Causes of vitamin B_{12} and folate deficiency

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Abstract

This review describes current knowledge of the main causes of vitamin B_{12} and folate deficiency. The most common explanations for poor vitamin B_{12} status are a low dietary intake of the vitamin (i.e., a low intake of animal-source foods) and malabsorption. Although it has long been known that strict vegetarians (vegans) are at risk for vitamin B_{12} deficiency, evidence now indicates that low intakes of animal-source foods, such as occur in some lacto-ovo vegetarians and many less-industrialized countries, cause vitamin B_{12} depletion. Malabsorption of the vitamin is most commonly observed as food-bound cobalamin malabsorption due to gastric atrophy in the elderly, and probably as a result of Helicobacter pylori *infection. There is growing evidence that gene polymorphisms in transcobalamins affect plasma vitamin B_{12} concentrations. The primary cause of folate deficiency is low intake of sources rich in the vitamin, such as legumes and green leafy vegetables, and the consumption of these foods may explain why folate status can be adequate in relatively poor populations. Other situations in which the risk of folate deficiency increases include lactation and alcoholism.*

Causes of vitamin B_{12} deficiency

Overview of vitamin B_{12} absorption and requirements

The most common causes of vitamin B_{12} (cobalamin) deficiency are inadequate dietary intake of the vitamin, and, primarily in the elderly, malabsorption due to gastric atrophy. Vitamin B_{12} absorption is a complex process, involving a series of steps that can be affected adversely by intestinal disease, infections, and medications (**table 1**). Cobalamin mixes with haptocorrin—a cobalamin-binding protein produced by salivary and esophageal glands—during chewing and swallowing of food. After its release from proteins in food by gastric acid and pepsin, it is bound to haptocorrin in the acid pH of the stomach, then released from haptocorrin by proteolytic enzymes in the alkaline pH of the small intestine, and bound to intrinsic factor secreted by the stomach. Subsequently, the vitamin is absorbed as a cobalamin-intrinsic factor complex by binding to specific receptors in the ileal mucosa. The complex is taken into lysosomes, where the cobalamin-intrinsic factor complex is released and intrinsic factor is degraded. The cobalamin is metabolized to its methyl and deoxyadenosyl forms, and released into plasma mainly in the methyl form. In plasma, cobalamin is transported and transferred to body cells bound to transcobalamin, which carries about 25% of the cobalamin in plasma. The remainder is bound to haptocorrin, the function of which is not well understood.

When the Recommended Dietary Allowance (RDA) for vitamin B_{12} was revised by the United States and Canada in 2002, it was based on the amount needed to

TABLE 1. Causes of vitamin B_{12} deficiency

Low dietary intake
Veganism
Lacto-ovo vegetarianism
Low animal-source food intake
Low stores and intake by breastfed infants
Malabsorption
Gastric atrophy and malabsorption from food
Pernicious anemia
Ileal disease
Chronic pancreatitis
Parasitic infections
Medications
Polymorphisms

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maintain normal serum vitamin B_{12} concentrations, and to sustain normal hematopoiesis. For adults, the RDA is 2.4 to 2.8 μg/day depending on gender and physiological status; for children aged 1 to 18 years, the RDA is from 0.9 to 2.4 μg/day. The median intake of the vitamin from food (not including supplements) in the United States is about 5 μg/day for men and 3.5 μg/day for women [1], but is considerably less where animal-source food intake is low. WHO and FAO, in determining their Recommended Nutrient Intakes (RNI) for vitamin B_{12}, accepted the approach and values developed by the Institute of Medicine, with minor differences such as setting an EAR and RNI for infants [2].

Bioavailability (efficiency of absorption) of the vitamin is assumed to be about 50% from the usual diet, but lower from sources containing high amounts (e.g., only 11% is absorbed from liver) [1]. Synthetic (crystalline) vitamin B_{12} is more efficiently absorbed: approximately 60% from a low dose. In healthy adults, about 50% of a 1-μg dose of vitamin B_{12} is absorbed from food, but only 20% of a 5-μg dose, and 5% of a 25-μg dose [3]. Although the intestinal receptors become saturated at high cobalamin intakes, about 1% of free vitamin B_{12} can be absorbed passively from a high dose when given alone, or 0.5% when the dose is given with food [4], a process that enables deficient individuals to be repleted by taking large oral doses of the crystalline form (i.e., 500 μg/day).

One aspect of vitamin B_{12} metabolism that is often misunderstood is the time that it takes to deplete body stores. This rate of depletion depends on the initial amount stored, and the efficiency of absorption from the diet and reabsorption from bile. Hence, it is possible to estimate the length of time it will take for the stores to be depleted under various scenarios [1]. Normal body stores are about 1 to 3 mg; the turnover of the vitamin in healthy persons is about 0.1% per day; and signs of deficiency appear when the pool drops below 300 μg. If there is no intake from food or supplements, and absorption is normal, a 1-mg store would meet the body's needs for 3 years, 2 mg for 5 years, and 3 mg for 6 years. In people with less efficient absorption of the vitamin from food because of gastric atrophy, these estimates would be reduced to 2, 3.6, and 4 years, respectively. Only if some of the vitamin is consumed daily, absorption is normal, and initial stores are reasonable, does it become possible to avoid symptoms for the ~25-year period that is often quoted as the time it takes for deficiency symptoms to appear.

TABLE 2. Vitamin B_{12} content of common animal-source foods[a]

Food	Serving	Vitamin B_{12} (μg)
Beef, ground, 20% fat	3 oz	2.3
Milk, cow, 2% fat	8 oz	0.9
Fish, cod, cooked	3 oz	0.9
Egg, chicken, boiled	1 egg	0.6
Liver, beef, cooked	3 oz	95[b]
Cheese, cheddar	1 oz	0.2

a. The adult RDA for vitamin B_{12} is 2.4 μg/day.
b. The efficiency of absorption from liver is approximately 11% compared with 50% for other foods [1].

Inadequate dietary intake

Food sources of vitamin B_{12}

Vitamin B_{12} is found only in animal-source foods, so intake is entirely dependent on the amount of animal-source foods in the diet, except where foods are fortified with the vitamin. **Table 2** provides information on the amount of vitamin B_{12} in some common animal-source foods. Other sources are fortified foods, and supplements. Although it has been suggested that foods such as spirulina, nori, kelp, chlorella, and algae provide useful amounts of the vitamin for vegans, it is likely that the cobalamins in these foods are not biologically active [5, 6]. In fact, they contain cobalamin analogs which may inhibit cobalamin-dependent enzymes [7] and cause further deterioration of vitamin B_{12} status [5]. More research is needed on whether foods prepared with bacterial fermentation can provide useful amounts of biologically active vitamin B_{12}, but to date there is little or no evidence that they can sustain adequate vitamin B_{12} status. To be biologically active, all parts of the cobalamin molecule must be present. The bacteria in the human large intestine probably produce some biologically active vitamin B_{12}, but since the bacteria are located below the ileum, the vitamin will not be absorbed from this source.

Strict vegetarians (vegans)

It is well known that strict vegetarians (vegans) are at high risk for vitamin B_{12} deficiency, and multiple case reports describe the clinical and biochemical symptoms of vitamin B_{12} deficiency in this group. Most of these cases are infants who were born to, and breastfed by, vegan mothers [8]. There are a few studies of larger groups of vegans. For example, of 110 adults and 42 children from a macrobiotic community in the United States, 51% of adults had low plasma cobalamin and 30% of adults and 55% of the children had elevated urinary methylmalonic acid (MMA) [9]. In healthy vegan adults in California (n = 25, age 20 to 60 years), 40% had evidence of vitamin B_{12} depletion based on either low plasma cobalamin (< 200 pg/mL), macrocytosis, or elevated serum MMA [10]. In a group of 131 German vegans aged 20 to 82 years who did not take vitamin B_{12} supplements, 40% of strict vegans had plasma cobalamin < 150 μg/mL, and approximately the same proportion had elevated plasma homocysteine

[11]. In a longer-term study of Dutch adherents to macrobiotic diets, low plasma cobalamin was prevalent in their infants and young children, accompanied by megaloblastic anemia, elevated urinary MMA, and low breastmilk concentrations of the vitamin [12]. In adolescence, those who had been fed macrobiotic diets during early childhood had a greater risk of vitamin B_{12} depletion, even though they had subsequently consumed omnivorous diets [13]. However, to what extent this was due to persistently lower intakes of the vitamin during the intervening years is uncertain.

Because about half of the vitamin excreted in bile is reabsorbed through enterohepatic circulation, it used to be believed that it took about 20 to 30 years for stores of the vitamin to become depleted in vegans [14]. However, as discussed above, individuals starting with normal stores and then changing to a vegan diet would develop signs of deficiency in only 6 years. This is consistent with observations in several studies. For example, in the German vegan study discussed above [11], vegan diets had been consumed for 7.1 years on average, and those who had been vegans for > 5 years had 1.8 times the rate of deficiency compared with those who had followed this diet for < 5 years. In the California study, the mean duration of veganism was 4.2 years, although on average the group had been following some type of vegetarian diet for a total of 12 years [10]. Few were taking supplements.

Lacto-ovo vegetarians

More recently, evidence has accumulated to reveal that lacto-ovo vegetarians are at greater risk of cobalamin depletion than omnivores. This important observation, which runs contrary to the traditional belief that only vegans are at risk for deficiency, suggests that people who avoid meat, or who consume relatively small amounts of animal-source foods, are at risk for vitamin B_{12} deficiency. Meat contains substantially more vitamin B_{12} (2.3 μg/100 kcal raw meat, and 1.3 μg/100 kcal cooked meat) than milk (0.6 μg/100 kcal). For example, in the Netherlands, 40% of pregnant lacto-ovo vegetarians failed to consume their Estimated Average Requirement (EAR) for vitamin B_{12}, compared with 6% of those who consumed small amounts of meat, and none of those who consumed a typical Western diet [15].

Several studies have shown low serum or plasma vitamin B_{12} concentrations in lacto-ovo vegetarians. The mean concentration in elderly Dutch lacto-ovo vegetarians was 273 pg/mL, significantly lower than in omnivores (350 pg/mL) [16]. In New Zealand, adults who never ate red meat, yet ate chicken or fish once a week or less, consumed only 1.6 μg/day of the vitamin (median intake) and 50% had a serum vitamin B_{12} concentration < 200 pg/mL [17]. Low intake of vitamin B_{12} due to avoiding meat, although dairy products were consumed, explained why one-third of a group of lacto-ovo vegetarians in England had low serum vitamin B_{12} concentrations [18]. Young "new vegetarians" recruited from a meditation center in Sydney, Australia, consumed dairy products and had avoided meat for only 7 years on average. However, 16% had vitamin B_{12} deficiency (plasma vitamin B_{12} < 200 pg/mL) and many more (number not stated) had "marginal" plasma concentrations [19]. Serum MMA concentrations were elevated in 0% of 79 middle-aged German omnivores, 5% of low consumers of meat (*n* not stated), 32% of lacto-ovo vegetarians ($n = 66$), and 43% of vegans ($n = 28$) [20].

We have analyzed the association between reported intake of animal-source foods and the prevalence of marginal (200–300 pg/mL) and deficient (< 200 pg/mL) plasma vitamin B_{12} concentrations in the US National Health and Nutrition Evaluation Survey (NHANES III) (Allen et al., not published). The data set has plasma cobalamin values for 12,020 individuals aged ≥ 4 years. Of those aged 19 to 50 years, 16% had deficient or marginal plasma vitamin B_{12}, compared with 43% of those who reported consuming meat or fish less than four times a month. This prevalence of deficiency is similar to that in the German lacto-ovo vegetarians described by Herrmann et al. [21]. The association between the frequency of meat consumption, but not dairy product or egg intake, and plasma vitamin B_{12} concentrations was highly significant ($p < .001$) in NHANES III.

How much animal-source food needs to be consumed to ensure adequate vitamin B_{12} status (assuming that absorption is normal)? Unfortunately there are few data on the association between dietary vitamin B_{12} intake, and even fewer on intakes of specific foods, and plasma vitamin B_{12}. The Institute of Medicine review of studies of individuals with low intake concluded that at least 1.5 μg/day must be consumed to prevent deficiency symptoms [1]. Among pregnant low meat-eaters in the Netherlands, those consuming 0.3 μg/day from meat + fish + eggs had significantly lower plasma vitamin B_{12} than those consuming 1.3 μg/day, who in turn had lower concentrations than those consuming 3.2 μg/day from these sources. Intakes from milk, yogurt, and cheese were similar [15].

Malabsorption

Malabsorption tends to cause vitamin B_{12} depletion more rapidly than does low dietary intake of the vitamin, and malabsorption combined with low intake causes the most rapid depletion. Deficiency symptoms appear within 2 to 5 years when malabsorption is due to lack of intrinsic factor or to intestinal abnormalities, because absorption of the enterohepatically recirculated vitamin is also impaired. However, when malabsorption is caused by poor release of the vitamin from food, as in gastric atrophy, the efficiency of enterohepatic recirculation may be normal, but the quantity reab-

sorbed is low once liver stores become depleted.

Malabsorption of vitamin B_{12} is usually measured by the Schilling test, which basically requires the patient to consume 1 μg of radioactively labeled synthetic cyanocobalamin in the fasted state, and to collect his or her urine for the subsequent 24 hours. A patient with malabsorption will excrete lower amounts of radioactivity in the urine. In most cases, the labeled vitamin is given in an aqueous solution, which will enable detection of malabsorption due to lack of intrinsic factor or intestinal problems, but will not test ability to absorb the vitamin from food. Thus, a better way to administer the test dose is to mix it with food, most commonly egg yolk, in an EYCAT (Egg Yolk Cobalamin Absorption Test) or "food-bound" Schilling test [22]. The food-bound test detects a much higher proportion of malabsorption, especially in groups such as the elderly, in whom gastric atrophy reduces gastric acid secretion and the ability to release the vitamin from food [23]. Unfortunately, few clinics in the world administer the food-bound test, and the common failure to detect existing malabsorption in other centers explains in part why even the crystalline Schilling test is rarely prescribed.

Malabsorption due to gastric atrophy

Atrophic gastritis is a common condition in the elderly. It is diagnosed by elevated serum gastrin and/or low serum pepsinogen I concentrations, or a low pepsinogen I:pepsinogen II ratio. Based on elevated serum gastrin, we observed a 30% prevalence of gastric atrophy in a representative group of 1,546 Californian Latinos aged ≥ 60 years [24]. Similarly, in Boston, USA, 31% of elderly (n = 359, age 60 to 99 years) had a low pepsinogen I:pepsinogen II ratio [25].

In atrophic gastritis, the stomach atrophy is accompanied by low or absent gastric acid secretion, and sometimes by bacterial overgrowth in the upper small intestine. Type A atrophic gastritis is relatively uncommon, mainly affects the body of the stomach, and is associated with pernicious anemia. Intrinsic factor secretion is low or absent so that both food-bound and supplemental vitamin B_{12} are poorly absorbed. Type B atrophic gastritis, where mainly the antrum is affected, is the most common type in the United States [25]. Since intrinsic factor secretion is usually relatively normal in type B, except in severely affected, long-term cases, only the food-bound form of the vitamin is poorly absorbed.

It is well documented that the elderly suffer from a high prevalence of vitamin B_{12} deficiency, even in industrialized countries. For example, in 548 surviving members of the original Framingham study in the United States, 40% had a serum cobalamin concentration < 350 pg/mL compared with 18% of younger controls [26]. Atrophic gastritis is one cause of the high prevalence of deficiency in elderly. In the 1,546 Californian Latinos aged ≥ 60 years, 16% had plasma vitamin B_{12} values in the marginal range (200–300 pg/mL), and 6% had deficiency (< 200 pg/mL) [24]. When elderly with deficient plasma vitamin B_{12} concentrations (n = 57) were compared with those with marginal (n = 68) and normal (n = 52) values, serum gastrin was inversely related to plasma vitamin B_{12} and significantly higher in the deficient group [24].

In the California Latinos with elevated serum gastrin (gastric atrophy), plasma vitamin B_{12} was significantly correlated with vitamin B_{12} intake from supplements + fortified food, but not from unfortified food, because synthetic (crystalline) vitamin B_{12} can be absorbed by individuals with gastric atrophy. Although the lack of gastric acid in this condition impairs the release of vitamin B_{12} from proteins in food, the intrinsic factor secretion is usually adequate until the gastric atrophy is very severe. This explains why the elderly can usually absorb vitamin B_{12} from fortified foods or supplements. For this reason the US-Canada RDA for vitamin B_{12} is not set higher for the elderly, who cannot absorb enough from food even if more is consumed, but rather it is recommended that the majority of their vitamin B_{12} should be obtained from fortified foods or supplements. Moreover, since about 1% of the vitamin is absorbed by passive transport, even in conditions where intrinsic factor is lacking, vitamin B_{12} repletion can be achieved by high dose oral supplementation (e.g., 500 μg/day).

Malabsorption due to pernicious anemia

Pernicious anemia is the final stage of an autoimmune disorder in which autoantibodies against H^+K^+-adenosine triphosphatase destroy parietal cells in the stomach. It was first described in the early 1800s, and diagnosed on the basis of neurological and gastric problems and anemia. In the late 1800s, it was observed that the condition was associated with atrophy of the gastric mucosa. In 1948, vitamin B_{12} was identified as the "anti-pernicious anemia principle." Pernicious anemia results from lack of gastric acid and intrinsic factor, and its prevalence is lower than commonly assumed. It can be detected by measuring anti-intrinsic factor antibodies in serum. Prevalence in Californian women was 4.3% in blacks and 4% in whites (n = 729, age ≥ 60 years) [27]. The prevalence may be higher in blacks than in whites [28].

Causes of vitamin B_{12} deficiency in developing countries

Typically the diets of populations in low-income countries, and especially those of the most disadvantaged groups, are low in animal-source foods because of their relatively high cost, lack of availability, and/or cultural and religious beliefs. It has become apparent that many such populations have a high prevalence of

deficient and marginal plasma vitamin B_{12} concentrations. **Table 3** provides a summary of some reported prevalences of low and marginal cobalamin values in Latin America. A similar situation is emerging in other poor regions of the world; in urban India 60% of plasma vitamin B_{12} values were < 150 pg/mL [29], and in Nepal, 49% of pregnant women had serum vitamin B_{12} < 225 pg/mL and 61% had elevated MMA [30]. In rural Kenya we found 31% deficient + 24% marginal plasma vitamin B_{12} concentrations in rural schoolchildren [31]. The new WHO prevalence data are provided in the report by McLean et al. [32] in this Supplement.

There is some evidence that an inadequate intake of vitamin B_{12}, rather than malabsorption, is the main cause of this high prevalence of deficiency, although the latter cannot be ruled out because it has not been assessed. In 94 lactating women in Guatemala City, usual vitamin B_{12} intake was correlated with, and the main predictor of, maternal plasma cobalamin concentrations ($r = -0.20$, $p < .05$) [33]. Replacement of breastmilk with cow's milk, which is much higher in vitamin B_{12}, predicted higher plasma cobalamin concentrations in a group of predominantly vitamin B_{12}-deficient Guatemalan infants [34]. Vitamin B_{12} intake was the only significant predictor of plasma vitamin B_{12} in 180 Guatemalan schoolchildren—60 each in deficient, marginal, and normal plasma vitamin B_{12} groups [35]. *Helicobacter pylori* infection and bacterial overgrowth were not significant predictors. The most definitive study to date was conducted in Kenyan schoolchildren enrolled in a school feeding intervention [31, 36]. At baseline, 69% of the 503 children had low or marginal plasma vitamin B_{12} concentrations. Those in the lowest tertile of animal-source food intake (0% to 1.3% of daily energy intake) had a six times greater risk of low serum cobalamin compared with those in the highest tertile of animal-source food intake (4% to 37% of daily kcal). The children were randomly assigned (by schools) to receive isocaloric daily snacks of maize and beans alone, maize and beans plus meat (60 g minced beef, an additional 1.3 μg/day of vitamin B_{12}), or milk (200 mL, an additional 1 μg/day of vitamin B_{12}), or to no intervention, for 2 years. At the end of 1 year of supplementation, the reduction in prevalence of deficient + marginal plasma vitamin B_{12} values was significantly greater in the meat- and milk-supplemented groups. At the end of 2 years, the prevalence of deficient + marginal concentrations fell from 56% to 4% in the meat-supplemented group, and from 41% to 9% in the milk-supplemented group. This study shows that the high prevalence of vitamin B_{12} deficiency in these Kenyan schoolchildren at baseline was predicted by their low intake of animal-source foods, and that a daily food supplement providing about the daily recommended intake (on about 50% of

TABLE 3. Prevalence (%) of deficient and marginal plasma vitamin B_{12} concentrations in Latin America[a]

First author	Ref.	Year	Location	Age (yr)	*N*	% < 200 pg/mL	% 200–300 pg/mL
Casterline	[33]	1997	Guatemala	0.25	113	12[b]	—
Anaya	[34]	2003	Guatemala	0.6–1.0	100	25	36
Allen	[40]	1995	Mexico	1.5–2.5	28	16	41
Black	[121]	1995	Mexico	1.5–2.5	219	10	33
Allen	[122]	2000	Mexico	1.5–2.5	128	11	29
Anaya	[123]	2004	Mexico	1–11	447	30	25
Garcia-Casal	[124]	2005	Venezuela	0–7	1,792	11	?
Casterline	[33]	1997	Guatemala	6–12	109	13	34
Rogers	[125]	2003	Guatemala	6–12	554	11	22
Allen	[40]	1995	Mexico	7–8	29	22	25
Garcia-Casal	[124]	2005	Venezuela	14–26, pregnant	1,283	61	?
Casterline	[33]	1997	Guatemala	25, lactating	113	13	33
Hertrampf	[126]	2003	Chile	Women	598	10	30
Anaya	[123]	2003	Mexico	Women	417	13	23
Black	[121]	1994	Mexico	Women	21	19	19
Black	[121]	1994	Mexico	Pregnant	42	19	43
Black	[121]	1994	Mexico	Lactating	20	30	25
Black	[121]	1994	Mexico	Men	22	27	14
King	[127]	1997	Guatemala	Men, women > 60	760	38	—
Olivares	[128]	2000	Chile	Men, 60–89	98	50	19
Olivares	[128]	2000	Chile	Women, 60–89	181	31	20

a. Deficient, < 200 pg/mL; marginal, 200–300 pg/mL.
b. Based on urinary MMA.

the days in each year) could be absorbed, and restored adequate vitamin B_{12} status.

Vitamin B_{12} deficiency in infants and young children

The liver of well-nourished newborn infants contains only 25 to 30 μg of vitamin B_{12}, and even less when the mother is vitamin B_{12} depleted during pregnancy [37]. At birth, concentrations of vitamin B_{12} in cord blood ($n = 173$) were strongly correlated with maternal levels in a Norwegian sample [38]. Higher maternal parity predicted lower infant plasma vitamin B_{12} at age 6 months. Even in well-nourished Norwegian infants, plasma cobalamin is low during the first 6 months of life; total plasma homocysteine and cobalamin are strongly correlated, but homocysteine and folate are not; and serum MMA tends to be elevated. The Norwegian investigators concluded that a substantial number of infants have insufficient vitamin B_{12} to support homocysteine remethylation in this well-nourished population [39].

Infants born to vitamin B_{12}-deficient mothers are at very high risk of developing deficiency because of their low stores of the vitamin at birth [37] and the fact that concentrations of the vitamin in their mothers' breastmilk will be inadequate [33, 40, 41]. Maternal plasma and breastmilk vitamin B_{12} concentrations are quite strongly correlated in most studies.

There are two larger-scale studies of the vitamin B_{12} status of vegetarian women and their children**.** In Boston, USA, participants included 64 women and 42 children (median age 3.9 years) [9]. Low serum cobalamin (< 220 pg/mL) was present in half of the women who had been strict vegetarians for 1 year, and in all after 5 years. Over half the children had high urinary MMA, one-third were stunted, and elevated MMA was associated with stunting. Vitamin B_{12} in the breastmilk averaged 0.3 μg/L in vegetarians ($n = 17$) and 0.5 μg/L in omnivores ($n = 6$) [42]. Women who had eliminated animal-source foods from their diet for only 4 to 5 years clearly secreted less vitamin B_{12} in their breastmilk. Maternal serum and breastmilk vitamin B_{12} concentrations were strongly correlated ($r = 0.79$, $p < .0001$), and inversely correlated with maternal urinary MMA ($r = –0.830$, $p < .0001$). Infant urinary MMA increased when milk vitamin B_{12} fell below 0.5 μg/L. These are the only data available on which to base a cutoff for adequate breastmilk vitamin B_{12} concentration. The Boston studies show that the onset of vitamin B_{12} deficiency is more rapid after animal-source foods are eliminated than is usually believed, and that breastfed infants are very vulnerable to poor maternal vitamin B_{12} status. This is likely to be the situation in many areas of the world where animal-source food intake is low.

In Mexico we observed that 62% of women at 6 to 8 months postpartum had low vitamin B_{12} concentrations in breastmilk, and milk and infant plasma levels were significantly correlated [40]. In Guatemala City, 31% of women had low vitamin B_{12} in breastmilk at 3 months postpartum [33]. In another study in Guatemala City, 24% of 127 infants aged 7 to 12 months had deficient (< 200 pg/mL), and 37% had marginal (200 to 300 pg/mL), plasma vitamin B_{12} concentrations. Surprisingly, plasma vitamin B_{12} was lower in those infants who consumed more breastmilk ($r = – 0.33$, $p < .0001$ with measured breastmilk intake, kcal/day) [34]. In a different group of infants ($n = 304$) aged 12 months in Guatemala City, deficient and marginal plasma vitamin B_{12} values were found in 30% and 20% of infants, and 36% and 32% of their mothers, respectively [43]. The main predictors of plasma cobalamin concentrations were higher maternal plasma vitamin B_{12}, higher intake of the vitamin from complementary foods (notably cow's milk), lower frequency of breastfeeding, and larger household size. The better vitamin B_{12} status of infants consuming more cow's milk may be due to the fact cow's milk vitamin B_{12} content is 10 times higher than that in breastmilk in well-nourished women, and probably closer to 20 times higher than in breastmilk of the vitamin B_{12}-depleted women in Guatemala City.

Infections

Helicobacter pylori *infection*

Helicobacter pylori is a gram-negative, spiral bacterium with four to six flagellae that causes acute and chronic gastritis, and over time, gastric atrophy. It is probably the main cause of gastric ulcers, and of gastric atrophy in the elderly. The bacteria reside at the interface of the gastric epithelial cells and the gastric mucosa. *H. pylori* uniquely produces urease, so it can be diagnosed by giving the patient a dose of isotopically labeled urea which the bacteria will hydrolyze to ammonia and water; and measuring the presence of labeled ammonia in exhaled breath. Diagnosis is also possible from detection of *H. pylori* antibodies in serum.

H. pylori infection is very widespread, and once gastric colonization has occurred, it persists throughout life unless treated. In developing countries and areas of poor sanitation, almost all infants and children may be infected by the first years of life [44]. In Western countries, the prevalence is closer to 20% in persons aged < 40 years, and 50% in those aged > 60 years. The standard treatment (antibiotics, bismuth, and acid suppressors) for 1 to 2 weeks is usually effective, but is often not provided unless there are clinical symptoms. In many people the infection is asymptomatic while others may develop acute gastritis.

Infection with *H. pylori* causes a series of changes in gastric function prior to the final stage of gastric atrophy. The initial infection causes transient hypochlorhydria and usually few or no clinical symptoms [45]. In some persons, the infection causes gastric inflam-

mation with elevated serum gastrin and pepsinogen and reduced somatostatin concentrations [46]. The elevated gastrin and reduced somatostatin then cause elevated acid secretion from gastric parietal cells [45], but over time (usually many years) some but not all infected persons develop peptic ulcer disease, gastric cancer, and eventually atrophic gastritis with low gastric acid secretion, and possibly inadequate production of intrinsic factor.

Since *H. pylori* infection eventually causes achlorhydria and gastric atrophy, it may be the major cause of food-bound vitamin B_{12} malabsorption and gastric atrophy in the elderly. However, proof of causality has been somewhat inconsistent; it is not usually known how long the infection has been present or the density of bacteria, and the different genotypes of the bacteria have different effects. Of vitamin B_{12}-deficient patients from California diagnosed with food-bound vitamin B_{12} malabsorption, 78% had serum antibodies against *H. pylori* compared with 42% of those with normal vitamin B_{12} absorption [47]. In another study in California, *H. pylori* infection was associated with food-bound cobalamin malabsorption, and 78% of subjects with severe malabsorption were infected [48]. *H. pylori* infection, gastrin levels, ethnic origin, and age were independent risk factors for food-bound vitamin B_{12} malabsorption. In 174 Guatemalan schoolchildren aged 8 to 12 years, 83% were infected so its prevalence was not different among those with deficient, marginal, and normal plasma vitamin B_{12} concentrations [35]. On average the infected children had elevated serum gastrin and pepsinogen concentrations, suggesting that they had gastric inflammation but not gastric atrophy at this life stage. However, elevated serum gastrin was associated with elevated serum MMA, perhaps indicating an early stage of vitamin B_{12} depletion. Few studies have measured the effect of *H. pylori* treatment on vitamin B_{12} status or absorption. An intervention trial in Turkey reported that *H. pylori* was present in 56% of 138 patients with vitamin B_{12} deficiency, but eradication of the bacteria improved anemia and serum vitamin B_{12} in 40% of the infected patients [49]. Overall, it is probable that *H. pylori* is a risk factor for vitamin B_{12} malabsorption, at least in adults and elderly who may have had long-term exposure to the bacteria. However, a review of 25 studies on this question concluded that the results are still inconclusive due to methodological differences among studies, and that *H. pylori* does not play a major role in the development of cobalamin deficiency [50].

Giardia lamblia *infection*

Giardia lamblia is a parasite that infects about 20% of the world's population, especially in areas of developing countries where sanitation is poor. The parasite cysts are ingested in contaminated food or water, and about three times more children are infected than adults. The parasite causes nausea, diarrhea, abdominal cramps, and weight loss, although there is substantial variation in the intensity of symptoms. In 29 Danish children with diarrhea caused by *Giardia*, one-third had poor absorption of vitamin B_{12} but this did not cause low plasma vitamin B_{12}, perhaps due to the short period of infection [51]. Twelve percent of *Giardia*-infected patients had vitamin B_{12} malabsorption in another study [52], but none in another [53]. Thus it is uncertain to what extent *Giardia* infection causes vitamin B_{12} deficiency, but any malabsorption would be expected to occur in longer-term and more severe infection.

Fish tapeworm infection

The fish tapeworm, *Diphyllobothrium latum*, occurs in Northern Europe, Latin America, Japan, and many other countries [54]. Infection with the parasite can occur due to eating raw or undercooked fish, such as salmon, from cold-water lakes and rivers. It used to be common around the Great Lakes in the United States, causing diphyllobothriasis in Jewish and Scandinavian fish consumers, although it is now rare in these locations. The fact that it causes vitamin B_{12} deficiency was first recognized in 1886 when it was identified as the cause of "fish tapeworm anemia" [55], which mimics pernicious anemia. The tapeworm competes for vitamin B_{12} with its human host, taking up large amounts of the vitamin from the intestinal lumen [56].

Bacterial overgrowth

Bacterial overgrowth refers to the presence of substantial numbers of bacteria in the upper intestine, which is normally relatively sterile. This condition occurs primarily as a result of low intestinal motility, with hypochlorhydria having less clinical importance [57]. Low gastrointestinal motility can result from gastrointestinal surgery, including gastric bypass, jejunal diverticulosis, and blind-loop syndrome after intestinal surgery, or structural abnormalities, including diverticulosis in the duodenum or jejunum.

Bacterial overgrowth impairs absorption of vitamin B_{12} from food. The mechanism may be competition with intestinal bacteria for the uptake of vitamin B_{12} [58], bacterial conversion of the vitamin to inactive analogs, and in hypochlorhydria, reduced absorption of the vitamin from food. In subjects with bacterial overgrowth due to hypochlorhydria, poor absorption of food-bound vitamin B_{12} was rapidly normalized by antibiotic (tetracycline) treatment [58].

Malaria

Plasma vitamin B_{12} concentrations were not different in Kenyan or Senegalese schoolchildren with malaria diagnosed based on serum antigens [31, 60].

HIV and AIDS

Plasma cobalamin concentrations are often low in

patients infected with the human immunodeficiency virus (HIV), and fall further with progression of the disease [61, 62]. Malabsorption of the vitamin is more likely among persons with gastrointestinal symptoms [63], and whose HIV infection is more advanced [64]. Of 310 HIV+ patients in the Baltimore-Washington DC area, followed for 9 years on average, those with low serum vitamin B_{12} (< 160 pg/mL) at baseline were free of AIDS for 4 years, compared with 8 years for those with normal values [65]. These differences persisted after removing from analysis those with more advanced disease at baseline and other relevant parameters. These investigators and others [62] concluded that low serum vitamin B_{12} concentrations preceded progression of HIV+ to AIDS, and predict more rapid progression. There is no evidence that vitamin B_{12} supplementation will slow progression of the disease.

Other medical problems

Because vitamin B_{12} is absorbed in the ileum, its absorption is impaired in several diseases and conditions in which ileal function is abnormal. Malabsorption occurs after surgery where > 60 cm of the ileum is removed [66], and in both tropical and nontropical sprue. Tropical sprue, as its name suggests, occurs mostly in the tropics and subtropics. Symptoms include diarrhea, malabsorption, vitamin B_{12} and folate deficiency, and anemia. Vitamin B_{12} malabsorption is probably caused by the ileal mucosa lesions. Studies in India [67] reported that between two-thirds and all of the patients with this condition have vitamin B_{12} deficiency. The resulting anemia responds to vitamin B_{12} and antibiotics. The prevalence of this condition has diminished so that it has "virtually disappeared from reports" [68].

Nontropical sprue, more commonly known as celiac disease or gluten enteropathy, is most common in Caucasians of European ancestry, and in 70% of cases affects women. It is caused by an allergic reaction to gluten in wheat, rye, oats, barley, and triticale. Inflammation of the small intestine causes flattening of the villi and loss of absorptive capacity. A gluten-free diet (which patients must consume for the rest of their lives) allows mucosal repair and improves vitamin B_{12} absorption compared with a diet containing gluten [69]. This condition might only occur if there are ileal lesions.

Zollinger-Ellison syndrome affects 6 out of 100,000 young adults, and is caused by a gastrinoma in the duodenum or pancreas that results in excessive amounts of gastric acid secretion secondary to high gastrin production. Vitamin B_{12} malabsorption occurs in these patients due to the low ileal pH, and can be relieved in the short-term by acid-suppressing drugs. However, long-term administration of these drugs, especially omeprazole, can cause food-bound vitamin B_{12} malabsorption and lower serum vitamin B_{12} concentrations in these patients [70].

Pancreatic insufficiency may also impair vitamin B_{12} absorption from food. The pancreas secretes trypsin and bicarbonate, which are required for release of vitamin B_{12} from R-binder in the intestine. Impaired trypsin secretion occurs in chronic pancreatitis in which vitamin B_{12} absorption is impaired in 40% of cases, and in cystic fibrosis in which absorption is almost always impaired [71, 72]. In spite of the clearly impaired absorption of the vitamin from food, it is uncommon to find vitamin B_{12} deficiency in patients with pancreatitis [73]. Administration of trypsin with vitamin B_{12}, or bicarbonate to increase ileal pH, can improve vitamin B_{12} absorption and status in these conditions.

Medications

The most commonly reported adverse effects of medications on vitamin B_{12} status are from drugs that impair gastric acid and pepsin secretion, and subsequently impair release of the vitamin from proteins in food. Frequently used medications of this type, for patients with gastric ulcers or gastroesophageal reflux disease, include H_2-receptor antagonists such as cimetidine, and proton pump inhibitors. Cimetidine inhibits both gastric acid and pepsin secretion [74, 75] and possibly secretion of intrinsic factor [75, 76]. The effect of cimetidine on vitamin B_{12} absorption is dose-related, with intakes > 1,000 mg/day reducing food-bound vitamin B_{12} absorption [74, 77], but not 400 mg/day [77]. Proton pump inhibitors, such as omeprazole and lansoprazole, strongly inhibit gastric acid secretion and subsequently food-bound vitamin B_{12} absorption [78]. A 20 mg/day dose of omeprazole reduced vitamin B_{12} absorption from a protein-bound source by about 70%, while 40 mg/day reduced absorption by about 90% [78]. Long-term omeprazole treatment of patients with Zollinger-Ellison syndrome did lower plasma vitamin B_{12} concentrations, especially in achlorhydric patients [70]. However, there are few indications that taking lower doses of acid-suppressing drugs often causes vitamin B_{12} depletion, possibly due to their not being taken for a long enough time.

Gene polymorphisms

Mild transcobalamin I deficiency has been associated with increased risk of low serum cobalamin concentrations and may explain 15% of low values [79]. A common polymorphism has been identified in the vitamin B_{12} carrier protein, transcobalamin II, where proline (P) is substituted for arginine [80]. In the relatively small groups of samples analyzed so far, prevalence is about 20% 259-arginine, 25% 259-proline arginine, and 50% heterozygotes [81-83]. Binding to

vitamin B_{12} and vitamin B_{12} receptor binding affinity are similar between the TC-II 259-proline and TC-II 259-arginine phenotypes. Mean total plasma vitamin B_{12} and homocysteine concentrations did not differ among those homozygous for the arginine or proline form of TC-II, but mean MMA concentrations were lower in the PP and PR groups compared with the RR group. It is possible that the PP genotype delivers cobalamin to tissues more efficiently [81]. Much remains to be learned about how polymorphisms might affect vitamin B_{12} status. None of six TC-II polymorphisms was associated with increased risk of neural tube defects (NTD) in a large sample of NTD mothers, fathers, and their affected children [84].

Juvenile pernicious anemia is an inherited disorder manifested by lack of intrinsic factor, and accompanied by cobalamin malabsorption. Malabsorption also occurs in Immerslund-Gräsbeck syndrome, due to abnormalities in the cubulin/amnionless complex. Defects in haptocorrin binding proteins do not produce abnormalities, although severe cobalamin deficiency can result from defects in the transcobalamin binding proteins.

Causes of folate deficiency

Overview of folate absorption and requirement

The generic term "folate" includes the many chemical forms of the vitamin. Folic acid is the synthetic form used as a food fortificant and vitamin supplement. It consists of a *p*-aminobenzoic acid molecule joined at one end to a pteridine ring and at the other to a single glutamic acid molecule. Most naturally occurring folates in food ("food folates") contain one to six additional glutamate molecules linked through a peptide bond to the γ-carboxyl group of glutamine. To be absorbed, the food folate polyglutamates must be hydrolyzed by conjugase in the brush border. When folic acid is given at high doses, absorption becomes less efficient due to saturation of the intestinal folate transport systems, but smaller amounts of the monoglutamate form are also absorbed by passive diffusion. In general, the bioavailability of food folate is about half that of the crystalline form. Folic acid is also more stable than food folate.

Folate stores in well-nourished adult men are ≈12 to 28 mg, and based on biopsies, the liver contains about half of the total. A substantial amount of folate is secreted in bile, with bile folate flux over five times higher than usual daily intake [85]—most of which is reabsorbed in the small intestine [86]. Renal reabsorption is also efficient and most urinary folate consists of cleavage products. Some fecal folate loss occurs, but some fecal folate derives from intestinal bacteria. Low intake or poor absorption of the vitamin is readily detected as low serum folate, followed by low erythrocyte folate, an increase in plasma homocysteine, and eventually megaloblastic anemia. It has been estimated that, based on tracer studies in humans, plasma and erythrocyte folate pools would fall by 50% in 100 to 130 days after dietary folate is removed, then to 75% of baseline in another 200 to 300 days, although other body pools turn over more slowly [85].

The recommended intake of folate by adults is based on that required to normalize erythrocyte folate, which is similar to the intake needed to maintain serum folate and suppress plasma homocysteine. The RDA increases from 150 to as much as 400 μg/day from age 1 year to 18 years, and is 400 μg/day for adults. WHO/FAO adopted the Institute of Medicine recommendations for folate [1].

Inadequate folate intake

The main dietary sources of folate include green leafy vegetables, legumes, some fruits, and fortified cereals and cereal products **(table 4)**. It is probable that these data underestimate the true dietary folate content, as past extraction or folate digestion procedures were not optimal. A tri-enzyme approach is now recommended for folate analysis in cereal grains, and gives substantially higher results than older methods [87, 88]. There are few data on folate intakes in different locations, but one would anticipate that status is poorer in populations who rely on unfortified wheat or rice as a staple, and consume low amounts of legumes and green leafy vegetables. This may explain why lower serum folate concentrations occur in some industrialized countries, such as Sweden, and in the United States and Chile before fortification. In contrast, 1 cup of cooked beans or 6 maize tortillas each provide about 70% of the RDA for folate for adult women, so the risk of deficiency may be low in populations consuming substantial amounts of maize and beans, such as in many countries in Latin America.

There has been a substantial amount of research on the association between level of dietary folate intake and folate status, mostly in the context of exploring links between folate intake and risk of NTD, and the need for folic acid fortification. A review of existing studies concluded that if usual dietary folate intake is low (≤ 100 μg/day), adding 300 μg/day of folic acid from fortified foods or supplements is adequate to maintain folate status even during pregnancy when folate requirements are substantially higher [1]. Mean plasma homocysteine concentrations in adults stabilize when folate intakes are ~300 μg/day and are not further lowered by intakes > 400 μg/day [89]. When 20 women consuming 281 mg from food were given supplemental doses of 100, 150, 200, and 400 μg/day of folic acid incrementally over 32 weeks, serum folate increased over the range of intake, but the maximum

TABLE 4. Folate content of common foods

Food	Serving size	Folate (μg DFE/serving)
Flour, white, enriched	1 cup	386[a]
Flour, whole wheat	1 cup	53
Corn meal, whole grain	1 cup	30
Rice, white, enriched, cooked	1 cup	180[a]
Rice, wild, cooked	1 cup	43
Spaghetti, enriched, cooked	1 cup	172[a]
Spaghetti, whole wheat, cooked	1 cup	7
Bread, white, 1 slice	0.88 oz	34[a]
Bread, whole wheat, 1 slice	1 oz	14
Tortilla, corn	1 medium (6 inch)	44
Beans, kidney, boiled	1 cup	230
Chickpeas, boiled	1 cup	282
Soybeans, boiled	1 cup	93
Potatoes, white, boiled	1 medium	15
Yam, boiled	1 cup	21
Cassava, raw	1 cup	56
Millet, cooked	1 cup	33
Cow's milk, 2% fat	8 oz	12
Beef, ground 20% fat, broiled	3 oz	8
Liver, beef, fried	3 oz	187
Chicken, light and dark, roast	3 oz	5
Egg, chicken, cooked	1 egg	22
Fish, cod, cooked	3 oz	7
Orange juice	8 oz	60
Mango	1 piece	29
Papaya	1 piece	115
Lettuce, raw	1 cup	31
Mustard green, boiled	1 cup	102
Spinach, boiled	1 cup	263

a. Fortified with folic acid and converted to dietary folate equivalents.

reduction in plasma homocysteine occurred with the 200 μg/day supplement [90]. The Institute of Medicine committee that set recommended folate intakes for the United States and Canada concluded that the risk of NTD increased below a Dietary Folate Equivalent (DFE) intake of 400 μg/day, but no reduction was apparent with higher intakes [1]. Data are lacking at higher intakes, however.

Folate bioavailability

It is generally accepted that the bioavailability of folate is ≈100% for folic acid supplements taken on an empty stomach; 85% for fortificant folic acid consumed in foods (based on absorption from fortified orange juice, as absorption from whole meals has not been assessed) [87]; and 50% from food [1, 91]. Because 85% of folic acid is absorbed when consumed with food, and 50% of food folate is absorbed, folic acid taken with food is 1.7 times more available than food folate. This means that the content of folate in fortified food should be calculated as: Dietary Folate Equivalents = actual μg of food folate + (1.7 × μg of folic acid) [1]. The validity of the 1.7 multiplier was supported by a recent long-term, controlled folate feeding study in women, where food folate bioavailability from a mixed diet was about 60% that of added folic acid [92].

There is little evidence that other nutrients or dietary constituents affect folate bioavailability, including dietary fiber [93], although organic acids in orange juice can reduce the activity of intestinal pteroylglutamate hydrolase, which has a pH optimum of 6 to 7, thus impairing absorption of heptaglutamate [94]. Although a naturally occurring inhibitor of pteroylglutamate hydrolase exists in the skin of some pulses, bean consumption did not reduce the bioavailability of heptaglutamates in humans [95]. In general, the activity of pteroylglutamate hydrolase exceeds that needed to

hydrolyze usual amounts of polyglutamyl folates consumed, and in most [96], although not all [97], studies the bioavailability of monoglutamyl and polyglutamyl folates is similar [96].

Losses during food preparation

Unlike folic acid, food folates are relatively unstable to oxidation and heat, so large losses can occur during food preparation and cooking. Boiling destroys 50% to 80% of the folate in green vegetables [98] and 50% of that in legumes [99]. Chopping and grinding spinach increases folate bioavailability [100], but ascorbic acid in foods improves folate stability [101]. There is inadequate information on the overall impact of these food preparation practices on folate status.

Increased requirements during lactation

Breastmilk folate concentrations are relatively well maintained even when maternal intake and status are poor. Thus lactation, especially if prolonged, may be a cause of folate depletion of some women in developing countries [102, 103]. Iron deficiency significantly reduces milk folate secretion in rats, but it is not clear whether this occurs in human subjects [104].

Alcoholism

Folate deficiency, as evidenced by low serum or erythrocyte folate and elevated homocysteine concentrations, is common in chronic alcoholic patients. In the United States prior to flour fortification with folic acid, and in Europe, folate deficiency occurred in the majority of those consuming > 80 g ethanol per day [105–108]. Alcoholics with liver disease have even poorer folate status than those who do not [107, 109]. In one study, 78% had low serum folate [109], and in another, 33% had a megaloblastic bone marrow [110].

Multiple factors contribute to causing folate deficiency in chronic alcoholism. These include low folate intake [107, 111], poor folate absorption due to impaired transcription of the intestinal folate carrier [112]; reduced liver uptake and storage [111, 113]; and increased urinary excretion [114]. In a micro-pig model, the onset of alcoholic liver disease was much more rapid when the animals were provided with a folate-deficient diet than when folate intake was adequate 115].

Polymorphisms

Several gene polymorphisms affect folate metabolism, as discussed by Finnell et al. elsewhere in this Supplement [116]. Some of these are associated with reduced folate absorption and poorer status, while others are not. A polymorphism (C1561T) in the gene encoding pteroylglutamate hydrolase reduces activity of the enzyme in vitro, but it is present in only about 0.5% of the population. It has been associated with folate status in some studies [117], but not others [118]. The 677C→T polymorphism in methyltetrahydrofolate reductase affects ≅ 10% to 30% of various population groups studied, mostly in industrialized countries, and the homozygote is associated with increased plasma homocysteine and a tendency to lower serum and erythrocyte folate concentrations [119]. In a recent study, only T/T (and not C/C) subjects had a significant inverse correlation between plasma homocysteine and erythrocyte 5-methyl folate concentrations, but the effect of dietary folate restriction on red blood cell folates was not different between the genotypes [120]. Current knowledge on the associations between gene polymorphisms and risk of NTD and other birth defects is reviewed by Finnell et al. elsewhere in this Supplement [116].

Medications

Medications known to impair folate status include methotrexate (a folate antagonist used to treat rheumatoid arthritis and inflammatory bowel disease), anticonvulsants (phenytoin, phenobarbital, and Dilantin), sulfasalazine for treatment of chronic ulcerative colitis, and pyrimethamine for treatment of malaria. Very large doses of nonsteroidal anti-inflammatory drugs (e.g., ≅ 3,900 mg/day) can have antifolate activity, but routine use is not known to impair folate status.

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