorectal cancer; CVD, cardiovascular disease; Hcy, homocysteine; MMA, methylmalonic acid; 5-mTHF, methyltetrahydrofolate; 5-MTHFR, methylenetetrahydrofolate reductase; NHANES, National Health and Nutrition Examination Survey; PLP, pyridoxal-5-phosphate; SAM, S-adenosylmethionine; SENECA, Study in Europe on Nutrition and the Elderly, a concerted action; SOD, superoxide dismutase.

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Bioavailability is usually defined as the proportion of micronutrient that is absorbed, then stored by tissues or used metabolically. Clear differences in bioavailability have been observed according to the type of food for a given B vitamin, on the one hand, and between B vitamins for a given food, on the other hand (Gregory, 1998; Tucker *et al.* 2000; Brouwer *et al.* 2001). Due to functional insufficiencies, the vitamin status of elderly individuals may be subject to higher variability than that of younger adults who are frequently taken as references. Estimation of how much of a B vitamin is required in the elderly must take into account all the potential age-related alterations. In particular, the declining of renal function with advancing age may become a relevant issue when the urinary excretion as well as the blood level of a vitamin, a vitamin metabolite or a biomarker is used as a proxy estimate of its absorption. For instance, serum homocysteine (Hcy) and methylmalonic acid (MMA) levels, which are used for the assay of folate and vitamin B<sub>12</sub> status, increase in renal insufficiency or hypovolaemia (Savage *et al.* 1994). Conversely, erythrocyte transketolase activity, which can serve as a biomarker of thiamine (vitamin B<sub>1</sub>) status, seems to decrease steadily over a range from 18 to 90 years (Rooprai *et al.* 1990).

Bioaccessibility must also be taken into consideration, since the release of B vitamins from the food matrix can be seriously impaired in age-related physiopathological situations.

Bioavailability assays in human subjects have been reviewed by Gregory & Quinlivan (2002) in the case of folate, which has been by far the most-studied B vitamin. The issue of biomarkers for B vitamins involved in one-C metabolism (vitamin B<sub>6</sub>, folate, vitamins B<sub>12</sub> and B<sub>2</sub>) has recently been addressed by Mason (2003). More general information on bioavailability (absorption, cell transport and metabolism), food distribution, stability (under physicochemical conditions or during food processing, storage and cooking), chemical structures and functions of B vitamins can be obtained from the Institute of Medicine, National Academy of Sciences (1998) and the *Handbook of Vitamins* (Rucker, 2001). The present review will consider mainly vitamins B<sub>1</sub> (thiamin), B<sub>2</sub> (riboflavin), B<sub>6</sub> (pyridoxine), folate and vitamin B<sub>12</sub> (cobalamin), since the other B vitamins (niacin, pantothenic acid and biotin) have been little studied in relation to ageing. As shown in Fig. 1, interactions may exist intracellularly between the metabolism of the different B vitamins.

### *B vitamin intake and status in the elderly population*

In a variety of countries, health and nutrition examination surveys have been used to estimate the dietary and total intake, as well as the status, of B vitamins in the elderly population. These include the third National Health and Nutrition Examination Survey 1988–1994 (NHANES III) and the elderly Framingham Heart Study cohort in the USA, and the National Diet and Nutrition Survey in the UK. Health and nutrition examination surveys in Europe also include the EURONUT Study in Europe on Nutrition and the Elderly, a concerted action (SENECA) in eleven European countries, the Dutch Nutrition Surveillance System in the Netherlands, the French Burgundy 'Etude du Statut VITaminique des Français' (ESVITAF) and

'Supplementation en Vitamines et Minéraux Antioxydants' (SU.VI.MAX) studies. However, a global assessment of the prevalence of B vitamin deficiency is rendered difficult by several factors. These include: (i) the unequal reliability of measurements of dietary intakes; (ii) discrepancies between status data based on different biomarkers and cut-off values; (iii) inter- and intra-method variations for vitamin assays; (iv) heterogeneity of the populations studied due to different regional origins or, especially as regards the elderly, to more or less extensive exclusions of individuals with certain diseases and treatments.

Other determinants of B vitamin deficiency in the elderly include:

The environment (institutionalisation (Essama-Tjani *et al.* 2000), hospitalisation, level of skilled nursing care, loneliness, income);

Gender (there may be a higher prevalence of B vitamin deficiency in elderly men than in women);

Lifestyle (smoking, sedentariness, excessive chronic consumption of alcohol), especially for vitamins B<sub>1</sub>, B<sub>6</sub> and folate (van den Berg *et al.* 2002);

Vegetarianism, especially for vitamin B<sub>12</sub>;

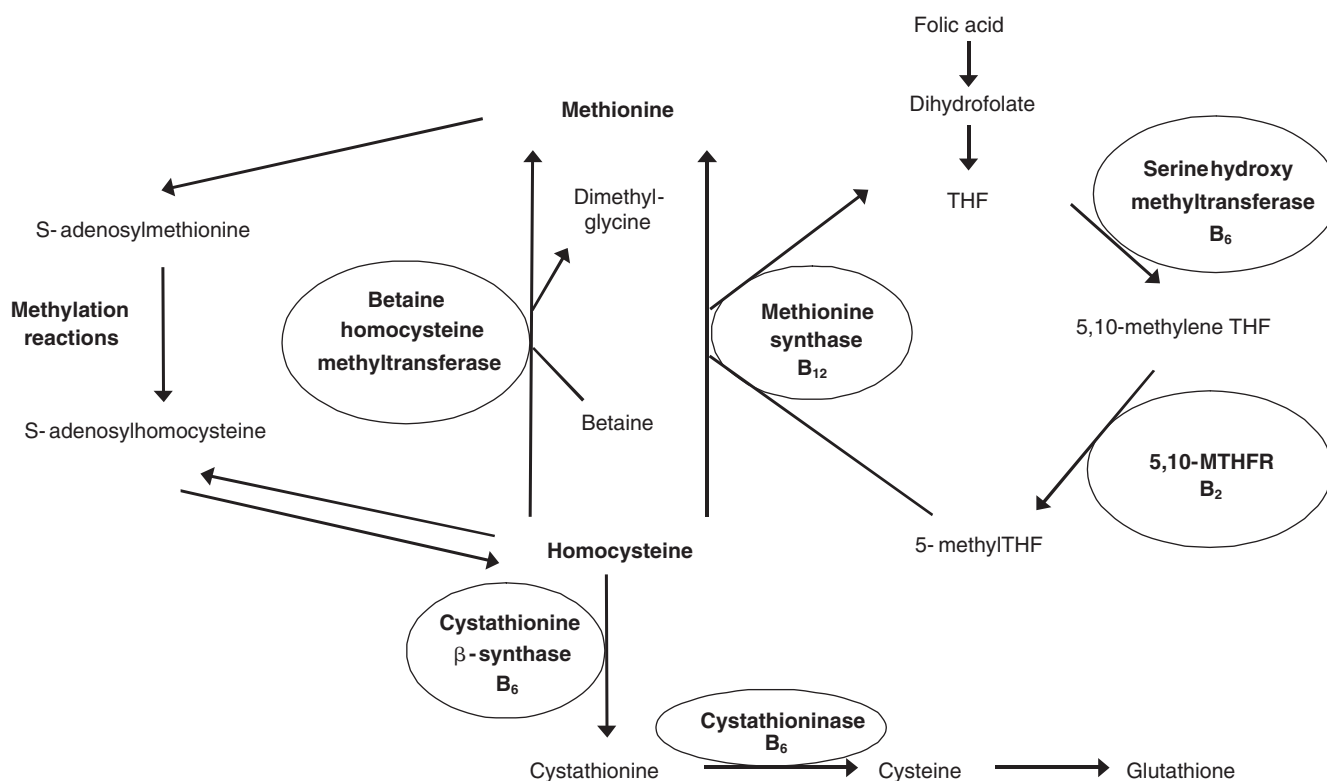
Genetic variations; for example, polymorphisms in the gene coding for methylenetetrahydrofolate reductase (MTHFR) (Meleady *et al.* 2003).

For instance, a reduced energy intake and vitamin bioavailability due to increased morbidity (chronic diseases and/or disability) and possibly concurrent drug consumption increase the risk for impaired B vitamin status in fragile elderly individuals (Desouza *et al.* 2002). Thus, controversy exists as to whether low plasma concentrations of B vitamins observed in the elderly strictly reflect changes with advancing age. Theoretically, the contribution of the ageing process *per se* to B vitamin deficiency should only be assessed in healthy elderly adults.

The intake of vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub> or folate below the recommended levels has been repeatedly reported in various groups of elderly individuals (Amorim Cruz *et al.* 1996; van der Wielen *et al.* 1996; Marshall *et al.* 2001). Regarding vitamin B<sub>12</sub>, very few, if any, of the elderly individuals studied, except those who were food-insecure, displayed insufficient intakes (Howard *et al.* 1998).

In a cross-sectional and 3-year longitudinal survey in New Zealand, the erythrocyte thiamin pyrophosphate concentration in elderly subjects, which was initially 32 % lower than in younger subjects, was further decreased by 20 % at the end of the study (Wilkinson *et al.* 2000). Such changes appeared to be related more to ageing than to comorbidities. Low thiamin status was also reported concurrent with apparently normal intake in Canadian free-living elderly subjects (Nichols & Basu, 1994). Ageing has been shown to be associated with a decreased passive diffusion permeability and a decreased affinity of the saturable transporter for thiamin despite an increased number of carriers (Gastaldi *et al.* 1992). Whether this may be related to the upregulation of intestinal transporters of vitamin B<sub>1</sub> by vitamin deficiency (also reported for vitamin B<sub>2</sub> and folate) remains to be elucidated.

The prevalence of poor riboflavin status in elderly individuals is between 16 and 45 % in most of the communities surveyed (Boisvert *et al.* 1993; Lopez-Sobaler *et al.* 2002).



**Fig. 1.** Metabolic interactions between folate and other B vitamins. Additionally, methionine synthase reductase which catalyses the NADPH-dependent reductive methylation of methionine synthase is a flavoprotein. It should also be noted that 5,10-methylenetetrahydrofolate is a substrate for purine synthesis via 10-formyl tetrahydrofolate and for thymidylate synthesis with the release of dihydrofolate. THF, tetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase.

Even more (77 % men, 79 % women) appeared to be at risk of riboflavin deficiency among free-living British elderly aged 68–90 years (Bailey *et al.* 1997). Moreover, Madigan *et al.* (1998) observed vitamin B<sub>2</sub> deficiency in elderly individuals with apparently adequate dietary intake. Such a paradox may indicate the possible malabsorption of vitamin B<sub>2</sub> in this population.

Using plasma pyridoxal-5-phosphate (PLP) as a biochemical marker, a high prevalence of vitamin B<sub>6</sub> deficiency was also observed in the studies of Bailey *et al.* (1997) and Madigan *et al.* (1998). An insufficient status of vitamin B<sub>6</sub> in 13 to 45 % of aged individuals was also suggested by many other studies using the same indicators or others (Selhub *et al.* 1993; Haller, 1999). Moreover, vitamin B<sub>6</sub> deficiency was found to increase with increasing age among the elderly population (Herrmann *et al.* 1999; Ravaglia *et al.* 2000). The ageing-dependent deficiency of vitamin B<sub>6</sub> could be due to changes at post-intestinal, cellular transport and/or metabolism levels rather than due to changes at the level of intestinal absorption (van den Berg, 1999). Alteration in the slowly exchanging vitamin B<sub>6</sub> pools, i.e. increased hydrolysis of PLP to pyridoxal before pyridoxal oxidation, has been reported in older rats. Higher vitamin B<sub>6</sub> catabolism in the elderly may lead to increased vitamin needs and reduced PLP-protein binding capacities. Moreover, plasma PLP concentrations in elderly individuals seem to be related to both the decrease in lean body mass

(mainly muscle protein) that occurs during ageing and the level of protein intake.

Assessments of folate status in the elderly have provided variable data. Various groups of elderly individuals such as the US Framingham original cohort (Selhub *et al.* 1993), free-living elderly Canadian residents (Quinn & Basu, 1996), Hispanics in New Mexico (Lindeman *et al.* 2000) and apparently healthy Chilean subjects (Olivares *et al.* 2000) were found to be folate deficient or at risk of deficiency. However, this was observed in less than 10 % of German and Northern Italian elderly (Herrmann *et al.* 1999; Ravaglia *et al.* 2000) while no differences occurred for plasma folate, as well as vitamins B<sub>6</sub> and B<sub>12</sub>, concentrations between Singapore Chinese groups aged 45–74 years (Saw *et al.* 2001). Human studies have suggested that ageing *per se* might have little effect on the activity of intestinal  $\gamma$ -glutamylhydrolase (conjugase), the enzyme responsible for the hydrolysis of dietary folate polyglutamates to folate monoglutamates, or on the absorption of the latter (Bailey *et al.* 1984). However, because the optimum pH for those activities is 6.5–7.0 and 6.0–6.3, respectively, digestive pathologies in the elderly (especially those eliciting pH modifications in the stomach and the upper intestine) could alter folate bioavailability (Kesavan & Noronha, 1983). Hypochlorhydria, that affects 10 to 40 % of the elderly, can decrease folate absorption, but normality can be recovered following acid administration (Russell, 2000).

Moreover, it is not currently known whether enterohepatic cycling, which represents an important pathway for the redistribution of folate to peripheral tissues (more than 80 µg/d), may be altered by ageing.

Hypochlorhydria impairs the dissociation of vitamin B<sub>12</sub> from food complexes and, subsequently, its release into the lumen of the small intestine. Type A or B atrophic gastritis is associated with pernicious anaemia or *Helicobacter pylori* infection, respectively. Pernicious anaemia, as well as gastrectomy, causes vitamin B<sub>12</sub> malabsorption. Although they may also be a source of B vitamins (Camilo *et al.* 1996), overgrowing gastrointestinal bacteria could capture cobalamin freed from food complexes (van Asselt *et al.* 1998). All of these conditions may explain why vitamin B<sub>12</sub> status is altered in a substantial fraction of elderly individuals although current recommendations for this vitamin are met (Baik & Russell, 1999). NHANES III data reported an age-dependent prevalence of low serum vitamin B<sub>12</sub> concentration (i.e. 9 % between 50 and 59 years; 13 % for ≥70 years), in agreement with other studies (Herrmann *et al.* 1999; Ravaglia *et al.* 2000). In the SENeca study, based on plasma cobalamin and MMA levels, only 25.7 % of apparently healthy, free-living Dutch elderly (74–80 years) were not cobalamin deficient (van Asselt *et al.* 1998). Increased serum MMA (and tHcy) levels were also reported in 49 % of normal Swedish individuals aged ≥70 years (Björkegren & Svärdsudd, 2001). A recent population-based study found that about 10 and 20 % of individuals living in Oxford (UK) and aged 65–74 and ≥75 years, respectively, were at high risk of vitamin B<sub>12</sub> deficiency; about 10 % of these vitamin B<sub>12</sub>-deficient individuals also showed folate deficiency (Clarke *et al.* 2003). Observations indicate that the serum concentration of vitamin B<sub>12</sub> is maintained at the expense of tissue stores. Thus, while a serum concentration below the classical cut-off value defining vitamin B<sub>12</sub> deficiency (120–180 pmol/l) indicates long-term depletion, a level above this value does not necessarily mean adequate vitamin B<sub>12</sub> status. Altered binding to plasma transcobalamin II proteins or an altered cellular metabolism of cobalamin may also occur during ageing (van Asselt *et al.* 1998).

### B vitamin deficiency and degenerative pathologies

An insufficient status of folate, cobalamin, vitamin B<sub>6</sub> and, to a lesser extent, vitamins B<sub>2</sub> and B<sub>1</sub>, is currently recognised as a risk factor for chronic diseases (cardiovascular diseases (CVD), cognitive and neuropsychiatric dysfunction and certain cancers) and can also exacerbate existing pathologies in the elderly.

#### *Hyperhomocysteinaemia*

Hcy re-methylation to methionine, and catabolism via the trans-sulfuration pathway, require folate, cobalamin, vitamin B<sub>6</sub> and riboflavin, which have proved to be more or less effective determinants of plasma fasting Hcy concentrations (tHcy normal range about 10 µmol/l). Hyperhomocysteinaemia is usually defined as a tHcy >15 µmol/l, although differences exist between the reported reference ranges. Its prevalence is about 5 % in the normal

population, but it seems to increase with age (Nygard *et al.* 1995). Prevalence of mild hyperhomocysteinaemia was 29.3 % in individuals aged 67 to 96 years from the original Framingham Heart Study cohort (Selhub *et al.* 1993) and 61 % in elderly hospitalised patients (Ventura *et al.* 2001). However, 72 % of tHcy in the latter group could be explained by variables other than age. Data from the NHANES III reported homocysteinaemia higher in males than in females at younger ages, but converging at older ages in both genders (Jacques *et al.* 1999a). Low serum folate levels have a central role in the pathogenesis of hyperhomocysteinaemia. For instance, in the elderly participants from the Framingham Heart Study cohort, mean tHcy was significantly higher in the lowest two deciles than in the highest decile of plasma folate concentrations. Serum vitamin B<sub>6</sub> and/or B<sub>12</sub> levels are also inversely associated with Hcy levels, but the relationship appears to be weaker than for folate. However, no correlation was found between tHcy and vitamin B<sub>12</sub> status in some studies (Hermann *et al.* 1999). The intake of folate and vitamin B<sub>6</sub>, but not vitamin B<sub>12</sub>, is generally inversely associated with tHcy (Selhub *et al.* 1993).

The meta-analysis of randomised trials estimates that folate supplementation (0.5–5 mg/d) can reduce tHcy by 25 % and that the addition of vitamin B<sub>12</sub> (0.5 mg/d) can reduce it by a further 7 %, with no further change following the addition of vitamin B<sub>6</sub> (16.5 mg/d) (Homocysteine Lowering Trialists' Collaboration, 1998). Nevertheless, such predictions may not be valid for the elderly, where baseline tHcy values often exceed the 10–15 µmol/l range. For instance, in a randomised, double-blind, placebo-controlled trial in healthy individuals aged 63–80 years, not deficient in vitamin B<sub>12</sub>, and who were made replete with folate and riboflavin, hyperhomocysteinaemia could be counteracted by a moderate vitamin B<sub>6</sub> supplementation (1.6 mg/d for 12 weeks; McKinley *et al.* 2001). Regarding the 677C→T (Ala222Val) variant of MTHFR, higher plasma folate and lower tHcy can occur in wild-type CC subjects when compared with TT and, to a lesser extent, CT subjects (de Bree *et al.* 2003). TT homozygotes require more dietary folate to achieve a plasma folate concentration identical to that in CC homozygotes. In a cross-sectional study of healthy subjects aged 19–63 years, mild hyperhomocysteinaemia typically associated with TT homozygosity occurred only with poor riboflavin status (McNulty *et al.* 2002). Folate and riboflavin could interact to lower tHcy by maximising the catalytic activity of MTHFR. However, it was recently suggested that this effect may be unrelated to MTHFR genotype (Moat *et al.* 2003). Moreover, while riboflavin intake could influence tHcy among non-supplement users in a population-based cohort (Jacques *et al.* 2001), supplemental riboflavin was not an effective tHcy-lowering agent in healthy elderly individuals with sub-optimal vitamin B<sub>2</sub> status (McKinley *et al.* 2002). The impact of other gene polymorphisms on tHcy has been reviewed by Lievers *et al.* (2003).

#### *Cardiovascular diseases*

Numerous cross-sectional and case-control studies have provided evidence for the probable existence of an associa-

tion between mildly elevated tHcy and CVD. The prevalence of hyperhomocysteinaemia is 13–47 % among patients with atherosclerotic disease (Malinow *et al.* 1998). A meta-analysis involving 5230 individuals from seventeen studies relating tHcy to coronary artery disease risk and eleven studies of folate effects on tHcy suggested that a 5  $\mu\text{mol/l}$  increment in homocysteinaemia is associated with a 60 % increased risk in men (Boushey *et al.* 1995). More recently, it was predicted that a 3  $\mu\text{mol/l}$  decrease in homocysteinaemia following an increased intake of folate would reduce the risk of IHD, deep-vein thrombosis and stroke by 16, 25 and 24 %, respectively (Wald *et al.* 2002). Data from prospective cohort studies are less strong than those from case–control studies in support of the relationship between elevated tHcy and the risk of CVD, especially in adults healthy at the study baseline. For instance, in the longitudinal Physician's Health Study (40–84-year-old men followed for 7.5 years), the individuals with the lowest 20 % of plasma folate or vitamin B<sub>6</sub> concentrations had a relative risk of 1.4 (95 % CI 0.9, 2.3) or 1.5 (95 % CI 1.0, 2.2), respectively, compared with those in the top 80 % (Chasan-Taber *et al.* 1996). Although not statistically significant, the results suggest that a low dietary intake and low plasma concentrations of folate and/or vitamin B<sub>6</sub>, on the one hand, and high tHcy, on the other hand, contribute to the risk of myocardial infarction. Other reports have reached a similar conclusion regarding atherosclerosis (Robinson *et al.* 1998). Some studies suggested that vitamin B<sub>6</sub> may offer independent protection against cardiovascular mortality (Folsom *et al.* 1998), but others did not (Medrano *et al.* 2000). These two reports also yielded contradictory results concerning vitamin B<sub>12</sub>. The reason for such a discrepancy between studies is not known, as study populations, age of subjects or follow-up time do not appear to explain the different findings. Data from NHANES III indicate that the association between serum folate and the risk of CHD differed significantly by age groups (Giles *et al.* 1998). They notably reported an inverse relationship only in adults aged 35–55 years, suggesting that the greatest potential for prevention might be achieved by increasing folate levels in young adults. A meta-analysis of data from forty case–control studies has indicated that individuals with the MTHFR 677TT genotype have a 16 % (odds ratio 1.05–1.28) higher risk of CHD compared with CC individuals. However, the result was significant in European populations unlike North American populations (Klerk *et al.* 2002). In the European Concerted Action Project, the TT genotype was found to confer a modest increase in vascular disease risk (Meleady *et al.* 2003). In the latter two studies, the risk increase was mainly mediated by low folate status.

#### *Cognitive and neuropsychiatric dysfunction*

Low dietary intakes or mild deficits of B vitamins have been associated with subtle or important neuropsychological impairment in the elderly.

Studies have suggested that low free thiamin levels in cerebrospinal fluid could be related to the risk for Parkinson's disease (Jimenez-Jimenez *et al.* 1999) and have found a relationship between vitamin B<sub>1</sub> levels in plasma or autopsied cerebral cortex and cognitive impairment in

patients with dementia of the Alzheimer's disease (AD) type (Gold *et al.* 1995). However, data on the effect of long-term administration of thiamin on the progression of AD are conflicting and no association seems to exist between cerebrospinal fluid thiamin levels and the risk for, or the progression of, this disease.

More frequently, low folate, vitamin B<sub>6</sub> and/or B<sub>12</sub> status-dependent hyperhomocysteinaemia in adults may be a risk factor for poor cognition, depression, dementia, AD and Parkinson's disease (Gonzalez-Gross *et al.* 2001). A positive correlation between the levels and mental health (assessed by the Mini-Mental State Examination) has been observed among the 5-year-follow-up of elderly participants in the European, multicentre, SENECA study. However, no such association was detectable in the Finale study, probably because the population size was almost one half that in the follow-up study, and represented the healthiest segment of the cohort (Eussen *et al.* 2002). The Canadian Study of Health and Ageing reported low serum folate levels independently associated with a higher risk of adverse cerebrovascular events among older, cognitively fragile individuals (Maxwell *et al.* 2002). Moreover, elderly individuals deficient in vitamin B<sub>12</sub> can develop neuropsychiatric abnormalities, such as depression, that are not seen in folate deficiency, suggesting effects specific to cobalamin metabolic, environmental or genetic factors (Tiemeier *et al.* 2002). Cognitive recovery after supplementation with pharmacological doses of vitamin B<sub>12</sub> has been demonstrated in patients with cobalamin deficiency (van Asselt *et al.* 2001). However, it depends on the duration of symptoms and the magnitude of neurological complications.

Community-based studies have shown that elevated tHcy may be an independent risk factor for cerebral infarction (Shimizu *et al.* 2002), as well as decreased cognitive performance in normally ageing individuals (Teunissen *et al.* 2003). Whether this condition precedes the onset of dementia, or results from dementia-related nutritional and vitamin deficiencies, is currently unknown. The first assumption is supported by the observation of a strong relationship between newly diagnosed dementia and AD with high tHcy measured 8 years before baseline in cognitively intact adults (Seshadri *et al.* 2002). Nevertheless, there are conflicting results as regards the existence of such an association between baseline hyperhomocysteinaemia and cognitive decline, as measured by changes in the Mini-Mental State Examination score (Kalmijn *et al.* 1999; Miller *et al.* 2003; Ravaglia *et al.* 2003). In particular, this association was not observed in centenarians, perhaps due to biological characteristics that are different from those of younger elderly individuals. A relationship between stroke and AD seems to exist and it has been suggested that elevated tHcy in patients with AD is rather related to vascular disease than to AD pathology (Miller *et al.* 2002). In the present study, low vitamin B<sub>6</sub> status was prevalent in AD patients, suggesting that it might also influence the pathogenesis or progression of the disease.

#### *Cancer*

There have been many investigations on the relationship between inadequate intake and/or status of B vitamins and

cancers, especially those of the gastrointestinal tract. Regarding vitamins B<sub>1</sub> and B<sub>2</sub>, the data are often contradictory and far from conclusive. Studies have suggested that, under certain circumstances, such as high fat intake or smoking, methyl-donor (for example, folate) and/or PLP deficiency may influence cell differentiation in the pancreas and contribute to the pathogenesis of pancreatitis and carcinogenesis (Stolzenberg-Solomon *et al.* 1999). Low dietary folate and/or vitamin B<sub>6</sub> have frequently been related to an increased occurrence of colorectal adenoma. Among the various components of vegetables and fruits, only soluble fibre and vegetables high in folate were found to be independently related to a lower risk of colorectal adenoma (Platz *et al.* 1997). A lower level of erythrocyte folate was additionally observed in colonic adenoma cases as compared with controls, however, with possible gender specificity (Bird *et al.* 1995). Supplementation with 2 mg folic acid/d for 3 months caused decreased colonic mucosal cell proliferation in patients with recurrent adenomatous polyps (Khosraviani *et al.* 2002). Moreover, the risk of adenoma formation, growth or recurrence could increase when high alcohol and low folate intakes are combined.

An inverse association of folate intake or blood folate with colorectal cancer (CRC) risk has also been reported (Giovannucci, 2002). A pooled analysis of nine prospective cohort studies suggested that a daily intake of 400–500 µg folate should be sufficient for minimising the risk of CRC. No clear differences in CRC risk apparently exist regarding the type of folate vitamins ingested (Konings *et al.* 2002). The NHANES I epidemiological follow-up study reported a significant relationship between folate intake and reduced colon cancer risk only among men and non-drinkers (Su & Arab, 2001). It also supported a synergistic interaction between folate and methionine intakes. Methionine may be beneficial through DNA methylation or the sparing of folate required for DNA synthesis and repair. Low intracellular levels of methionine cause an increased S-adenosylhomocysteine:S-adenosylmethionine (SAM) ratio, thus stimulating MTHFR and allowing 5-methyltetrahydrofolate (5-mTHF) to provide Hcy with a methyl group to form methionine (Finkelstein, 1998). However, if the 5-mTHF level is insufficient, cell methylation reactions can be affected. Only a few studies have reported directly on methionine intake and the existence of an inverse association with CRC is not clearly demonstrated. Individuals with 'methyl-poor' diets (for example, high in alcohol and low in folate and methionine) are at markedly higher risk of colorectal adenoma or CRC compared with those with 'methyl-rich' diets (Fuchs *et al.* 2002).

The TT genotype for the C677T variant of MTHFR may confer a relatively lower risk of CRC or adenoma than the CC or CT genotypes, provided that it is concurrent with a 'methyl-rich' diet (Ma *et al.* 1997) or high vitamin B<sub>6</sub> and folate intake (Le Marchand *et al.* 2002). Under conditions of low folate intake, the TT variant has been linked to reduced levels of plasma folate, aberrant DNA methylation in leucocytes and an increased risk of CRC, especially in older populations, possibly due to age-related disturbances in folate metabolism (Shannon *et al.* 2002). The association of other polymorphisms in the MTHFR, methionine syn-

thase or cystathionine β-synthase gene with CRC has been suggested, but remains to be established.

Prospective case-cohort studies suggest an inverse relationship between dietary folate and lung cancer (Voorrips *et al.* 2000). However, no association was found between folate intake, or serum and erythrocyte levels and non-small-cell lung cancer (Jatoi *et al.* 2001). A significant relationship between lung cancer incidence and serum vitamin B<sub>6</sub>, B<sub>12</sub> or tHcy, but not folate, was reported by Hartman *et al.* (2001). The risk of breast cancer was the highest with low folate, and possibly vitamin B<sub>6</sub>, plasma levels, particularly among women with high alcohol consumption (Zhang *et al.* 2003). A large, multi-ethnic community-based, case-control US study of invasive cervical cancer, at least 6 months after completion of cancer treatment, reported a substantially and significantly higher risk for women in the upper three quartiles of homocysteinaemia, but no association was found with circulating folate (Ziegler *et al.* 2002). Whether elevated tHcy is a risk factor for carcinogenesis or a tumour marker in cancer patients remains to be demonstrated. The C677T variant in the MTHFR gene may increase the risk for several cancers, except for lung cancer, in elderly men, especially those with a low folate and a high alcohol intake (Heijmans *et al.* 2003).

### Mechanisms of the effects of age-associated B vitamin deficiency

#### *Alteration of methylation reactions*

Chronic nutritional deficiencies in B vitamins, choline (betaine) and/or methionine can perturb the complex regulatory network maintaining normal one-C metabolism and Hcy homeostasis (see Fig. 1). 5-mTHF synthesis occurs from one-C units provided by serine or from methyl groups of choline while *de novo* synthesis of choline via SAM uses methyl groups from methionine. Altering the metabolism of one of these pathways results in compensatory changes in the others. For instance, in addition to the previously mentioned interaction between methionine and 5-mTHF, folate deprivation may cause a higher use of methyl groups from choline and an increased dietary requirement for choline (Niculescu & Zeisel, 2002).

Folate deficiency can affect the configuration and structural stability of DNA through two potential pathways: the transfer of methyl groups from 5,10-methyleneTHF to uracil and the conversion of the latter to thymine for DNA synthesis and repair. Additionally, it may cause misincorporation of uracil into DNA, leading to double strand breaks and chromosomal damage. Folate can also affect gene promoter activity by regulating cellular levels of SAM, the substrate of DNA methyltransferases that contribute to the high conservation of the methylation patterns of genomic cytosine residues after cell replication (Duthie *et al.* 2002). Methylation of cytosine residues, often within the promoter regions of genes, is essential for the control of gene transcription and genetic stability. Increased serum and tissue levels of S-adenosylhomocysteine, concomitant with chronic hyperhomocysteinaemia, have been shown to inhibit DNA methyltransferases and thus to contribute to DNA hypomethylation (James *et al.* 2002). In contrast to

DNA hypermethylation, DNA hypomethylation may cause the overexpression of genes (for example, proto-oncogenes), and lead to cancer in mitotic cells while in post-mitotic cells (for example, neurones), it may promote cell death (Mattson *et al.* 2002). Genetic polymorphism can act synergistically with nutritional deficiencies to perturb methylation reactions. For instance, TT homozygotes for the C677T variant of MTHFR have been shown to display genomic hypomethylation in peripheral leucocytes (Stern *et al.* 2000). Additionally, imbalance between biological methylation and nucleotide synthesis at the MTHFR reaction level may be important in folate-related carcinogenesis (Choi & Mason, 2002).

Ageing, as well as most chronic diseases, is partly caused by damage to both nuclear and mitochondrial DNA. Ageing, marginal deficiencies in folate or vitamin B<sub>12</sub> and elevated tHcy are accompanied by spontaneous chromosome damage, as measured by micronucleus formation in blood and epithelial cells. Normalisation of DNA methylation by folate repletion seems to be delayed in older women with moderate folate depletion (Rampersaud *et al.* 2000). Double-blind placebo-controlled intervention studies conducted over 4 months indicated that chromosomal damage and DNA hypomethylation are minimised when erythrocyte folate concentration is higher than 700 nmol/l, while micronucleus formation is the lowest when the plasma concentration of vitamin B<sub>12</sub> is above 300 pmol/l and plasma tHcy is below 7.5 µmol/l (Fenech, 2002). Moreover, folate insufficiency and ageing in rats have been shown to be concurrent with non-enzymic modification of aspartyl and asparaginyl residues in proteins and peptides (Ghandour *et al.* 2002). This may be related to the decreased activity of protein-L-isoaspartyl methyltransferase, since this enzyme allows re-isomerisation, and restoration of the original α-peptide linkage by catalysing the transfer of the methyl group of SAM to altered L-isoaspartyl sites of proteins.

#### *Pro-oxidative effects*

B vitamins could provide protection against oxidative stress either by decreasing pro-oxidant Hcy in plasma and/or more directly by scavenging reactive oxygen species. Oxygen-dependent auto-oxidation of Hcy occurs when added to serum or in the presence of transition metals (Fe<sup>2+</sup>, Cu<sup>2+</sup>), leading to homocysteine formation and the generation of superoxide anions, O<sub>2</sub><sup>-</sup> and hydroxyl radicals, ·OH. Hcy bound to LDL may facilitate their oxidation through this mechanism (Olszewski & McCully, 1993). It also may promote atherogenesis through the effects of oxidised LDL on monocyte recruitment and macrophage differentiation, as well as on the formation of lipid-laden foam cells (Duell & Malinow, 1997). Additionally, Hcy auto-oxidation may contribute to endothelial dysfunction: O<sub>2</sub><sup>-</sup> production can interact with the endothelium-derived relaxing factor NO to form peroxynitrite, which in turn leads to the inactivation of the biological functions of NO (Zhang *et al.* 2000). High circulating tHcy lowered the endothelium-dependent vasomotor response in healthy older individuals (Hirsch *et al.* 2002b) and this effect could be reversed by intracellular O<sub>2</sub><sup>-</sup> scavengers (Lang *et al.* 2000). Also, the methionine-induced impairment of resistance vessel dilatation to

acetylcholine or bradykinin in human subjects was prevented by the oral administration of antioxidant vitamins (Raghuveer *et al.* 2001). However, there was also evidence for oxidative stress-independent endothelial dysfunction after oral methionine loading in human subjects (Nightingale *et al.* 2001).

As regards total antioxidant capacities or oxidative damage, different magnitudes of tHcy increase may account for the discrepant observations *in vivo*. Impaired endothelium-dependent relaxation of coronary resistance vessels, as well as increased carotid arterial permeability and initiation of arterial stiffening, in folate-depleted, hyperhomocysteinaemic rats was associated with increased oxidative (Cu–Zn + Mn superoxide dismutase (SOD) activity) and glycooxidative (pentosidine) stress (Symons *et al.* 2002). Elevated tHcy in patients seemed to evoke an adaptive increase in the activity of the antioxidant enzymes glutathione peroxidase and, to a lesser extent, SOD in the circulation (Moat *et al.* 2000). However, a 4-week folate-depletion period gave rise to decreased glutathione peroxidase and SOD activities and increased lipid peroxidation in rat liver (Huang *et al.* 2001).

Long-term (1-year) supplementation with pharmacological doses of folic acid improved arterial endothelial function in hyperhomocysteinaemic healthy volunteers (Woo *et al.* 2002). In patients suffering from CVD, this treatment decreased tHcy, fibrinogen, malondialdehyde and von Willebrandt factor levels, and increased plasminogen, anti-thrombin III, GSH, glutathione peroxidase and SOD levels concurrently. Nevertheless, there is currently no general consensus regarding the ability of Hcy-lowering treatments to increase plasma antioxidant activity or to improve endothelial function. In particular, it has been suggested that the impaired bioavailability of NO in hyperhomocysteinaemia is secondary to the accumulation of the endogenous NO synthase inhibitor asymmetric dimethylarginine (Sydow *et al.* 2003).

Moreover, the administration of 5-mTHF (5 mg/d for 6 weeks) to patients with coronary artery disease improved flow-mediated dilatation without affecting their homocysteinaemia, thus suggesting the involvement of a Hcy-independent mechanism (Doshi *et al.* 2002). A similar mechanism could be responsible for the restoration of forearm blood flow by a high concentration of 5-mTHF after the stimulation of endothelium-dependent vasodilatation in patients with familial hypercholesterolaemia (Verhaar *et al.* 1998). Based on the hypothesis of impaired NO availability, folate might increase the NO level by reducing the O<sub>2</sub><sup>-</sup> level in a dose-dependent manner. Folate was actually found to react with free radicals at an almost diffusion-controlled rate, thus forming a delocalised molecular radical, and to inhibit microsomal lipid peroxidation in a concentration-dependent way (Joshi *et al.* 2001). Studies have also suggested antioxidant properties for vitamins B<sub>2</sub> and B<sub>6</sub> in rats submitted to oxidant-mediated inflammatory lung injury (Seekamp *et al.* 1999) and exercise-induced oxidative stress (Benderitter *et al.* 1996), respectively.

Many factors associated with brain ageing (i.e. cerebral microangiopathy, endothelial dysfunction, impaired NO activity, oxidant damage to neurones, altered methylation

reactions) are related to hyperhomocysteinaemia and altered one-C metabolism due to folate deficiency. The ability of the latter condition to exacerbate dopamine depletion, neuronal degeneration and motor dysfunction induced by environmental toxins suggests a mechanism whereby dietary folate may influence the risk for Parkinson's disease (Duan *et al.* 2002). Additionally, a relationship between increased Hcy concentrations and (E)-4-hydroxy-2-nonenal, a neurotoxic product of lipid peroxidation in the ventricular fluid and brain, has been observed in patients with AD (Selley *et al.* 2002).

### Prevention of B vitamin deficiency during ageing

Cereals, vegetables, legumes and fruits on the one hand, and meats, fish and dairy products, on the other hand, contribute mainly to B vitamin intake in the populations of developed countries. Offal (mainly liver) contains potentially important sources of B vitamins, but it is a lower contributor to total intake because it is consumed in relatively small amounts. Fruit and vegetables constitute essential sources in a multitude of vitamins (apart from vitamin B<sub>12</sub>), and other biologically active micronutrients: natural antioxidants, carotenoids, flavonoids, minerals, etc. Aiming to prevent chronic diseases (Potter, 2002), experts of nutrition–health programmes of Western countries recommend the consumption of at least five foods of fruit or vegetable origin daily. However, the percentage of individuals following this recommendation may be limited, suggesting an important risk of micronutrient deficiency in the population. For instance, while the recommended dietary allowance is 400 µg dietary folate equivalents/d, NHANES III reported a median intake of folate from food amounting to about 250 µg/d in the US adult population from 1988 to 1994 (Institute of Medicine, National Academy of Sciences, 1998). The increased consumption of vegetables and citrus fruit increases plasma folate levels and diminishes tHcy in healthy volunteers (Brouwer *et al.* 1999; Broekmans *et al.* 2000).

To reduce the risk of neural-tube defects in the newborn by increasing folate intake in women of childbearing age, manufacturers of cereal-grain foods have been required to add folic acid in several countries; for example, at a concentration of 140 µg/100 g product in the USA. Additionally, a significant fraction of the general population (for example, about 40 % of North Americans) is already consuming nutritional supplements. These are mainly as vitamin and mineral supplements (Ervin *et al.* 1999) that usually provide around 400 µg folate, 3 mg vitamin B<sub>6</sub> and 9 µg vitamin B<sub>12</sub>/d, i.e. about 127, 183 and 375 % of the recommended dietary allowances, respectively (McKay *et al.* 2000). The total daily intake of folate increased by about 323 µg of food folate equivalents in the American population exposed to folate fortification since 1998, i.e. about twice more than supplemental intake initially predicted (Choumenkovitch *et al.* 2002). Thus, the prevalence of individuals with a folic acid intake above the upper tolerable intake level (1 mg folic acid/d) increased from 1.3 to 11.3 % in supplement consumers exposed to the fortification. Additionally, the supply of micronutrients by vitamin and mineral supplements may be highly variable and

thereby be harmful. For instance, the long-term increased intake of vitamin A may promote the development of osteoporotic hip fractures in post-menopausal women (Feskanich *et al.* 2002).

Since the start of folic acid fortification, a steady increase in the indicators of plasma folate status has been observed in various groups of the American, Chilean and Canadian populations, including the elderly, who did not use vitamin supplements (Jacques *et al.* 1999b; Hirsch *et al.* 2002a). Folate intake via a multivitamin supplement formulated at about 100 % daily value for most vitamins and minerals was found to be of further benefit among free-living older adults already consuming a diet fortified with folic acid in improving B-vitamin status and reducing tHcy (McKay *et al.* 2000). However, interest in increased folate intake on the risk of chronic diseases needs to be balanced against concerns on the general lack of data about safety of continuous high intakes (Meltzer *et al.* 2003). Although folic acid, as well as the other B vitamins except vitamin B<sub>6</sub> and niacin, is generally considered as safe in therapeutic use, there are currently insufficient data from human or animal investigations to define a safety upper limit for B vitamins (Expert Group on Vitamins and Minerals, 2003). Recently, it has been reported that long-term high folic acid supplementation may reduce N digestive function in aged rats (Achon *et al.* 2002). Additionally, it is worth considering that folate supplementation and fortification may potentiate the growth and progression of colon lesions, in the context of advanced colorectal tumorigenesis (Lamprecht & Lipkin, 2003) and, more generally, diminish the effectiveness of drugs frequently used by the elderly. Moreover, the Canadian folic acid food fortification has not been concurrent with a significant decline in the prevalence of vitamin B<sub>12</sub> insufficiency (Ray *et al.* 2002). It has been suggested that an increase in folate status could precipitate or worsen neurological dysfunction in vitamin B<sub>12</sub>-deficient individuals. Macrocytic anaemia, one of the first detectable clinical signs of vitamin B<sub>12</sub> deficiency, can resolve following folic acid supplementation, so that neurological problems are the first signs to develop (Quinlivan *et al.* 2002). Concurrent supplementation or fortification with vitamin B<sub>12</sub>, especially in the elderly, might reduce concerns about adverse side effects from the consumption of high levels of folic acid and be also effective at the lowering of tHcy (Flood *et al.* 2001). However, the exact dose at which this occurs remains currently unknown, in part due to the difficulty of assessing vitamin B<sub>12</sub> deficiency. Moreover, whereas oral administration of crystalline cyanocobalamin seems to be an effective treatment for food-cobalamin malabsorption, it remains unknown whether it is the same regarding vitamin B<sub>12</sub> from fortified food (Russell *et al.* 2001).

The question as to whether elderly individuals have specific needs for certain B vitamins cannot be answered easily. Data on women aged 60–85 years (Kauwell *et al.* 2000) suggest the need to recommend a higher daily intake of folate for that group of the population, as compared with younger women usually taken as references for defining recommended dietary allowances. However, owing to difficulties in performing repeated depletion–repletion studies



in older adults, data on specific recommendations are scarce for this age group. Moreover, recommended intakes of several B vitamins are usually expressed relative to energy intake, which is lower in older than in younger individuals. Despite some quantitative differences, the recommendations established in different countries generally agree on the specific requirements of B vitamins for the elderly population. Based on the use of biochemical indicator cut-off values, the daily requirements of B vitamins for elderly individuals have been judged either similar to (vitamins B<sub>1</sub>, B<sub>2</sub>, folate, vitamin B<sub>12</sub>) or higher than (vitamin B<sub>6</sub>) those for young adults (for instance, see Institute of Medicine, National Academy of Sciences, 1998; AFSSA & CNERNA-CNRS, 2001). However, the use of supplements of vitamin B<sub>12</sub> or vitamin B<sub>12</sub>-fortified food products has been advised for individuals aged over 50 years by the US Institute of Medicine, National Academy of Sciences (1998).

Indices of wellbeing were improved in elderly women with marginal thiamin deficiency following supplementation with high doses of thiamin (Smidt *et al.* 1991). Vitamin B<sub>6</sub>, cobalamin and/or folic acid treatment, as well as the intake of multivitamin mix, increased serum vitamin levels while lowering serum MMA and tHcy levels in the elderly (Tucker *et al.* 2000; Björkegren & Svärdsudd, 2001; McKinley *et al.* 2001). In healthy elderly subjects with erythrocyte glutathione reductase activity coefficient and plasma PLP values indicative of insufficient vitamin B<sub>2</sub> and B<sub>6</sub> status, respectively, supplementation with physiological doses of riboflavin also improved both biochemical markers (Madigan *et al.* 1998). These results confirmed the biochemical interdependency of these two vitamins, but also suggested that only riboflavin was limiting. However, in a similar study, riboflavin, unlike folic acid, did not appear to be an effective tHcy-lowering agent despite the metabolic dependency of tHcy on vitamin B<sub>2</sub> (McKinley *et al.* 2002). In individuals with baseline concentrations of 12 µM and 12 nM for Hcy and folate, respectively, there appears to be a tHcy-lowering effect plateauing with supplemental doses of folic acid of 400–500 µg/d (Bailey *et al.* 2003). However, nutrient-dense foods containing physiological amounts of various micronutrients decreased tHcy and MMA concentrations in free-living Dutch frail elderly individuals during a 17-week randomised controlled intervention trial (de Jong *et al.* 2001). Currently, the amount of folate required to enhance body stores to levels that would be protective against chronic diseases is unclear and, consequently, it remains unknown whether natural foods can or cannot deliver such an amount. The ongoing large-scale randomised controlled intervention trials (for example, NORVIT, VITATOPS, SEARCH, VITAL, SU.FOL.OM3) that are underway in several countries should shed light on the effect of folic acid and other B vitamin supplementation on chronic disease occurrence. However, these secondary intervention trials in patients will bring limited information, if any, on the actual protective effects of B vitamins against chronic diseases in healthy adults. So far, a change in the food practices of individuals toward a balanced diet, rich in varied fruit and vegetables, remains much preferable to the intake of enriched foodstuffs or to the intake of additional multivitamin mix (Bernstein *et al.* 2002).

## Conclusions

There is currently good epidemiological evidence that the elderly population is at risk of insufficient status of several B vitamins. This has been associated with an increased risk of degenerative diseases including vascular diseases, cognitive and neurological dysfunction and cancers. However, there are differences between the data currently available due to many factors. These include the diversity of follow-up duration, the population sample size and ethnic origin. Other factors are the health and B vitamin status of the enrolled subjects and the parameters used to evaluate their dysfunctioning, the possible adjustment for covariates, the availability of baseline data, accuracy of metabolic markers and cut-off values used for determining B vitamin status, and the precision of estimates of micronutrient intake through dietary questionnaires and current food composition tables. Further longitudinal prospective studies before there are signs of health alteration are particularly needed to demonstrate that subtle B vitamin deficiency or concurrent alteration of biochemical indices (for example, homocysteinaemia) is causal to the aetiology of each of these pathologies.

Moreover, there is still progress to be made as regards *in vivo* assessments of the bioavailability of naturally occurring B vitamins in human subjects, with consideration for current food processing and cooking conditions (Wright *et al.* 2003). Also, possible alterations in the mechanisms of folate bioavailability in elderly individuals, on the one hand, and consequences of folate deficiency, on the other, deserve investigation at the molecular level using animal and cell-culture models. The metabolic fate and cellular effects of high doses of folic acid will shed light on the assumed risk of folic acid fortification and/or supplementation with vitamin and mineral mixtures. Regarding vitamin B<sub>6</sub>, studies are needed to determine the mechanisms underlying the apparently increased requirement of elderly individuals for this vitamin. Finally, future research projects should consider B vitamins, other than folate, vitamins B<sub>6</sub>, B<sub>12</sub> and B<sub>2</sub>, which have yet been little studied because of the current poor understanding of their public health significance.

Overall, data from future studies should allow us to better define whether proper nutrition, i.e. as micronutrient-dense food, starting at a younger age, has a protective effect against degenerative diseases and whether elderly individuals deserve particular attention regarding their B vitamin status.

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