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Bioavailability of Vitamin B12

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Abstract: Vitamin B12 deficiency is common in people of all ages who consume a low intake of animal-source foods, including populations in developing countries. It is also prevalent among the elderly, even in wealthier countries, due to their malabsorption of B12 from food. Several methods have been applied to diagnose vitamin B12 malabsorption, including Schilling's test, which is now used rarely, but these do not quantify percent bioavailability. Most of the information on B12 bioavailability from foods was collected 40 to 50 years ago, using radioactive isotopes of cobalt to label the corrinoid ring. The data are sparse, and the level of radioactivity required for *in vivo* labeling of animal tissues can be prohibitive. A newer method under development uses a low dose of radioactivity as ^{14}C-labeled B12, with measurement of the isotope excreted in urine and feces by accelerator mass spectrometry. This test has revealed that the unabsorbed vitamin is degraded in the intestine. The percent bioavailability is inversely proportional to the dose consumed due to saturation of the active absorption process, even within the range of usual intake from foods. This has important implications for the assessment and interpretation of bioavailability values, setting dietary requirements, and interpreting relationships between intake and status of the vitamin.

Key words: vitamin B12, bioavailability, absorption, cobalamin

Introduction

Vitamin B12 deficiency, long thought to be prevalent only in the elderly or strict vegetarians, has now emerged as a common global problem. Some reviews put the prevalence of deficiency and marginal status at 30–80 % in many countries, starting from infancy and affecting both genders throughout the lifespan [1]. Since dietary sources of the vitamin are limited to animal-source foods (ASF) and fortified foods, deficiency is associated with low intakes of these foods. Indeed, even ovo-lacto vegetarians have lower serum B12 concentrations than omnivores [2]. Low ASF intake is common in the majority of poorer countries, due to lack of availability, the cost of ASF relative to income, and sometimes because of cultural or religious beliefs. Older persons, starting at around the age of 60 years, are at high risk of becoming deficient or depleted in the vitamin, probably due to impaired absorption of the vitamin from food [3]. Even in wealthier populations in the United States and Europe, approximately 20 % of elderly are deficient or depleted [4, 5]. The prevalence must be much higher in elderly in developing countries but there have been few studies of the situation.

The adverse health consequences of vitamin B12 deficiency are relatively well documented in the clinical setting. They include impaired neurological and cognitive function, megaloblastic anemia if severe, and biochemical abnormalities including low serum B12 and holotranscobalamin (holoTC), elevated plasma total homocysteine (tHcy), and elevated serum and urinary methylmalonic acid (MMA) [6]. There are likely to be many other health effects including increased risk of neural tube defects [7] and bone loss [8]. Certainly women with very low intakes or status during pregnancy and/or lactation give birth to infants who are at risk of severely delayed develop-

ment, a situation exacerbated by exclusive breastfeeding because of the reduced concentration of the vitamin in breast milk [9]. Much less is known about the adverse effects of the chronically poor vitamin B12 status in populations that consume low intakes throughout life.

The recommended dietary intake for vitamin B12 is around 2.0 to 2.5 ug/day for adults in most countries, which assumes 50 % bioavailability from the diet [10]. The enterohepatic recirculation of the vitamin is efficient in healthy individuals, amounting to approximately 1 μg/day.

Mechanism of absorption

Many factors affect the absorption of this vitamin, as described in detail elsewhere [11]. The initial steps in its digestion include release of the vitamin from its binding to proteins in food through the action of gastric acid and binding of the free B12 to haptocorrin in the stomach. In the upper small intestine the B12 is released and forms a complex with intrinsic factor that is secreted by the stomach. The transport of the vitamin across the epithelial cells of the ileum has become better understood recently [12]. Cobalamin bound to intrinsic factor enters the apical surface of the cell through binding to an intrinsic factor-binding receptor (cubam). Bound to cubam and intrinsic factor, the cobalamin is transported into endosomes in which the receptor is released and recycled to the cell surface. The cobalamin-intrinsic factor complex then enters the cellular lysosomes where the cobalamin is released from intrinsic factor, followed by exit at the basolateral membrane bound to MRP-1 (multidrug resistance protein 1), an energy-requiring process. The free cobalamin is transported on transcobalamin as holotranscobalamin in serum, from which it is taken up by cells throughout the body via surface transcobalamin receptors.

There is some evidence that vitamin B12 absorption in young infants occurs by a different mechanism. In human milk the vitamin is tightly bound to haptocorrin, where it is present at about 100 times the amount in human serum. Release from haptocorrin is always difficult in the normal gastrointestinal environment; haptocorrin is resistant to proteolytic enzymes [13]. In young infants this situation is exacerbated by their low gastric pH, pepsin, and secretion of intrinsic factor. The limited data suggest that the vitamin is absorbed as a B12-haptocorrin complex in the first months of life [14]. The haptocorrin in the intestine may provide the advantage of antimicrobial activity, against Escherichia coli for example [15]. In cow's milk the vitamin is present on transcobalamin, from which absorption may occur more readily, although this has not been studied.

Measurement of bioavailability

Most of the quantitative research on vitamin B12 absorption was conducted some 40 to 50 years ago, and was summarized by Chanarin [16]. The earlier investigators used radioactively labeled vitamin B12, labeling the cobalt molecule in the corrinoid ring with 100 nCi to 1 uCi of ^{57}Co, ^{58}Co, or ^{60}Co. The approach used in most studies was to feed a dose of the labeled B12 and calculate absorption based on recovery of the unabsorbed isotope in feces [17], while in other studies, whole-body retention of an oral dose was measured at a time when the unabsorbed isotope had been excreted in feces [18]. Either approach provides a quantitative estimate of B12 absorption; i. e., percent of the administered dose absorbed.

For many years the qualitative Schilling test [19] was the standard clinical approach for detecting malabsorption of the vitamin. The method involves giving an oral dose of ^{57}Co-labeled vitamin B12 followed by a large intramuscular dose (1 mg) of unlabeled vitamin B12 in order to saturate the tissues with the vitamin and ensure that a measurable amount of isotope will appear in the urine during the first 24 hours. If less than 10 % of the oral dose appeared in urine, this was diagnosed as malabsorption. Most often this test was used to diagnose malabsorption in the elderly, in whom deficiency due to malabsorption becomes more common, thought to be caused by poor gastric acid production and difficulty in releasing the vitamin from the proteins to which it is bound in food. Unfortunately many clinicians did not realize that many elderly, probably most of them, can absorb the free vitamin given in Schilling's test but not the vitamin in food, so that the diagnosis of malabsorption was missed. The correct method would have been to give the oral dose of the vitamin bound to proteins such as albumin, or in egg yolk (in a version of the test known as the egg-yolk cobalamin absorption test or EYCAT), which correctly identified many more elderly as having malabsorption [20]. For several reasons including the use of radioactivity, need to collect a complete urine sample for 24 hours (often difficult in elderly people), cost of the test, and its failure to diagnose malabsorption when conducted incorrectly, Schilling's test has virtually disappeared from standard clinical

practice [21]. Since the test was qualitative in nature it was not useful for deriving percent bioavailability of the vitamin.

Another qualitative test of B12 absorption has been developed recently, namely the C-CobaSorb test [22]. The assay is based on measuring the amount of cyanocobalamin carried on holotranscobalamin (holoTC) in plasma before and after a test dose of the vitamin, administered as cyanocobalamin. HoloTC is the form in which newly absorbed B12 is transported from the intestine to the cells of the body. The method has been described in volunteers consuming three, 9-ug doses of the vitamin over one day's time and measurement of the change in cyanocobalamin-holoTC during the next 24 hours. This test may be useful for detecting malabsorption but does not provide a quantitative estimate of bioavailability.

Published data on vitamin B12 bioavailability from foods

Some of the relatively sparse amount of information available on B12 bioavailability from foods is provided in Table I [17, 18, 23–25]. From this information we can see that the percent absorbed from foods containing large amounts of the vitamin, such as liver, is far less (11 %) than from foods providing smaller amounts (around 20–35 % from fish and eggs, and ≥ 60 % from chicken and mutton). There are no data on bioavailability from red meats other than mutton. Information on bioavailability of the vitamin from food is critical for calculating its dietary requirement. The committee that set the current values for dietary vitamin B12 requirements in the United States and Canada reviewed the available data on percent absorption of the vitamin from various food sources and concluded that 50 % would be a conservative estimate of its bioavailability from food and its enterohepatic circulation in bile, and 60 % from supplements containing less than 5 ug [10]. Bioavailability from foods or supplements high in the vitamin would be lower. FAO-WHO accepted the U.S. Institute of Medicine's (IOM) recommendations for their Recommended Nutrient Intake values.

A few investigators have compared usual intake from specific foods or the whole diet against serum or plasma concentrations of the vitamin, with the idea that this would reveal relative availability of B12 from different sources and the level of intake required to attain adequate or plateau serum B12 concentrations. In a large study of adults (age 26 to 83 years) who did not take B12 supplements and resided in the northeastern United States, within each quintile of vitamin B12 intake plasma B12 concentrations were highest for fortified cereals and dairy products, followed by other foods [26]. The increase in plasma B12 (pmol/L) for each doubling of intake was 24 for fortified cereal, 39 for dairy products, and only 12 for meat, fish, and poultry, suggesting that bioavailability might be lowest from the latter group. Plasma B12 plateaued at an intake of 10 ug per day. Others have noted that a daily intake of 4 to 7 ug from foods is consistent with steady concentrations of indicators of vitamin B12 status in healthy young adults [27]. These observations should be interpreted cautiously because bioavailability from foods rich in the vitamin may have been overestimated, making the intake needed to optimize status appear higher than it would need to be had foods lower in the vitamin been consumed (see below). More studies of this question are needed.

Table I: Selected studies on vitamin B12 bioavailability from foods

Reference	Method	Food	ug B12	Bioavailability (%)
18	^{60}Co, body retention	Mutton	0.9 3.0 5.1	56–77 76–89 40–63
17	^{57}Co, fecal excretion	Chicken	0.6 1.3 1.9	65 63 61
18	^{60}Co, body retention	Liver	38	11
26	^{57}Co, fecal excretion	Eggs	< 2	24–36
24	^{57}Co, fecal excretion	Trout	2.1–13.3	17–25
25	^{57}Co, body retention	Milk	0.25	25

Assessment of bioavailability using ^{14}C-labeled vitamin B12

As described above, older studies that quantified bioavailability of B12 used cobalt isotope-labeling methods. The dose of radioactivity used to measure absorption in humans (100 nCi to 1 uCi) made it necessary to give relatively high doses to animals for *in vivo* labeling of eggs or meat, and limited the application of this method to human studies. Recently a method has been developed for producing the ^{14}C-labeled vitamin by growing *Salmonella enterica* in a medium containing ^{14}C-dimethylbenzimidazole (DMB) [28]. This results in cobalamin that is labeled in the DMB ring, rather than the cobalt in the corrinoid ring. Mass spectrometry revealed that the labeled ^{14}C-B12 is chemically pure. The advantage of using ^{14}C-B12 as the tracer is that the radioactivity can be measured using the highly sensitive method of accelerator mass spectrometry (AMS), so that lower doses (≈30 nCi) can be used in human feeding trials and detected in microliter-volumes of plasma or urine for a longer period of time. We are currently using this form of labeled vitamin B12 to measure its bioavailability from chicken eggs labeled *in vivo* by injecting hens once a day with the labeled vitamin over a four-day period (Garrod *et al.,* unpublished data). Bioavailability is then measured in humans after feeding them a single dose of labeled eggs and collecting urine and feces for the next eight days.

The use of the vitamin labeled in the DMB ring has revealed a previously unknown fact about vitamin B12 metabolism in the gastrointestinal tract, which is that the unabsorbed vitamin is degraded – probably by intestinal bacteria [28]. This became evident from the high percentage of the oral radioactive dose that is excreted in urine – ranging among subjects from about 5 to 60 % (Garrod *et al.,* unpublished data). This is far higher than the < 1 % of dose excreted when ^{57}Co is used as the label, and in fact the low urinary excretion of the cobalt label is the reason why in Schilling's test, a one-milligram dose of the unlabeled vitamin is given to "flush" enough of the label out of tissues into urine so that it can be measured more accurately. Even then, a urinary excretion of 2 % of an oral dose in food is accepted as signifying normal absorption [29]. Preliminary investigation into the nature of the radioactive compound in urine showed it to be associated with polar compounds, and that it is not intact ^{14}C-B12 or ^{14}C-DMB [28]. This indicates that when ^{14}C-labeled B12 is used in bioavailability studies, bioavailability should be calculated as [^{14}C in the dose – (^{14}C in feces + ^{14}C in urine)], since the label in neither feces nor urine is derived from absorbed B12.

The inverse relationship between dose and percent bioavailability

Preliminary results using the ^{14}C method to measure vitamin B12 bioavailability from eggs support the older observations that the percentage of vitamin absorbed depends greatly and inversely on the amount of vitamin given in the oral dose (unpublished data). This is due to saturation of the B12-intrinsic factor receptors in the ileum. The dose-bioavailability relationship, illustrated in Figure 1, suggests that 50 % of a one-microgram dose given in meat or water is absorbed, but less than 10 % of a 10-ug dose and only ≈5 % of a 20-ug dose [16]. This means that the average maximum amount absorbed can only be about 1.5 ug over the range of intake of 5 to 50 ug, and explains why very high oral doses have to be given to ensure that enough B12 is absorbed by people with pernicious

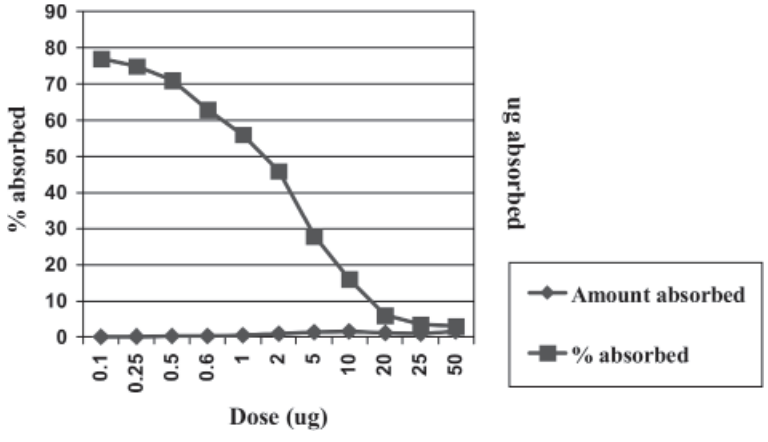

Figure 1: Relationship between dose of vitamin B12 ingested, the percent absorbed and the amount absorbed, in human subjects. Data from Chanarin [16]. Figure reproduced from Reference 1.

anemia (an autoimmune condition in which antibodies to intrinsic factor are produced). Approximately 1 % of a high dose can still be absorbed by passive diffusion, meaning that 5 ug per day can be absorbed from a 500-ug supplement, for example.

There are several implications of the fact that the active transport mechanism is saturated by doses of vitamin B12 within the range of amounts usually consumed in meals. First, it is not possible to assign one value for percent bioavailability from a specific food, unless it is stated in reference to a specific dose e. g. 50 % bioavailability of a one-microgram dose. Second, when comparing intake data to serum concentrations, it is necessary to "correct" for the lower percent absorbed from rich sources of the vitamin such as liver; failure to make this correction may make it appear that dietary requirements to achieve normal or plateau serum vitamin B12 concentrations are higher than they really are. This may partially explain the estimate that intakes need to be 4 to 7 ug/day to achieve stable B12 status biomarkers [22], but further work is needed on this question. Third, it is likely that bioavailability will be higher from foods or supplements ingested several hours apart rather than taken at one time, since a high dose does not impair absorption from a subsequent dose taken about 4 hours later [18].

Opportunities for future research

The further development and validation of the new ^{14}C-B12 absorption test may make it possible to replace the former Schilling's test. Importantly, Schilling's test could only diagnose subjects as having malabsorption or normal absorption of the free or food-bound vitamin, but was not useful for the quantitative assessment of bioavailability. In reality, many elderly and other individuals with gastrointestinal abnormalities are likely to have some degree of impaired B12 absorption but not complete malabsorption; they are at risk of developing deficiency over time that could be prevented by consuming fortified foods or low-dose supplements, but they do not require intramuscular injections. The new method should also be applied to study populations at higher risk of B12 malabsorption due to long-term chronic infection with *Helicobacter pylori* or bacterial overgrowth, such as occurs commonly in developing countries, and to determine whether treatment for these conditions improves subsequent absorption. Finally, there is still doubt about the ability of elderly with gastric atrophy to absorb the vitamin from fortified flour, which in part explains the reluctance of flour fortification programs to include vitamin B12 even in the face of the high global prevalence of this nutrient deficiency. We are currently using the ^{14}C-B12 approach to evaluate this question in individuals with low gastric acid secretion.

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