Conclusions of a WHO Technical Consultation on folate and vitamin B_{12} deficiencies

All participants in the Consultation

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Preamble

Folate and vitamin B_{12} deficiencies occur primarily as a result of insufficient dietary intake or, especially in the case of vitamin B_{12} deficiency in the elderly, poor absorption. Folate is present in high concentrations in legumes, leafy green vegetables, and some fruits, so lower intakes can be expected where the staple diet consists of unfortified wheat, maize, or rice, and when the intake of legumes and folate-rich vegetables and fruits is low. This situation can occur in both wealthy and poorer countries. Animal-source foods are the only natural source of vitamin B_{12}, so deficiency is prevalent when intake of these foods is low due to their high cost, lack of availability, or cultural or religious beliefs. Deficiency is certainly more prevalent in strict vegetarians, but lacto-ovo vegetarians are also at higher risk for inadequate intakes. If the mother is folate-depleted during lactation, breastmilk concentrations of the vitamin are maintained while the mother becomes more depleted. In contrast, vitamin B_{12} concentrations in breastmilk can be markedly lower in vitamin B_{12}-depleted women. The impact of gene polymorphisms on folate and vitamin B_{12} status and requirements in a population will vary depending on the underlying prevalence in that population. Although not well understood, gene polymorphisms almost certainly affect the risk of adverse pregnancy outcomes. Folic acid and vitamin B_{12} in synthetic form are absorbed at about twice the efficiency as the food forms, especially in lower doses.

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The consultation agreed on conclusions in four areas:

» Indicators for assessing the prevalence of folate and vitamin B_{12} deficiencies
» Health consequences of folate and vitamin B_{12} deficiencies
» Approaches to monitoring the effectiveness of interventions
» Strategies to improve intakes of folate and vitamin B_{12}

Indicators for assessing and monitoring vitamin status

Prevalence of deficiencies

The recent review by WHO showed that the majority of data on the prevalence of folate and vitamin B_{12} deficiencies are derived from relatively small, local surveys, but these and national survey data from a few countries suggest that deficiencies of both of these vitamins may be a public health problem that could affect many millions of people throughout the world. Low blood concentrations of the vitamins occur across population groups and in countries in various stages of development. Inclusion of folate and vitamin B_{12} status assessment in more representative national surveys is recommended, with standardization of the methods used to measure blood vitamin concentrations, and the application of universal cutoffs such as those proposed in this Consultation.

Inadequate intake of folate and vitamin B_{12} leads to low serum or plasma concentrations of both vitamins, and elevated plasma homocysteine. In addition, inadequate folate intake causes low red blood cell folate, and elevated urinary or serum methylmalonic acid (MMA) occurs in vitamin B_{12} deficiency. Serum holotranscobalamin II is a more recently proposed additional measure of vitamin B_{12} status. Thus, several feasible and reasonably good indicators of status are available for both vitamins.

Biochemical and hematological indicators of status

Serum/plasma or erythrocyte concentrations of folate and serum/plasma concentrations of vitamin B_{12} provide an accurate picture of a population's status. The most practical and least expensive measurements at the population level are serum or plasma folate and vitamin B_{12}.

MMA and homocysteine assays have limitations in that they are more expensive, and the results can be affected by poor renal function. Furthermore, interpretation of homocysteine concentrations requires knowledge of both folate and vitamin B_{12} status as well as that of vitamin B_6 and riboflavin, since deficiencies of any of these vitamins can contribute to elevated plasma homocysteine.

Anemia due to folate and vitamin B_{12} deficiency is relatively uncommon worldwide, but can best be detected by elevated mean corpuscular volume (MCV) in a complete blood count (CBC). It is important to note that iron deficiency tends to lower MCV more readily than it is raised by folate or vitamin B_{12} deficiency, so high MCV may be a more sensitive measure of deficiency of these vitamins in populations where iron deficiency is less prevalent.

Cutoff values that indicate deficiency

The Consultation recognized the lack of universally accepted cutoffs to define deficiency of either vitamin, and expressed the need for consensus on this issue. There are several reasons for this situation.

Folate and vitamin B_{12} concentrations can differ depending on the method used for assessment, including various radioimmunoassays and microbiological assays. Values frequently differ most in the lower range of concentrations where it is critical to have accurate and precise values. The microbiological assay is inexpensive but may be difficult to perfect initially. There is a need for international reference materials and more interaction and communication among laboratories regarding these analyses so that population prevalences of deficiency can be correctly determined and compared. The serum or plasma vitamin B_{12} cutoff used to diagnose vitamin B_{12} deficiency has gradually increased over time, because initially it was based on detection of neurological consequences of deficiency, then later on other indicators including elevated plasma homocysteine and MMA. However, the public health significance of the impact of applying these cutoffs is not fully understood, and requires further study.

Appropriate cutoffs for pregnancy are uncertain, since serum/plasma concentrations of both vitamins decline over the course of pregnancy and recover at delivery.

Despite these caveats, the Consultation arrived at a consensus on the cutoffs that should be used at this time for assessing the nutritional status of populations. They are derived from the US NHANES III (National Health and Nutrition Examination Survey) [1], based on the plasma vitamin concentrations below which plasma metabolites become elevated (total homocysteine concentration for folate and MMA for vitamin B_{12}). In addition, these cutoffs are consistent with data in the Institute of Medicine report [2] on recommended intakes of folate and vitamin B_{12}, in which blood vitamin concentrations were used to determine Estimated Average Requirements (EAR).

The concentrations suggested for defining folate and vitamin B_{12} deficiencies based on metabolic indicators are:

> < 10 nmol/L (4 ng/mL)* for serum folate and
> < 340 nmol/L (151 ng/mL) for RBC folate.

< 150 pmol/L (203 pg/mL)** for plasma vitamin B_{12}.

Health consequences of folate and vitamin B_{12} deficiencies

Folate deficiency

Hematology

There is strong evidence that folate deficiency causes megaloblastic anemia. In regions where folate deficiency is more common during pregnancy and lactation, a few studies have associated megaloblastic anemia with prolonged lactation and multiple pregnancies.

Pregnancy outcomes

There is strong evidence of an inverse association between blood folate concentrations and risk of low birthweight. Folic acid supplementation increased birthweight in studies in Africa and India. This is an important issue for developing countries.

There is some evidence of an inverse association between blood folate concentrations during pregnancy and the risks of placental abruption and delivering preterm or small-for-gestational-age infants.

Birth defects

There is strong evidence of a causal association between low maternal folate intake or lower folate status and increased risk of neural tube defects (NTD)—and for a protective effect of folic acid supplementation or consumption of fortified foods, in the periconceptional period, against NTD. This association has been confirmed in two randomized clinical trials, in large-scale supplementation trials in China, and from postfortification data available from countries such as

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* Conversion factor used for folic acid: 2.265 [2].
** Conversion factor used for vitamin B_{12}: 0.737 [2].

Canada, Chile, and the United States.

There is moderately strong evidence of an association between the MTHFR 677C→T gene variant, which affects folate metabolism, and increased risk of NTD. However, it is probable that there are other genotypes that are at special risk for adverse birth outcomes, yet which may respond positively to folic acid supplementation.

Some evidence exists to support an association between folate status and the risk of other birth defects such as orofacial clefts and heart defects.

Cognitive function

Evidence for an association between serum/plasma folate concentrations and cognitive function is weaker in children than in adults. However, some studies have reported lower scores in schoolchildren with low folate status [3].

In adults, there is moderate evidence that low folate status is associated with cognitive impairment and some measures of dementia, but evidence for a beneficial effect of folic acid supplementation on cognitive performance or dementia is inconsistent. In addition, there is moderately convincing evidence that low serum or red blood cell folate concentrations are associated with either a higher prevalence or a longer duration of depression.

Vitamin B_{12} deficiency

Hematology

There is little evidence that widespread vitamin B_{12} deficiency at the population level increases the prevalence of anemia, either in the elderly or in people consuming low amounts of animal-source foods. In contrast, megaloblastic anemia is common when vitamin B_{12} deficiency is more severe, such as in clinical cases of pernicious anemia (defined as vitamin B_{12} deficiency due to malabsorption caused by absence of intrinsic factor) and in some infants exclusively breastfed by mothers who are strict vegetarians.

Pregnancy outcomes

There is inadequate information on the relationship between poor maternal vitamin B_{12} status and adverse pregnancy outcomes. The observed association between vitamin B_{12} deficiency and recurrent abortions may only apply to women with pernicious anemia.

There is a moderate level of evidence that maternal vitamin B_{12} status correlates with neonatal vitamin B_{12} status, and strong evidence that maternal B_{12} status affects concentrations of the vitamin in breastmilk.

Birth defects

There is moderately convincing evidence that low maternal vitamin B_{12} status is associated with an increased risk of NTD.

Evidence for an association between maternal vitamin B_{12} status and other birth defects, such as orofacial clefts and heart defects, is weaker than the evidence for an association of these defects with maternal folate status.

Child development

The importance of adequate vitamin B_{12} status for child development is clear. Children who suffer developmental delays as a result of severe vitamin B_{12} deficiency tend not to fully recover. In severe deficiency, as seen in children who are raised under macrobiotic conditions,* all developmental milestones are delayed.

School-aged children with poor vitamin B_{12} status had lower scores at school and lower cognitive performance in the two studies on this question.

Neuropathy and sustained spinal cord degeneration

There is strong evidence that severe vitamin B_{12} deficiency causes neuropathy in cases of pernicious anemia, and in medical conditions such as gastrectomy and ileal resections. The clinical sequelae that lead to neurological damage in pernicious anemia are rarely seen in cases of dietary B_{12} deficiency, except in young infants (particularly those exclusively breastfed by mothers who are strict vegetarians), nor are the serum concentrations of the vitamin B_{12} as low as are seen in patients with pernicious anemia.

Cognitive function

Severe vitamin B_{12} deficiencies cause memory and cognitive impairment at all ages.

There is weak evidence of an association between low blood vitamin B_{12} and cognitive function, although inconsistent results among studies may be due to problems with assessment. Although studies to date have failed to show consistent positive effects of oral vitamin B_{12} supplementation on cognitive function, there is some evidence of improvement in status after vitamin B_{12} injections in both deficient adults and elderly.

Homocysteine

There is strong evidence that plasma homocysteine is a predictor of folate and vitamin B_{12} status.

There are strong associations between elevated plasma homocysteine and adverse pregnancy outcomes such as preeclampsia, placental abruption, preterm delivery, and low birthweight, but there is uncertainty about the direction of effect. For example, the relation-

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* Typically a macrobiotic diet consists of grain cereals (mainly rice), vegetables, pulses (mainly soy), and sea vegetables, and only very small amounts of fruits and products of animal origin [4].

ship between higher risk of preeclampsia and elevated homocysteine is not understood, and may or may not be explained by poor folate or vitamin B_{12} status.

Elevated plasma homocysteine was correlated with cognitive decline and cerebral atrophy in several studies, and predicted the subsequent development of dementia in cognitively intact middle-aged and elderly individuals. Controlling for folate and vitamin B_{12} status in analysis of such trials may not be appropriate, since poor folate and vitamin B_{12} status may be acting through elevated plasma homocysteine. Evidence is still needed on the specific benefits of lowering plasma homocysteine by folic acid and vitamin B_{12} interventions in these conditions.

There is moderately convincing evidence of an association between elevated plasma homocysteine and such conditions as cardiovascular disease, cerebrovascular disease, cognitive decline, and bone dysfunction. However, intervention trials to date have not had sufficient power to demonstrate clear effects of reducing homocysteine on cardiovascular disease overall, although there may be an effect on stroke. More definitive results will require meta-analysis of the data from current and future trials.

Recommendations for monitoring the efficacy and effectiveness of intervention programs

Establishing baseline data

At a minimum, on a random cohort of samples of the population groups of interest, appropriately stratified by age, socioeconomic status, and other relevant factors, usual dietary intakes of vitamin B_{12} and folate and their sources, and prevalence of inadequate intakes of vitamin B_{12} and folate (i.e., intake below the EAR) should be assessed using methods described elsewhere [5]. This is essential for predicting the potential impact of fortifying specific food vehicles, and the impact of the intervention on intake.

Similarly, on a random cohort of blood samples collected from the population groups of interest, stratified by age, status, and other relevant factors, the prevalence of deficiency should be assessed by measuring folate in serum/plasma (or red blood cells) and vitamin B_{12} concentrations in serum/plasma.

If possible, a complete blood count (CBC) should be performed on the baseline samples for measurement of mean corpuscular volume (MCV), the most sensitive hematological indicator of vitamin B_{12} and folate deficiencies.

Metabolic markers of folate and vitamin B_{12} deficiency, homocysteine, and MMA, could be measured, if feasible, at least on a representative subset of the population.

Baseline data should be collected on the prevalence of NTD. The methods used to collect this information must be documented, since the methods will affect the estimated prevalence values and the ability to compare them across populations.

Monitoring

Monitoring the impact of interventions

After a predetermined period of at least 6 months after the start of the intervention and preferably annually thereafter, intake and status assessment need to be repeated using the same indicators and methods as at baseline. This will provide information on change in prevalence of deficiency and status over time, and reflect whether changes in intake are stable over time.

A change in the highest quintile of MCV and the lowest quintile of folate or vitamin B_{12} can indicate prevalence of deficiency at baseline in populations with a low prevalence of iron deficiency, and evaluate response to intervention.

Monitoring the safety of interventions

Vitamin B_{12} intake is assumed to be safe at any level, but monitoring plasma levels of cyanocobalamin and levels of nonfunctional analogs of vitamin B_{12} in those who have very high plasma vitamin B_{12} levels from supplements, especially children, may be useful when cyanocobalamin intake is high. Homocysteine and MMA are not useful for detecting excessive intakes.

While stimulation of cancer growth, suppression of killer cell activity, reversal of antiepileptic drug effect, and unpredictable epigenetic and embryo rescue outcomes have been proposed as possible adverse effects of excessive folic acid exposure, none of these effects have reached a level of certainty so as to be recommended for surveillance, except for cancer.

Measurement of unmetabolized folic acid concentrations in serum was proposed as a way of detecting whether folic acid is being consumed in excess of requirements, but so far this has not been used to monitor safety in any survey.

Monitoring of interventions to control folate and/or vitamin B_{12} deficiencies should include measurements of folic acid and vitamin B_{12} intake to make sure that their consumption does not exceed the safe upper level.

The higher frequency of food-bound vitamin B_{12} absorption in the elderly will limit the ability of food sources to improve their status. Persons with less severe deficiency are more likely to have normal vitamin B_{12} absorption from food and supplements.

Strategies to improve folate and vitamin B$_{12}$ intakes

General introduction

The overarching and long-term strategy recommended for the control of folate and B$_{12}$ deficiency is the consumption of a diet that meets the recommended intakes of these vitamins. However, in populations where it is unlikely that diet will provide recommended intakes of these nutrients, strategies such as supplementation and fortification should be considered.

For safety reasons, when deciding the best way of delivering additional folic acid and/or vitamin B$_{12}$—either by supplementation or by fortification—the main criterion to be taken into consideration is the need to target the population group to be reached.

Furthermore, programs to increase the consumption of folate among women of reproductive age to 400 µg/day may benefit countries where the NTD rate has been determined to be higher than 0.6/1,000 live births based on surveillance methods that can detect most NTD cases. Such programs should collect data on both folate and vitamin B$_{12}$ status, and monitor NTD rates during the selected interventions.

These conclusions do not consider the potential benefits of lowering homocysteine through folate or vitamin B$_{12}$.

Currently available approaches

Diet

The best strategy to improve folate and vitamin B$_{12}$ intakes is to promote the consumption of foods rich in folate and vitamin B$_{12}$. Advocacy programs will facilitate identification of local food sources of the vitamins and promote their use. However, where foods rich in folate and vitamin B$_{12}$ are not affordable or available, supplementation and/or fortification may be considered.

Supplementation

Before selecting a supplementation strategy, it is recommended that clear goals and target populations be set, e.g., reduction in the prevalence of folate and/or vitamin B$_{12}$ deficiency, NTD rates, megaloblastic anemia, and adverse pregnancy outcomes. In situations where other ongoing supplementation programs exist, the incorporation of folic acid and vitamin B$_{12}$ in the supplements can avoid duplication of costs and efforts.

Criteria for considering supplementation programs should include the existence of other supplementation programs, an effective health system, and a strong public education component.

The usual recommendation for supplemental folic acid is 0.4 mg/day. The ideal amount of supplemental vitamin B$_{12}$ is less clear. Although the recommended daily intake is 2.4 µg/day, many elderly persons appear to require supplement doses many times higher to achieve optimum vitamin B$_{12}$ status, probably due to limited absorption. The supplements can be given daily, or as a larger dose less frequently, e.g., 0.4 mg folic acid daily versus 5 mg once weekly. They can be provided as tablets or in powdered form with other micronutrients. The intervention must cover a large proportion of the targeted population to assure a significant public health impact. To be successful, supplementation interventions require sustained and strong education programs for health providers and the general public, social marketing, social mobilization, and advocacy efforts.

The advantages of a supplementation program are the ability to target at-risk population groups, cost-effectiveness, and the possibility of its being nested into an already existing program. On the other hand, such programs require additional costs for promotion, education, and advocacy, and their success relies on a well-functioning health care system to deliver the supplements. Furthermore, the sustainability of supplementation programs may be dependent on the ability of the target population to pay for the supplements, which may not be possible in some populations, in which case their cost must be defrayed by local or international institutions. The greatest limitations of supplementation programs may be lack of compliance and unacceptability of the supplements due to cultural factors, if motivation programs are ineffective or not sustained. Prior to embarking on a supplementation program, it is important to document through appropriate social marketing studies that the proposed motivation programs are effective in the community of interest.

Food fortification

The criteria for fortification strategies are 1) a high prevalence of inadequate folate and/or vitamin B$_{12}$ intakes; 2) evidence of deficiency; 3) a high burden of deficiency-related disease such as NTD; 4) absence of circumstances that would make targeted supplementation likely to be successful; and 5) a widely available and highly consumed food vehicle that can be produced centrally. In many countries there are ongoing food-fortification programs to which folic acid and vitamin B$_{12}$ may be added, or their content adjusted to become adequate for the intended purpose. However, the effective amount needed for vitamin B$_{12}$ has not been satisfactorily defined, because availability may be affected by poor absorption, especially among the elderly. Furthermore, food-fortification programs are passive interventions that are cost-effective, self-sustaining, and do not require extensive social marketing and promotion. However, there is no assurance that all target groups will receive adequate amounts of the fortificants. Where food fortification is universal, which may be the case in industrialized countries, there may be concern about the benefits and safety of

fortified food, as unfortified foods are not likely to be available.

The level of folic acid or vitamin B_{12} that should be added to food can be calculated based on the prevalence of inadequate intakes in a sample of the target population(s). Details of how to formulate and regulate fortified foods have been established by WHO/FAO [5]. Some elderly may need more vitamin B_{12} than is supplied by fortification of staples, but most can absorb the synthetic form of the vitamin.

Specific issues

Prevention of NTD

Programs to increase the consumption of folate among women of reproductive age to 400 µg/day may benefit countries where the NTD rate is higher than 0.6/1,000 live births. To reduce the risk of NTD for women capable of becoming pregnant, the recommendation is to take 400 µg of folic acid daily from fortified foods, supplements, or both, in addition to consuming food folate from a varied diet beginning at least 1 month prior to conception.

The decision to choose between supplementation and fortification to deliver additional folic acid should be driven by the need to target women at risk for becoming pregnant. With that in mind, the main criteria to meet when considering a supplementation program are a high rate (80%) of planned pregnancies, easy access to the health care system, a strong public health infrastructure capable of sustained promotional campaigns, and availability of social marketing interventions known to be effective in the targeted area. Where the conditions to implement folic acid supplementation are not met, targeted fortification should be considered.

Vitamin B_{12} deficiencies

Population groups who consume low amounts of animal-source foods, not just strict vegetarians, are at high risk for vitamin B_{12} deficiency. Subgroups at special risk for deficiency are pregnant or lactating women, infants, and young children with low vitamin B_{12} intake. The elderly often have difficulty in absorbing vitamin B_{12} from animal-source foods. There is evidence that most elderly can absorb synthetic vitamin B_{12} in supplements and in foods, but those with more severe malabsorption may require higher amounts.

Folic acid supplements or fortification programs can mask the megaloblastic anemia of severe B_{12} deficiency such that the condition is not detected through hematological changes, and neurological deterioration can progress. Providing vitamin B_{12} as well as folic acid can help to reduce this problem. However, pernicious anemia is a medical condition and should be treated as such, and will not be responsive to the low vitamin B_{12} doses in supplements or most fortification programs.

There is some evidence that there is a potential harmful effect of high folate intakes in people with low vitamin B_{12} status.

Research needs

Causes and contributing factors

» Examine potential role of bacterial overgrowth, tropical sprue-like conditions, and possibly *Helicobacter pylori* as a cause of vitamin B_{12} deficiency.
» Determine health/disease status of subjects voluntarily consuming large amounts of oral folic acid or vitamin B_{12} supplements.

Assessment and monitoring of interventions (efficacy/effectiveness and safety)

» Further define the validity of chosen cutoffs adding new status indicators as they are developed.
» Determine and test criteria that define safe levels of folic acid intake, including avoidance of exceeding safe upper levels, and focusing attention on upper deciles for folate and vitamin B_{12} status.
» Develop indicators of adverse functional consequences of folate and vitamin B_{12} deficiencies.
» Study folic acid accumulation and its consequences in adults and children with high levels of folic acid after fortification or supplementation.
» Study accumulation of cyanocobalamin and nonfunctional cobalamin analogs and their consequences in adults and children with high cobalamin levels after fortification or supplementation.
» Assess effects of genetic polymorphisms on cutoff points for folate and vitamin B_{12} sufficiency and deficiency.
» Develop new analytic tools to fully explore gene–nutrient and gene–gene interactions.
» Establish objective criteria to assess early neurological impairment caused by vitamin B_{12} deficiency (e.g., neurophysiological measurements).

Health consequences

» Examine relationships between folate status and pregnancy outcomes, including birthweight, placental abruption, prematurity, and small-for-gestational-age infants in both developed and developing countries.
» Examine relationships between folate status and risk of birth defects other than NTD, such as orofacial clefts and heart defects.
» Assess effects of folate and vitamin B_{12} interventions

on risk of birth defects other than NTD.

» Assess persistent effects of folate and vitamin B_{12} interventions during gestation on late-onset disease risk.

» Examine folate and vitamin B_{12} metabolism and/or status in relation to malaria, HIV, and hemolytic anemia.

» Examine the association between folate deficiency and risk of megaloblastic anemia in pregnancy and lactation, especially with prolonged lactation and multiple pregnancies in both developed and developing countries.

» Examine possible risk of infection related to high vitamin B_{12} level (e.g., vitamin B_{12}, which may enhance microbial growth).

» Determine the link between low folate and "mood" or depression, in terms of prevalence or duration.

» Determine whether poor folate and/or vitamin B_{12} status increases the risk of preeclampsia via hyperhomocysteinemia.

» Assess the effect of folate and vitamin B_{12} status on gene expression and cancer risk.

» Determine the link between low folate and/or vitamin B_{12} status and cognitive impairment at all ages.

» Examine interference of increased folic acid intake on the efficacy of therapeutic drugs (e.g., antifolate antimalarials, anticonvulsants, methotrexate).

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