Water-soluble Vitamins: Research Update

Eugene J. Bruno, Jr, MHS and Tim N. Ziegenfuss, PhD*

Address
*Huntington College of Health Sciences, 1101 S. Milliken, STE A, Ontario, CA 91761, USA.
E-mail: gbruno@hchs.edu

Current Sports Medicine Reports 2005, **4**:207–213
Current Science Inc. ISSN 1537-890x

For more than 50 years, the Food and Nutrition Board of the National Academy of Sciences has been reviewing nutrition research and defining nutrient requirements for healthy people, referred to as the recommended dietary allowances (RDA). As new nutrition research is published, the importance of vitamins as vital nutrients is underscored, and new physiologic roles and applications to human health are examined and considered with regard to updating the RDA. Each year a substantial amount of research is published on vitamins. This article examines and summarizes noteworthy research published on individual water-soluble vitamins (excluding vitamin C) in the past 12 months, provides relevant background information on these vitamins, and offers critical reviews as appropriate.

Introduction

Since the pioneering research on vitamin A by E.V. McCollum and coworkers nearly a century ago, the significant role that vitamins play in human health has been recognized. This role is evidenced in the word *vitamin*, which is derived from *vita* (life) as well as *amine* (containing nitrogen; the first vitamins discovered contained nitrogen). Today, current understanding of vitamin literature encompasses a broad range of coenzymatic roles in metabolism and biochemical functioning. Of recent significance is their role in the prevention of degenerative diseases, beyond classic deficiency disease states. This role has been highlighted by recommendations from many in the nutrition and medical community that all adults should use a multivitamin supplement on a daily basis. In an effort to stay abreast of this rapidly developing field of study, this article reviews noteworthy research published on water-soluble vitamins (excluding vitamin C) over the past year.

Folate

Whitney *et al.* [1] describe folate as a water-soluble B vitamin, whose chief function in the body is DNA synthesis and therefore new cell formation. Folate performs this role as part of the coenzymes tetrahydrofolate and dihydrofolate. Deficiency symptoms include large-cell type anemia, a smooth, red tongue, mental confusion, weakness, fatigue, irritability, and headache, and elevated homocysteine levels. Bailey *et al.* [2] identify folate as the form of this nutrient naturally occurring in foods, whereas folic acid is the form found in supplements and fortified foods. The 1998 recommended dietary allowance (RDA) for adults is 400 µg daily, and significant food sources include fortified grains, leafy green vegetables, legumes, seeds, and liver [1]. Table 1 provides a synopsis of the coenzyme forms and chief functions of folate, as well as each of the nutrients in this review.

Neural Tube Defects

Folate is well known for its role in reducing the risk of neural tube defects (NTD). NTD may result in anencephaly or spina bifida, which are devastating and sometimes fatal birth defects. Prior to the fortification of foods with folic acid, the risk of NTD in the United States was estimated to be one per 1000 pregnancies [3]. When women consumed folic acid supplements in addition to a varied diet during the periconceptional period, a 60% to 100% decrease in NTD cases were demonstrated in randomized trials [4]. As a result of these and other studies, the US Public Health Service recommended that all women capable of becoming pregnant consume 400 µg of folic acid daily to prevent NTD.

Folate recommendations not followed

In an international retrospective cohort study of neural tube defects, Botto *et al.* [5•] presented data from over 13 million births in Europe and Israel from 1988 to 1998, identifying cases of NTD and policies and recommendations regarding folic acid. Research results demonstrated no detectable improvement in the trends of incidence of NTD from issuing recommendations on folic acid supplementation. Researchers concluded that a reasonable strategy would be to integrate food fortification of folic acid with a more significant implementation of recommending supplements. This is important because the neural tube is fully developed 22 to 28 days after conception, but many women are not aware they are pregnant until after this time. Starting folic acid supplements after this period is therefore assumed to be too late to protect against birth defects.

Table 1. Water-soluble vitamins, coenzyme forms, and chief functions

Vitamin	Coenzyme forms	Chief functions
Folate (folic acid)	Tetrahydrofolate, dihydrofolate	DNA synthesis and therefore new cell formation
Vitamin B1 (thiamin)	Thiamin pyrophosphate	Assist in energy metabolism
Vitamin B2 (riboflavin)	Riboflavin-5'-phosphate, flavin adenine dinucleotide	Involved in the release of energy from nutrients in all body cells
Vitamin B3 (niacin/nicotinic acid, niacinamid/nicotinamide)	Nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate	Key role in energy metabolism
Vitamin B6 (pyridoxine, pyridoxal, pyridoxamine)	Pyridoxal 5'-phosphate, pyridoxamine 5'-phosphate	Amino acid and fatty acid metabolism, converts tryptophan to niacin and to serotonin, helps make erythrocytes
Vitamin B12 (cobalamin)	Methylcobalamin, 5-deoxyadenosyl cobalamin	Role in new cell synthesis, helps maintain nerve cells, reforms the folate coenzyme, helps break down some fatty acids and amino acids

Folate consumption during pregnancy and breast cancer risk

Although most published research regarding folate consumption during pregnancy is associated with positive outcomes, a report by Charles *et al.* [6] presented data that folate supplementation during pregnancy increased the risk of maternal breast cancer. These researchers followed-up on a large trial of folate supplementation in pregnancy from the 1960s [7], examining the association between folate status and death, as well as analyzing the effects of folate supplementation.

Oakley and Mandel [8] criticized the study by Charles *et al.* [6], pointing out that the results were nonstatistically significant, and that the randomized controlled trial sought to evaluate the effect of antenatal folate consumption and pregnancy outcomes, not breast cancer. They believe that the most likely explanation for the reported association is chance. Oakley and Mandel [8] also described a population based study by Shrubsole *et al.* [9], which contrasts the findings of the study by Charles *et al.* [6]. In the study by Shrubsole *et al.* [9], of 1321 cases and 1382 controls, dietary folate was inversely associated with breast cancer (OR 0.71, 95% CI 0.56–0.92).

Homocysteine-related Disorders

Elevated plasma homocysteine (Hcy) levels are implicated in the development of atherosclerotic and venous thromboembolic disease [10], with NTD and early pregnancy loss [11], and possibly chronic renal failure [12] and pre-eclampsia [13]. Furthermore, a substantial body of research has demonstrated the efficacy of folate in lowering Hcy, and in reducing the risk for atherosclerotic disease [14–16].

Folate, carotenoids, and coronary artery disease in Central and Eastern Europe

A study by Connor *et al.* [17] lends further credibility to the relationship between coronary artery disease (CAD), Hcy, and folate. Researchers surveyed coronary mortality and diet in Central and Eastern European countries, concluding that a diet low in foods containing folate and carotenoids (beta-carotene and lutein/zeaxanthin) may be a major contributing factor to increased coronary risk observed in the countries of Central and Eastern Europe.

Folate and coronary stenting

Conversely, the results of a recent study by Lange *et al.* [18] on folate therapy after coronary stenting were particularly unusual. In this study 636 patients who had undergone successful coronary stenting were randomly assigned to receive folic acid, vitamin B6 and vitamin B12, or a placebo. Compared with the placebo group, those receiving the B vitamins had significantly smaller minimal luminal diameter ($P = 0.008$), significantly greater late luminal loss ($P = 0.004$), a significantly higher rate of restenosis ($P = 0.05$), and a significantly higher rate of repeated target-vessel revascularization ($P = 0.05$).

Based on the findings of Lange *et al.* [18], Herrmann [19] noted that vitamin B therapy may be harmful in certain clinical conditions. However, it should be noted that Lange *et al.* [18] clearly indicated that their data did not provide any evidence that folate therapy for the primary or secondary prevention of CAD is potentially harmful; nor that their data be interpreted to mean that multivitamin supplementation should be discontinued in patients after coronary stenting.

Homocysteine, folate, pre-eclampsia, and black women

Recent research by Patrick *et al.* [20] provided new data about a relationship between Hcy and pre-eclampsia in black women. Compared with black women with normal pregnancy, white women with pre-eclampsia, and white women with normal pregnancy, researchers found that black women with pre-eclampsia had elevated Hcy concentrations. In addition, folic acid concentrations were lower in black women compared with white women, but plasma

Hcy was inversely related to folic acid only among black women with pre-eclampsia ($P = 0.01$).

Homocysteine, folate, and dementia

Previous research has examined the role of Hcy in vascular disease, and epidemiologic evidence [21] has raised the possibility that vascular risk factors might play a role in the pathogenesis of vascular dementia (VaD) and Alzheimer disease (AD). In new research by Quadri *et al.* [22•], subjects in the lowest folate tertile were found to have significantly higher adjusted odds ratios for mild cognitive impairment and dementia, whereas hyperhomocysteinemia was significantly associated with dementia and AD. Quadri *et al.* [22•] concluded that relative folate deficiency may precede AD and VaD onset, and that hyperhomocysteinemia might also be an early risk factor for cognitive decline in the elderly.

Homocysteine and depression

Low blood folate and vitamin B12 concentrations have previously been found in patients with major depression [23,24]. In recent research by Tolmunen *et al.* [25], serum Hcy concentrations were found to be elevated in 36.7% of the depressed men and in 26.4% of the nondepressed men. Those in the upper tertile for serum Hcy had a more than twofold higher risk of being depressed than did those in the lowest tertile for serum Hcy. The same researchers also found a cross-sectional association between low folate intake and depression.

Folate and colorectal cancer

According to Martínez *et al.* [26], epidemiologic studies indicate that higher intakes or blood concentrations of folate are associated with a lower risk of colorectal neoplasia. Recent research conducted by Martínez *et al.* [26] found a lower recurrence of colorectal adenomas in subjects with higher intakes and plasma concentrations of folate. Lower Hcy and higher vitamin B6 intake were additional markers involved in folate metabolism that were also associated with lower odds of recurrence.

Homocysteine and fracture risk

Two recent studies have demonstrated that increased Hcy levels significantly raise the risk of both hip fracture and other broken bones resulting from osteoporosis. van Meurs *et al.* [27] found that 2406 subjects aged 55 or older with the highest Hcy levels almost doubled their risk of fracture. Given the ability of folic acid therapy to reduce Hcy levels, the researchers suggested that additional studies are needed to assess whether the use of folic acid therapy will reduce the risk of fracture.

Likewise, McLean *et al.* [28] found that the risk of hip fracture nearly quadrupled in men in the top quartile of Hcy levels and nearly doubled in the top 25% of women. The researchers concluded that Hcy concentration is an important risk factor for hip fracture in older persons, and may be modifiable by dietary intake of folic acid and vitamins B6 and B12; although more research in needed. Table 2 provides a summary of the research presented herein on folate, as well as each of the nutrients in this review.

Vitamin B1

Vitamin B1, or thiamin, is described by Whitney *et al.* [29] as part of the coenzyme thiamin pyrophosphate, whose chief function it is to assist in energy metabolism. Beriberi is the classic vitamin B1 deficiency disease, and deficiency symptoms include enlarged heart, cardiac failure, muscular weakness, apathy, poor short-term memory, confusion, irritability, and anorexia and weight loss. The 1998 RDA is 1.2 mg for men, and 1.1 mg for women [29]. Significant food sources include whole grain, fortified or enriched grain products, moderate amounts in all nutritious food, and pork [29].

Thiamin, diabetes, and heart disease

As noted by Babaei-Jadidi *et al.* [30], diabetes increases the risk of heart disease two- to threefold in men and three- to fivefold in women. The increased risk is linked to high serum levels of cholesterol and triglycerides. In research conducted on diabetic rats, Babaei-Jadidi *et al.* [30] found that high-dose thiamin therapy (70 mg/kg) prevented diabetes-induced increases in plasma cholesterol and triglycerides but did not reverse the diabetes-induced decrease of HDL. Thiamin also normalized food intake of diabetic rats. The researchers suggested that thiamin supplementation should be considered as a part of nutritional therapy to counter diabetic lipid disorders and vascular complications.

Vitamin B2

The physiologic activity of vitamin B2, or riboflavin, is due to is coenzyme forms, riboflavin-5'-phosphate (flavon mononucleotide) and flavin adenine dinucleotide [31]. This activity is chiefly involved in the release of energy from nutrients in all body cells [32]. Ariboflavinoisis is the riboflavin deficiency disease, and deficiency symptoms include inflamed eyelids, sensitivity to light and reddening of the cornea, sore throat, cracks and redness at the corners of the mouth, painful, smooth, purplish red tongue, and skin inflammation characterized by lesions covered with greasy scales [32]. The 1998 RDA is 1.3 mg for men, and 1.1 mg for women [33]. Significant food sources include dairy products, whole grain or enriched grain products, and liver [33].

Riboflavin and migraine prophylaxis

In 1994 and 1998, an open-label pilot study [34] and a subsequent randomized controlled trial [35], respectively, demonstrated the efficacy of high-dose riboflavin treatment (400 mg/d) in migraine prophylaxis. In 2004, Boehnke *et al.* [36] demonstrated similar results when migraine sufferers using 400 mg/d of riboflavin for 3 months experienced signifi-

Table 2. Water-soluble vitamin research summaries

Study	Vitamin	Research summary
Botto *et al.* [5•]	Folate	Folate recommendations to prevent neural tube defects not being followed in parts of Europe
Charles *et al.* [6]		Folate supplementation during pregnancy increased the risk of maternal breast cancer
Conner *et al.* [17]		Diet low in foods containing folate and carotenoids may be a major contributing factor to increased coronary risk Central and Eastern Europe
Lange *et al.* [18]		Patients on folate therapy after coronary stenting had smaller minimal luminal diameter, greater luminal loss, higher restenosis, and a higher rate of repeated revascularization
Patrick *et al.* [20]		Black women with pre-eclampsia had elevated Hcy concentrations and lower folic acid concentrations
Quadri *et al.* [22•]		Subjects in the lowest folate tertile had significantly higher adjusted odds ratios for mild cognitive impairment and dementia; hyperhomocysteinemia was significantly associated with dementia and Alzheimer disease
Tolmunen *et al.* [25]		Subjects in the upper tertile for serum total Hcy had more than twofold higher risk of being depressed than those in the lower tertile; researchers also found cross-sectional association between low folate intake and depression
Martínez *et al.* [26]		Lower recurrence of colorectal adenomas was shown in subjects with higher intakes and plasma concentrations of folate
van Meurs *et al.* [27], McLean *et al.* [28]		Increased Hcy levels significantly raise the risk of both hip fracture and other broken bones resulting from osteoporosis
Babaei-Jadidi *et al.* [30]	Vitamin B1	High-dose thiamin therapy in rats prevented diabetes-induced increases in plasma cholesterol and triglycerides but did not reverse the diabetes-induced decrease of HDL
Boehnke *et al.* [36]	Vitamin B2	Migraine sufferers using 400 mg/d of riboflavin for 3 months experienced significantly fewer migraines
Liu *et al.* [37••]		Malnutrition (riboflavin deficiency, protein malnutrition, and iron deficiency) in the first few years of life leads to antisocial and aggressive behavior throughout childhood and late adolescence
Groenen *et al.* [41]	Vitamin B3	Risk of spina bifida associated with inadequate maternal intake of iron, magnesium, and niacin
Morris *et al.* [42]		People consuming more than 22.4 mg/d of niacin in the lowest intake group
Friso *et al.* [46•]	Vitamin B6	Low plasma PLP concentrations were found to be inversely related to major markers of inflammation and independently associated with increased CAD risk
Groenen *et al.* [51]	Vitamin B12	Marginal maternal vitamin B12 status increases the risk of an offspring with spina bifida; at the lowest vitamin B12 concentration, the risk of spina bifida was increased 3.5-fold
Tucker *et al.* [52••]		Men and women with vitamin B12 levels lower than 148 pM had significantly lower average BMD
Stone *et al.* [53]		Lower levels of serum vitamin B12 were associated with more rapid hip bone loss in elderly women

BMD—bone mineral density; CAD—coronary artery disease; Hcy—homocysteine; HDL—high-density lipoprotein cholesterol; PLP—pyridoxal 5'-phosphate.

cantly fewer migraines, averaging two per month during treatment with riboflavin in contrast to four per month prior to the study ($P < 0.05$).

Early nutrient deficiency and adolescent violence

According to research by Liu *et al.* [37••], malnutrition in the first few years of life leads to antisocial and aggressive behavior throughout childhood and late adolescence. The signs of malnutrition were angular stomatitis (cracking in the lips and corners of the mouth, predominantly a sign of riboflavin deficiency, but can also reflect niacin deficiency) hair dyspigmentation (protein malnutrition), sparse, thin hair (a sign of protein-energy malnutrition), and anemia (iron deficiency). There was a dose-response relationship between degree of malnutrition and degree of externalizing behavior at ages 8 and 17 years. Low IQ mediated the link between malnutrition and externalizing behavior at ages 8 and 11 years.

Vitamin B3

The term vitamin B3 refers to niacin (nicotinic acid) and niacinamide (nicotinamide). The body converts niacin into

niacinamide, the major form of vitamin B3 in the blood. The two coenzymatic forms of vitamin B3 are nicotinamide adenine dinucleotide (NAD) and NAD phosphate (NADP) [38]. The chief functions of these coenzymes are their key role in energy metabolism [39]. The classic vitamin B3 deficiency disease is pellagra, and deficiency symptoms include blurred vision, diarrhea, abdominal pain and vomiting, an inflamed, swollen, smooth, bright red tongue, depression, apathy, fatigue, loss of memory and headache, and a bilateral symmetrical skin rash on areas exposed to sunlight [40]. In sensitive individuals, supplemental intake of niacin specifically (but not niacinamide) may cause a "niacin flush" of the skin characterized by a burning, tingling, and itching sensation. These effects are reversed if niacin the niacin dosed is reduced or discontinued [38]. The 1998 RDA is 16 mg for men, and 14 mg for women. Significant food sources include milk, eggs, meat, poultry, fish, whole grain and enriched grain products, nuts and all protein-containing foods [38].

Spina bifida and maternal intake of iron, magnesium, and niacin

As previously discussed, the risk of neural tube defects due to inadequate maternal intake of folate is well established. Research by Groenen *et al.* [41] now suggests that the risk of spina bifida may also be higher with inadequate maternal intake of iron, magnesium and niacin. Compared with control mothers, case mothers had significantly lower intakes of iron (6%), magnesium (6%), and niacin (4%). Risk of spina bifida was 2.5 times higher for mothers in the lowest quartile of niacin intake.

Niacin and Alzheimer disease

The three "Ds" of the niacin-deficiency disease pellagra are well known: dementia, diarrhea, and dermatitis. Morris *et al.* [42] investigated whether variation in intake of niacin in the usual diet is also linked to neurodegenerative decline. The results demonstrated that people consuming more than 22.4 mg of niacin daily were 80% less likely to suffer Alzheimer disease than those in the lowest intake group.

Vitamin B6

The three forms of vitamin B6 are pyridoxine, pyridoxal, and pyridoxamine, all of which can be converted to the coenzyme pyridoxal 5'-phosphate (PLP) [43]. PLP and pyridoxamine 5'-phosphate (PMP) are used in amino acid and fatty acid metabolism. They also help convert tryptophan to niacin and serotonin, as well as helping to make erythrocytes [44]. Deficiency symptoms include small cell-type anemia, depression, confusion, abnormal brain wave pattern, convulsions, and scaly dermatitis [44,45]. At daily doses in excess of 1000 mg for extended periods, painful neurologic symptoms (sensory neuropathy) may develop [45]. The 1998 RDA for adults is 1.3 mg. Significant food sources include meats, fish, poultry, potatoes, legumes, noncitrus fruits, fortified cereals, liver, and soy products [44].

Vitamin B6 and coronary artery disease

From a cardiovascular disease risk perspective, vitamin B6 is primarily known for its role in reducing Hcy. According to Friso *et al.* [46•], low concentrations of PLP are also associated with high C-reactive protein (CRP) concentrations; and low PLP and elevated high-sensitivity CRP and fibrinogen, are related to higher risk of CAD. Friso *et al.* [46•] evaluated the relationship between PLP and acute-phase reactants in affecting CAD risk and the risk of CAD relative to low plasma PLP. The results indicated that low plasma PLP concentrations were inversely related to major markers of inflammation and independently associated with increased CAD risk.

Vitamin B12

Vitamin B12, also known as cobalamin, is part of the coenzymes methylcobalamin and 5-deoxyadenosyl cobalamin [47]. The chief functions of these coenzymes include their role in new cell synthesis, helping to maintain nerve cells, reforming the folate coenzyme, and helping to break down some fatty acids and amino acids [48]. The relationship between vitamin B12 and folate is an important one. Vitamin B12 removes a methyl group to activate the folate coenzyme, and when folate gives up its methyl group the vitamin B12 coenzyme becomes activated. The stomach secretes a binding protein called intrinsic factor that attaches to vitamin B12 and facilitates its absorption in the small intestine and into the bloodstream [48,49]. The classic vitamin B12 deficiency disease is pernicious anemia, which is caused by a lack of intrinsic factor, rather than by inadequate dietary intake. Deficiency symptoms include large cell-type anemia, a smooth sore tongue, fatigue, degeneration of peripheral nerves progressing to paralysis, and skin hypersensitivity. The 1998 RDA for adults is 2.4 µg. Significant food sources include animal products, such as meats, fish, poultry, shellfish, milk, cheese and eggs, as well as fortified cereals [47].

Vitamin B12 and spina bifida

An inadequate intake of vitamin B12 may also be a risk factor in NTD [50]. In a recent report by Groenen *et al.* [51], blood samples were taken from 45 mothers and their children with spina bifida, and from 83 mothers and their healthy children to assess vitamin B12 levels. In the case mothers, vitamin B12 levels were 21% lower than control mothers. Groenen *et al.* [51] concluded that marginal maternal vitamin B12 status increases the risk of an offspring with spina bifida. At the lowest vitamin B12 concentration ($\leq$ 185 pmol/L), the risk of spina bifida was increased 3.5-fold.

Vitamin B12 and osteoporosis

The role of calcium and vitamin D in the prevention of osteoporosis is well established. Two recent studies suggest that vitamin B12 may also play a role in reducing the risk of osteoporosis.

Vitamin B12 and bone mineral density in men and women

Tucker *et al.* [52••] examined the association between vitamin B12 and bone mineral density (BMD) in 2576 adults. Men and women with vitamin B12 levels lower than 148 pM had significantly lower average BMD ($P < 0.05$). Researchers concluded that "because low vitamin B12 status is prevalent and easily preventable, more attention should be given to this vitamin in the treatment and prevention of osteoporosis."

Vitamin B12 and bone mineral density in elderly women

Research conducted by Stone *et al.* [53] examined whether low serum vitamin B12 levels were associated with more rapid bone loss in elderly women. Researchers archived baseline serum samples and measured hip bone mineral density in subjects during 2- and 6-year follow-up examinations. After adjusting for age, weight, and clinic site, women with the lowest levels of B12 (< 280 pg/mL) experienced significantly more rapid hip bone loss than women with higher levels of B12 (> 280 pg/mL). They concluded that lower levels of serum vitamin B12 were associated with more rapid hip bone loss in elderly women, and that long term use of multivitamins may be an effective means of reducing B12 deficiency.

Conclusions

Water-soluble vitamins are indispensable for the development, normal growth, and functioning of the human body. Epidemiologic evidence generally supports the relationship between a diet rich in water-soluble vitamins and enhanced overall health. Given their high nutrient content, and the possibility that they contain yet-to-be-identified micronutrients, the most prudent recommendation at this point regarding water-soluble vitamins is to consume copious quantities of fruits and vegetables. However, given the unfortunate, yet inherent compliance problems with this approach, careful supplementation with B complex vitamins appears warranted for most adults. In this regard, daily supplementation with these vitamins at the level of the RDA is suggested. Supplementation above the RDA should be done under the auspices of a knowledgeable physician, dietitian, or other qualified healthcare provider.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Whitney EN, Cataldo CB, Rolfes SR: *Understanding Normal and Clinical Nutrition*. Belmont, CA: Wadsworth/Thompson Learning; 2002:322–328.
2. Bailey LB, Moyers S, Gregory III JF: **Folate.** In *Present Knowledge in Nutrition*, edn 8. Edited by Bowman BA, Russell RM. Washington, DC: ISLI Press; 2001:214–229.
3. Food and Nutrition Board, Institute of Medicine: *Folic Acid. Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, Vitamin B-6, Vitamin B-12, Pantothenic Acid, Biotin, and Choline.* Washington, DC: National Academies Press; 1998:193–305.
4. Higdon J: *An Evidence-Based Approach to Vitamins and Minerals.* New York: Thieme; 2003:6–14.
5. • Botto LD, Lisi A, Robert-Gnansia E, *et al.*: **International retrospective cohort study of neural tube defects in relation to folic acid recommendations: are the recommendations working?** *BMJ* 2005, **330**:571.

Retrospective cohort study with data from over 13 million births in Europe and Israel from 1988 to 1998, identifying cases of neural tube defects and policies and recommendations regarding folic acid; as well as data showing no detectable improvement in incidence of NTD resulting from issuing recommendations on folic acid supplementation.

6. Charles D, Ness AR, Campbell D, *et al.*: **Taking folate in pregnancy and risk of maternal breast cancer.** *BMJ* 2004, **329**:1375–1376.
7. Hall MH: **Folic acid deficiency and congenital malformation.** *J Obstet Gynaecol Br Commonw* 1972, **79**:159–61.
8. Oakley GP, Mandel JS: **Commentary: folic acid fortification remains an urgent health priority.** *BMJ* 2004, **329**:1376.
9. Shrubsole MJ, Jin F, Dai Q, *et al.*: **Dietary folate intake and breast cancer risk: results from the Shanghai Breast Cancer Study.** *Cancer Res* 2001, **61**:7136–7141.
10. Ray JG: **Meta-analysis of hyperhomocysteinemia as a risk factor for venous thromboembolic disease.** *Arch Intern Med* 1998, **158**:2101–2106.
11. Nelen WL: **Hyperhomocysteinaemia and human reproduction.** *CCLM* 2001, **39**:758–763.
12. Hoffer LJ: **Methods for measuring sulfur amino acid metabolism.** *Curr Opin Clin Nutr Metab Care* 2002, **5**:511–517.
13. Dekker GA, de Vries JI, Doelitzsch PM, *et al.*: **Underlying disorders associated with severe early-onset preeclampsia.** *Am J Obstet Gynecol* 1995, **173**:1042–1048.
14. **Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. Homocysteine Lowering Trialists' Collaboration.** *BMJ* 1998, **316**:894–898.
15. Brattstrom L: **Vitamins as homocysteine-lowering agents.** *J Nutr* 1996, **126(4 Suppl)**:1276S–1280S.
16. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG: **A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes.** *JAMA* 1995, **274**:1049–1057.
17. Connor S, Ojeda L, Sexton G, *et al.*: **Diets lower in folic acid and carotenoids are associated with the coronary disease epidemic in central and eastern Europe.** *J Am Diet Assoc* 2004, **104**:1793–1799.
18. Lange H, Suryaprana H, De Luca G, *et al.*: **Folate therapy and in-stent restenosis after coronary stenting.** *N Engl J Med* 2004, **350**:2673–2681.
19. Herrmann HC: **Prevention of cardiovascular events after percutaneous coronary intervention.** *N Engl J Med* 2004, **350**:2708–2710.
20. Patrick TE, Powers RW, Daftary AR, *et al.*: **Homocysteine and folic acid are inversely related in black women with preeclampsia.** *Hypertension* 2004, **43**:1279–1282.
21. Breteler MMB: **Vascular risk factors for Alzheimer's disease: an epidemiologic perspective.** *Neurobiol Aging* 2000, **21**:153–160.
22. • Quadri P, Fragiacomo C, Pezzati R, *et al.*: **Homocysteine, folate, and vitamin B-12 in mild cognitive impairment, Alzheimer disease, and vascular dementia.** *Am J Clin Nutr* 2004, **80**:114–122.

Recent research showing the lowest folate tertile were found to have significantly higher adjusted odds ratios for mild cognitive impairment and dementia, whereas hyperhomocysteineemia was significantly associated with dementia and Alzheimer disease.

23. Ghadirian A, Ananth J, Engelsmann F: **Folic acid deficiency and depression.** *Psychosomatics* 1980, **21**:926–929.

24. Wolfersdorf M, Konig F: **Serum folic acid and vitamin B-12 in depressed inpatients [in German].** *Psychiatr Prax* 1995, **22**:162–164.
25. Tolmunen T, Hintikka J, Voutilainen S, *et al.*: **Association between depressive symptoms and serum concentrations of homocysteine in men: a population study.** *Am J Clin Nutr* 2004, **80**:1574–1578.
26. Martínez M, Henning S, Alberts D: **Folate and colorectal neoplasia: relation between plasma and dietary markers of folate and adenoma recurrence.** *Am J Clin Nutr* 2004, **79**:691–697.
27. van Meurs JBJ, Dhonukshe-Rutten RAM, Pluijm SMF, *et al.*: **Homocysteine levels and the risk of osteoporotic fracture.** *BJM* 2004, **350**:2033–2041.
28. McLean RR, Jacques PF, Selhub J, *et al.*: **Homocysteine as a predictive factor for hip fracture in older persons.** *BJM* 2004, **350**:2042–2049.
29. Whitney EN, Cataldo CB, Rolfes SR: *Understanding Normal and Clinical Nutrition.* Belmont, CA: Wadsworth/Thompson Learning; 2002:310–313.
30. Babaei-Jadidi R, Karachalias N, Kupich C, *et al.*: **High-dose thiamine therapy counters dyslipidaemia in streptozotocin-induced diabetic rats.** *Diabetologia* 2004, **47**:2235–2246.
31. Rivlin RS: **Riboflavin.** In *Present Knowledge in Nutrition,* edn 8. Edited by Bowman BA Russell RM. Washington, DC: ISLI Press; 2001:191–198.
32. Higdon J: *An Evidence-Based Approach to Vitamins and Minerals.* New York: Thieme; 2003:27–32.
33. Whitney EN, Cataldo CB, Rolfes SR: *Understanding Normal and Clinical Nutrition.* Belmont, CA: Wadsworth/Thompson Learning; 2002:314–316.
34. Schoenen J, Leneaerts M, Bastings E: **High dose riboflavin as a prophylactic treatment of migraine: results of an open pilot study.** *Cephalalgia* 1994, **14**:328–329.
35. Schoenen J, Jacquy J, Lenaerts M: **Effectiveness of high-dose riboflavin in migraine prophylaxis. A randomized controlled trial.** *Neurology* 1998, **50**:466–470.
36. Boehnke C, Reuter U, Flach U, *et al.*: **High-dose riboflavin treatment is efficacious in migraine prophylaxis: an open study in a tertiary care centre.** *Eur J Neurol* 2004, **11**:475–477.
37.•• Liu J, Raine A, Venables PH, Mednick SA: **Malnutrition at age 3 years and externalizing behavior problems at ages 8, 11, and 17 Years.** *Am J Psychiatry* 2004, **161**:2005–2013.

Birth cohort study tracking subjects through adolescence, demonstrating malnutrition (riboflavin deficiency, protein malnutrition, and iron deficiency) in the first few years of life leads to antisocial and aggressive behavior.

38. Whitney EN, Cataldo CB, Rolfes SR: *Understanding Normal and Clinical Nutrition.* Belmont, CA: Wadsworth/Thompson Learning; 2002:316–319.
39. Rivlin RS: **Riboflavin.** In *Present Knowledge in Nutrition,* edn 8. Edited by Bowman BA Russell RM. Washington, DC: ISLI Press; 2001:199–206.
40. Higdon J: *An Evidence-Based Approach to Vitamins and Minerals.* New York: Thieme; 2003:15–22.
41. Groenen PMW, van Rooij IALM, Peer PGM, *et al.*: **Low maternal dietary intakes of iron, magnesium, and niacin are associated with spina bifida in the offspring.** *J Nutr* 2004, **134**:1516–1522.
42. Morris MC, Evans DA, Bienias JL, *et al.*: **Dietary niacin and the risk of incident Alzheimer's disease and of cognitive decline.** *J Neurol Neurosurg Psychiatry* 2004, **75**:1093–1099.
43. McCormick DB: **Vitamin B-6.** In *Present Knowledge in Nutrition, edn 8.* Edited by Bowman BA Russell RM. Washington, DC: ISLI Press; 2001:207–213.
44. Whitney EN, Cataldo CB, Rolfes SR: *Understanding Normal and Clinical Nutrition,* Belmont, CA: Wadsworth/Thompson Learning; 2002:321–322.
45. Higdon J: *An Evidence-Based Approach to Vitamins and Minerals.* New York: Thieme; 2003:48–55.
46.• Friso S, Girell D, Martinelli N, *et al.*: **Low plasma vitamin B-6 concentrations and modulation of coronary artery disease risk.** *Am J Clin Nutr* 2004, **79**:992–998.

A case control study demonstrating that low concentrations of PLP are also associated with high CRP concentrations; and low PLP and elevated hsCRP and fibrinogen are related to higher risk of coronary artery disease.

47. Higdon J: *An Evidence-Based Approach to Vitamins and Minerals.* New York: Thieme; 2003:56–64.
48. Whitney EN, Cataldo CB, Rolfes SR: *Understanding Normal and Clinical Nutrition.* Belmont, CA: Wadsworth/Thompson Learning; 2002:328–330.
49. Stabler SP: **Vitamin B-12.** In *Present Knowledge in Nutrition,* edn 8. Edited by Bowman BA Russell RM. Washington, DC: ISLI Press; 2001:230–240.
50. Kirke PN, Molloy AM, Daly LE, *et al.*: **Maternal plasma folate and vitamin B12 are independent risk factors for neural tube defects.** *Q J Med* 1993, **86**:703–708.
51. Groenen PMW, van Rooij IALM, Peer PGM, *et al.*: **Marginal maternal vitamin B12 status increases the risk of offspring with spina bifida.** *Am J Obstet Gynecol* 2004, **191**:11–17.
52.•• Tucker KL, Hannan MT, Qiao N, *et al.*: **Low plasma vitamin B12 is associated with lower BMD: the Framingham Osteoporosis Study.** *J Bone Miner Res* 2005, **20**:152–158.

Framingham Offspring Osteoporosis Study analysis demonstrates that men and women with vitamin B12 levels lower than 148 pM had significantly lower average BMD ($P < 0.05$).

53. Stone KL, Bauer DC, Sellmeyer D, Cummings SR: **Low serum vitamin bB12 levels are associated with increased hip bone loss in older women: a prospective study.** *J Clin Endocrinol Metab* 2004, **89**:1217–1221.