Neglected tropical diseases and vitamin B₁₂: a review of the current evidence

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Vitamin B₁₂ deficiency is an urgent public health problem that disproportionately affects individuals in low- and middle-income settings, where the burden of neglected tropical diseases (NTDs) is also unacceptably high. Emerging evidence supports a potential role of micronutrients in modulating the risk and severity of NTDs. However, the role of vitamin B₁₂ in NTD pathogenesis is unknown. This systematic review was conducted to evaluate the evidence on the role of vitamin B₁₂ in the etiology of NTDs. Ten studies were included in this review: one study using an *in vitro*/animal model, eight observational human studies and one ancillary analysis conducted within an intervention trial. Most research to date has focused on vitamin B₁₂ status and helminthic infections. One study examined the effects of vitamin B₁₂ interventions in NTDs in animal and *in vitro* models. Few prospective studies have been conducted to date to examine the role of vitamin B₁₂ in NTDs. The limited literature in this area constrains our ability to make specific recommendations. Larger prospective human studies are needed to elucidate the role of vitamin B₁₂ in NTD risk and severity in order to inform interventions in at-risk populations.

Keywords: cobalamin, infection, neglected tropical diseases, nutrition, vitamin B₁₂

Introduction

Neglected tropical diseases (NTDs)

NTDs are a group of 17 infectious diseases that affect more than 2.7 billion people globally and are endemic in more than 149 countries.^{1,2} Neglected tropical diseases (NTDs) include viral, bacterial, protozoan and helminthic infections. Chronic infections from NTDs can result in extensive morbidity and disability,³ reduced quality of life, work productivity, education attainment and social well-being^{4–6} and may increase the severity and risk of death from coinfections.⁵

NTDs disproportionately affect the world's poorest populations in endemic tropical countries. In Africa, NTDs account for approximately 73% of the total burden of disease and 71% of deaths on the continent.⁷ In Latin America and the Caribbean, 8.8% of the population is affected by at least one NTD.⁵ Women and young children are at the highest risk of infection due to greater exposure, poor sanitation and barriers to accessing treatment.

Nutrition and immunity

The vicious cycle of malnutrition and infection was first noted more than 50 years ago, with malnutrition as both a risk factor and consequence of infection. Hookworm infection and schistosomiasis are well-established culprits in iron-deficiency anemia

due to gastrointestinal blood loss and micronutrient supplements are frequently given in combination with deworming treatment.^{8,9}

Micronutrient deficiencies, including vitamins and minerals, influence host innate and adaptive immune responses to infections, including macrophage, lymphocyte and metabolic functions^{10,11} and the risk and severity of infectious diseases.¹² Vitamins such as A, D, E, folate and vitamin B₁₂ confer protection against oxidative stress, enhance immune cell proliferation and differentiation, regulate epithelial integrity and improve antigen presentation.^{12–24} For example, vitamin A deficiency impairs both humoral and cell-mediated immunity and increased susceptibility to infectious diseases.¹² Minerals such as zinc, iron and copper are also critical for the host immune system.^{20–25} For example, zinc is involved in the regulation of innate and adaptive immune responses and influences lymphocyte maturation, cytokine production and the generation of free radicals while maintaining normal macrophage and natural killer cell activity.²⁶ Emerging evidence supports a role of micronutrient deficiencies in the risk and severity of NTDs,²⁷ although the effects of micronutrient supplementation in NTDs have not been established.

Vitamin B₁₂

Vitamin B₁₂ deficiency (<148.0 pmol/L) is a major public health problem worldwide.²⁸ Inadequate vitamin B₁₂ status leads to

reduced DNA synthesis, genomic hypomethylation and chromosomal aberrations.²⁹ In addition, vitamin B₁₂ deficiency during pregnancy and early childhood has been associated with an increased risk of adverse pregnancy outcomes³⁰ and impaired psychomotor and cognitive development.^{31,32}

The classic cause of vitamin B₁₂ deficiency is pernicious anemia, an autoimmune disease that destroys parietal cells, which are required for intrinsic factor (IF)-mediated vitamin B₁₂ absorption.³³ In settings where consumption of animal products is inadequate or intestinal infections are common, the prevalence of vitamin B₁₂ deficiency is high.³⁴ The prevalence of vitamin B₁₂ deficiency have been reported to be as high as 40% in Latin America,³⁵ 70% in Africa³⁶ and 70 to 80% in South Asia.^{37,38} Regions with a high prevalence of vitamin B₁₂ deficiency are also the regions with the greatest NTD burden.

In the absence of sufficient vitamin B₁₂, impaired DNA synthesis can lead to bone marrow aplasia and consequent cytopenia, including lymphopenia.^{39,40} Although there are limited data on vitamin B₁₂ and immunity, some studies have demonstrated associations between vitamin B₁₂ status and leukocyte cell counts. Studies from Japan reported lower CD8 T-cell counts among patients with pernicious anemia compared with healthy controls and that patients with vitamin B₁₂ deficient anemia had improvements in CD8 T-cell and natural killer cell counts after administration of methyl-cobalamin injections.^{19,41} In another cohort study from Turkey, patients with pernicious anemia given daily intramuscular vitamin B₁₂ injections had significantly increased total leukocyte counts, CD8 T-cell counts, natural killer cell activity and immunoglobulins.⁴² The restoration of immune cells by vitamin B₁₂ interventions may be due to the role of vitamin B₁₂ in DNA synthesis, although the mechanisms have not been identified.

Despite these immune cell associations, specific mechanisms of vitamin B₁₂ in the host immune response to infection by NTDs have not been elucidated. Given the role of vitamin B₁₂ in immune cell cytotogenesis, host vitamin B₁₂ status may impact the risk, severity and clinical presentation of NTDs.

The high coprevalence of vitamin B₁₂ deficiency and NTDs in low- and middle-income settings and the increasing evidence that micronutrients may play a role in influencing the risk and severity of NTDs have stimulated interest in vitamin B₁₂ research. Further, vitamin B₁₂ supplementation represents a potential adjunct for NTD prevention and treatment. However, there is a lack of data on the efficacy of vitamin B₁₂ interventions in alleviating the risk and severity of NTDs. No reviews to date have been conducted to examine the role of vitamin B₁₂ in NTDs. A review is warranted to summarize existing data on vitamin B₁₂ and NTDs

to inform screening and targeted interventions for prevention and treatment.

The objective of this review was to examine the evidence on the role of vitamin B₁₂ in NTDs. Specifically, we hypothesize that vitamin B₁₂ deficiency increases the risk and severity of NTD infection and that existing NTD infections can lead to vitamin B₁₂ deficiency. We examine evidence from animal and *in vitro* studies, observational studies and intervention trials. We then discuss research gaps and implications for the development of interventions and public health approaches to reduce the risk and severity of NTDs, with emphasis on low- and middle-income settings.

Methods

Search strategy and selection process

We conducted a structured literature search using the MEDLINE electronic database. Relevant Medical Subject Heading (MeSH) terms were used to identify published studies through 27 September 2015. The search strategy and MeSH terms used are provided in Table 1 and the findings are summarized in Figure 1.

The initial inclusion criteria for abstracts in this review were the inclusion of data on vitamin B₁₂ status or intake and inclusion of data on at least one of the 17 NTDs identified by the World Health Organization.⁴³ The following biomarkers of vitamin B₁₂ status were included: serum and plasma vitamin B₁₂ concentrations, holotranscobalamin (holoTC) and methylmalonic acid (MMA). The included NTDs were protozoan (i.e., Chagas disease, human African trypanosomiasis [sleeping sickness], leishmaniasis), bacterial (i.e., Buruli ulcer, leprosy [Hansen's disease], trachoma, endemic treponematoses [yaws]), helminthic or metazoan worm (i.e., cysticercosis/taeniasis, dracunculiasis [guinea-worm disease], echinococcosis, foodborne trematodiasis, lymphatic filariasis, onchocerciasis [river blindness], schistosomiasis, soil-transmitted helminthiasis) and viral (i.e., dengue/chikungunya, rabies).

The available abstracts of all studies were searched, full-text articles were extracted and reviewed and the following inclusion criteria were applied: availability of data on vitamin B₁₂ intake or status and at least one of 17 NTDs, and reported association between vitamin B₁₂ and an NTD-related outcome. *In vitro* studies, animal studies, observational cross-sectional studies, case-control studies, cohort studies, randomized trials, interventions, quasi-randomized trials and uncontrolled trials were included. Sources were retrieved, collated, indexed and assessed for

Table 1. Search strategy: Medical Subject Heading (MeSH) terms

(Neglected diseases[MeSH] OR parasitic diseases[MeSH] OR protozoan diseases[tw] OR dengue[tw] OR A aegypti[tw] OR rabies[tw] OR lyssavirus[tw] OR echinococcosis[tw] OR hydatid*[tw] OR Chagas*[tw] OR trypanosoma*[tw] OR trypanosomiasis [tw] OR sleeping sickness [tw] OR HAT[tw] OR leishmani*[tw] OR trematode infections[mesh] OR trematod*[tw] OR filariasis[tw] OR onchocerciasis[tw] OR schistosom*[tw] OR helminth* [tw] OR taenia*[tw] OR cysticercosis[tw] OR tapeworm[tw] OR ascaris*[tw] OR whipworm[tw] OR hookworm [tw] OR buruli ulcer[tw] OR mycobacterium infections[MeSH] OR Leprosy[tw] OR Hansen*[tw] OR trachoma[tw] OR egyptian ophthalmia[tw] OR yaws[tw] OR bejel[tw] OR treponema*[tw]) AND (b-12[all fields] OR B12[tw] OR vitamin B12[MeSH] OR Vitamin B 12 Deficiency[MeSH] OR cobalamin*[tw] OR transcobalmin*[tw] OR transcobalamins[MeSH] OR methylmalon*[tw])

Figure 1. Search strategy. A diagrammatic representation of the retrieval strategy used for identifying and selecting studies for inclusion in the final analysis.

vitamin B₁₂ and NTD outcome data. An additional search was conducted to find review articles, which were examined to cross-reference other relevant studies. Standardized data tables were created and used to extract and summarize key information from observational and experimental studies. As part of this protocol, publication date, author names, study design, setting, target population, methods, definitions of exposures and outcomes, main findings and study limitations were recorded. For each individual outcome we assessed the quality of the evidence, risk of bias, heterogeneity, precision of effect estimates and potential and residual confounding.

Results

The structured literature search yielded 516 articles that were reviewed for potential inclusion in this review. After 479 articles were excluded (missing abstracts, $n=263$; reviews or meta-analyses, $n=31$; case reports, $n=27$; missing data on NTDs, $n=85$; missing data on vitamin B₁₂, $n=20$; laboratory methods, $n=1$; missing data on vitamin B₁₂, NTDs, or the association between vitamin B₁₂ and NTDs, $n=157$), 37 full-text articles were extracted for further review. After excluding 27 articles that did not meet the aforementioned inclusion criteria (non-English language, $n=1$; laboratory methods, $n=1$; missing data on vitamin B₁₂, $n=3$; missing data on NTDs, $n=9$; no data on the associations between vitamin B₁₂ and NTDs, $n=13$), 10 studies

were included in this review. These included one *in vitro*/animal study, eight observational human studies (six cross-sectional, one case-control and one cohort study) and one ancillary analysis conducted within an intervention trial. NTDs that were examined in association with vitamin B₁₂ included helminths (hookworm [$n=4$], roundworm [$n=1$], trematode [$n=1$] and multiple soil-transmitted helminths [$n=1$]), Chagas disease ($n=1$) and leprosy ($n=2$). The structured literature search is summarized in Figure 1 and findings from these studies are summarized in detail in Tables 2–4.

Helminths

Helminths are intestinal parasitic worms, whipworms and hookworms. Helminthic infections affect more than 1 billion people worldwide (7.8% of the global population is infected with hookworms, 14.5% with *Ascaris lumbricoides* and 8.3% with *Trichuris trichiura*).⁵⁴ Pregnant women, children and elderly populations are at higher risk of helminthic infections.⁵⁵ Helminthic infections have been associated with malnutrition due to micronutrient malabsorption, blood loss and inflammation.^{3,55–57}

Six observational studies identified in this review examined the role of vitamin B₁₂ in helminth infections in humans. Study populations were based in regions with high helminth infection rates: South Asia (Thailand, India and Bangladesh), Latin America (Panama and Cuba) and Oceania (Papua New Guinea).

Table 2. Evidence of vitamin B₁₂ and NTDs from observational human studies

Reference	Study design	Sample (N)	Methods	Exposure	Outcome	Main findings
Shield et al. ⁴⁴ ; Eastern Highland Province, Papua New Guinea	Baseline data from intervention	367 incarcerated individuals	Blood and fecal samples collected; height, weight, anemia status, iron status, and liver/spleen size measured	Time of incarceration; diet: iron (13.1 mg/d), energy (2407 cal/d), protein (75.6 g/d), folate (160.41 mg/d) and vitamin B ₁₂ (12.9 mg/d)	Serum vitamin B ₁₂ ; helminthic infection (<i>N. americanus</i> , <i>A. lumbricoides</i> , <i>T. trichiura</i>); egg count in stool	No significant association was found between hookworm egg count and serum vitamin B ₁₂ status
Núñez Fernandez et al. ⁴⁵ ; Havana, Cuba	Case-control	55 children: group 1, 15 high parasitemia cases; group 2, 20 low parasitemia cases; group 3, 20 controls	Stool samples taken from 2063 people to identify cases and controls; fasting venous blood drawn from selected cases and controls	<i>T. trichiura</i> infection determined by egg count in stool using the Kato-Katz method; high parasitemia >10 000 eggs/g feces, low parasitemia <5000 eggs/g feces	Plasma vitamin B ₁₂	Mean vitamin B ₁₂ concentrations were not significantly different between the high-infection group, low-infection group and control group (232.6±85.6 pmol/L vs 261.0±90.0 vs 247.8±89.9; p>0.05)
Lindstrom et al. ⁴⁶ ; Matlab, Bangladesh	Baseline data from a randomized clinical trial	740 pregnant women (body mass index <18.5 kg/m ²)	Pregnancy determined by urine test and confirmed by ultrasound; anthropometric, socioeconomic and physical examination data; pregnancy history; food security scores and stool samples collected at 8 weeks gestation; venous blood collected at 14 weeks gestation	<i>Ascaris</i> (67%) and <i>Trichuris</i> infection (43%); determined from stool samples	Plasma vitamin B ₁₂ : deficiency <150 pmol/L; anemia: hemoglobin <110 g/L	<i>Ascaris</i> infection was associated with a higher prevalence of vitamin B ₁₂ deficiency (p>0.05). Women with <i>Ascaris</i> infection had significantly higher odds of vitamin B ₁₂ deficiency (OR 1.49 [95% CI 1.06 to 2.09], p<0.05); 28% of anemic women were also vitamin B ₁₂ deficient
Osei et al. ⁴⁷ ; Tehri Garhwal District, India	Cross-sectional	499 school children (6–10 y of age)	Primary caretakers of children recorded age, sociodemographic data and morbidity for diarrhea, fever, cough, runny nose and vomiting; weight and height measured; nonfasting whole blood drawn and stool samples collected (n=437)	Helminthic infection (<i>A. lumbricoides</i> , hookworm, <i>T. trichiura</i> , <i>T. saginata</i>); determined by microscopic analysis of eggs in stool	Serum vitamin B ₁₂ : low <300 pmol/L, moderate deficiency 150–300 pmol/L, severe deficiency <150 pmol/L	20.2% of children had helminthic infection: 9.4% <i>A. lumbricoides</i> , 7% hookworm, 1.6% <i>T. trichiura</i> and 1.6% <i>T. saginata</i> ; 17.4% of children had low vitamin B ₁₂ concentrations; mean vitamin B ₁₂ concentrations were not significantly different between infected and noninfected individuals with any type of helminth (p>0.05)

Continued

Table 2. Continued

Reference	Study design	Sample (N)	Methods	Exposure	Outcome	Main findings
Scatliff et al. ⁴⁸ ; Bocas del Toro Province, Panama	Cohort	209 children (1–5 y of age)	Demographic, health, anthropometric and infection status data collected at baseline by questionnaire; dietary intake measured at baseline and 3 and 5 months by Food Frequency Questionnaire; blood collected at 5 months in a subset of participants (n=65)	Dietary vitamin B ₁₂ intake: adequate intake (defined by the WHO): 1–3 y old ≥ 0.9 $\mu\text{g/day}$, 4–6 y old ≥ 1.2 $\mu\text{g/day}$; ascariasis: eggs/g feces; diarrhea: episodes/month	Vitamin B ₁₂ status: serum vitamin B ₁₂ : deficient <150 pmol/L, marginal 150–221 pmol/L, adequate >221 pmol/L	<i>Ascaris</i> infection intensity (eggs/g of feces) did not significantly differ between adequate and inadequate vitamin B ₁₂ intake groups (23 265 $\pm$ 3955 eggs/g of feces vs 24 349 $\pm$ 6247; $p>0.05$); children with adequate dietary intake of vitamin B ₁₂ had significantly more episodes of diarrhea per month (1.8 ± 0.2 vs 1.4 ± 0.3 ; $p=0.04$) compared with children with inadequate dietary intake
Jaroonsesama et al. ⁴⁹ ; Ayutthaya Province, Thailand	Cohort	10 patients with <i>F. buski</i> infection	Pretreatment: blood was collected, carbohydrate absorption tested by D-xylose tolerance test, protein absorption tested by a protein-loss test, vitamin B ₁₂ absorption tested by a modified Schilling test and a jejunal biopsy taken for histologic analysis; tetrachloroethylene given to patients for <i>F. buski</i> infection; stool samples collected before and after treatment	<i>F. buski</i> infection: Stoll's egg count in stool; tetrachloroethylene treatment (4 mL)	Pretreatment: serum vitamin B ₁₂ status, reference 200–1000 pg/mL; vitamin B ₁₂ absorption, Schilling test, normal absorption $\geq 50\%$. Posttreatment: <i>F. buski</i> egg count in stool	80% (n=8) of participants had vitamin B ₁₂ absorption below the referent range; serum vitamin B ₁₂ was below the referent range in 6 of 9 participants; 1 of 8 participants had histologic evidence of damaged jejunal villi
Karat and Rao ⁵⁰ ; Tamil Nadu Province, India	Cross-sectional	904 leprosy patients	Skin smears obtained by Wade slit-skin technique; bone marrow films taken and stained with Leishman's stain and Ziehl–Neelsen stain; peripheral blood collected and a single fresh stool sample was collected	Leprosy status: presence of <i>M. leprae</i> on skin smears; leprosy stage: determined by bacterial index using the Ridley Scale (lepomatous, tuberculoid, borderline, indeterminate, duration of leprosy infection, treatment for leprosy); hookworm status: egg presence in fecal samples	Serum vitamin B ₁₂ concentrations; changes in bone marrow cell morphology: grade I (minimal changes)–grade IV (severe megaloblastic changes)	Lepromatous group had significantly higher mean serum vitamin B ₁₂ compared with other clinical groups (i.e., tuberculoid, borderline and indeterminate groups) ($p<0.05$); among males, the mean serum vitamin B ₁₂ was lower in the lepomatous group (236.4 $\mu\text{g}/100$ mL [SE 7.6]), tuberculoid group (226.9 $\mu\text{g}/100$ mL [SE 13.2]), borderline group (208.1 $\mu\text{g}/100$ mL [SE 16.0]) and indeterminate group (250.6 $\mu\text{g}/100$ mL [SE 24.5])

Continued

Table 2. Continued

Reference	Study design	Sample (N)	Methods	Exposure	Outcome	Main findings
Karat and Rao ⁵¹ ; Tamil Nadu Province, India	Cross-sectional	321 adult males with lepromatous leprosy	Methods described above	Leprosy status: presence of <i>M. leprae</i> on skin smears, leprosy bacterial index (negative, 0.01–0.05, 0.6–1.0, 1.1–2.0, >2.0), leprosy infection duration, leprosy treatment duration (short treatment <1 y, long treatment >1 y)	Serum vitamin B ₁₂ concentrations	<p>compared with the general population (260.9 µg/100 mL [SE 9.7]); among females, serum vitamin B₁₂ was lower in the tuberculoid group (228.5 µg/100 mL [SE 15.9]), borderline group (250.0 µg/100 mL [SE 30.4]) and indeterminate group (167.8 µg/100 mL [SE 13.7]) compared with general population (274.0 µg/100 mL [SE 11.2]); among males, the tuberculoid and borderline groups had significantly higher megaloblastic changes in bone marrow ($p<0.05$); 18% of male and 16.6% female cases had megaloblastic bone marrow</p> <p>Higher bacterial index was associated with an increase in serum vitamin B₁₂ ($p<0.05$); mean serum vitamin B₁₂ concentrations were higher among late disease patients compared with early disease patients (226.8 µg/100 mL [SE 22.4] vs 209.8 [SE 15.21]; $p<0.05$); mean serum vitamin B₁₂ concentrations were significantly lower in the long-term treatment group compared with late disease patients (200.8 µg/100 mL [SE 11.26] vs (226.8 [SE 22.4]; $p<0.05$))</p>

Helminths investigated in these observational studies included *Necator americanus*, *A. lumbricoides*, *T. trichiura*, *Fasciolopsis buski*, *Taenia saginata* and *Enterobius vermicularis*. Most of the observational studies found that vitamin B₁₂ status was not associated with a significantly increased risk or severity of helminth infection. A quasi-randomized trial was conducted in Papua New Guinea to investigate the effect of iron supplementation and anthelmintic drugs on hematologic status in male inmates at a correctional institution. Prior to intervention, an ancillary analysis was conducted at baseline to examine the impact of incarceration time and prison diet (containing 12.9 mg/day vitamin B₁₂) on the prevalence of helminthic infections. The study revealed no significant associations between serum vitamin B₁₂ concentrations and hookworm egg counts.⁴⁴ Similarly a case-control study in Cuba investigating the nutritional consequences of *T. trichiura* infection in at-risk children reported that vitamin B₁₂ concentrations did not significantly differ between patients with *T. trichiura* infection and uninfected patients (232.6 vs 261.0 pmol/L; $p>0.05$).⁴⁵ A cross-sectional study of school-age children from India was conducted to assess the burden of vitamin deficiencies; no statistically significant differences in mean vitamin B₁₂ concentrations between individuals with any type of helminth (*A. lumbricoides*, hookworm, *T. trichiura*, *T. saginata*; $p>0.05$) infection and uninfected individuals were found.⁴⁷ A cohort study of children (1–5 y) from Panama was conducted to identify predictors of serum vitamin B₁₂ concentrations and vitamin B₁₂ intake, including *A. lumbricoides* infection.⁴⁸ Although an increased frequency of diarrheal episodes was associated with lower vitamin B₁₂ concentrations, *A. lumbricoides* infection intensity (eggs/g stool) was not significantly associated with serum vitamin B₁₂ levels.

Two observational analyses reported the prevalence of impaired vitamin B₁₂ status and absorption among individuals with helminthic infections. A cross-sectional analysis was conducted at baseline prior to intervention in the Maternal and Infant Nutrition Interventions in Matlab (MINIMat) randomized trial among pregnant women in rural Bangladesh. At baseline, *Ascaris* infection (i.e., stool samples positive for *Ascaris* parasites) was associated with vitamin B₁₂ deficiency (<150.0 pmol/L; $p<0.05$). Furthermore, in women, *A. lumbricoides* infection was associated with significantly higher odds of vitamin B₁₂ deficiency (<150.0 pmol/L) compared with women without *Ascaris* infection (odds ratio [OR] 1.49 [95% confidence interval {CI} 1.06 to 2.09], $p<0.05$) after adjusting for socioeconomic status and food security.⁴⁶ In a cross-sectional study of patients with *F. buski* in Thailand, the effect of *F. buski* infection on intestinal absorption disorders was assessed. Although no specific associations were determined, 80% ($n=8/10$) of patients infected with *F. buski* had vitamin B₁₂ absorption levels below the reference range of absorption ($\geq 50\%$) as determined by a modified Schilling test.⁴⁹ Six of the nine participants had low serum vitamin B₁₂ concentrations (<200.0 pg/mL). One patient had histologic evidence of damaged jejunal villi, suggesting intestinal damage as a potential mechanism for impaired vitamin B₁₂ absorption in *F. buski* infection.⁴⁹

An intervention was conducted in Spain to examine the effects of different anthelmintic treatments on clinical outcomes in 86 children with *Giardia lamblia*, *Cryptosporidium parvum* or *E. vermicularis*. In ancillary analyses of data, investigators examined

changes in plasma vitamin B₁₂ concentrations before and after treatment.⁵² The mean serum vitamin B₁₂ concentrations significantly increased after 3 months of antiparasitic treatment (630.57 ± 200.97 vs 667.97 ± 181.55 pg/mL; $p=0.002$).⁵²

Other NTDs

Three studies reported associations between vitamin B₁₂ and other NTDs, including Chagas disease ($n=1$) and leprosy ($n=2$). Chagas disease is caused by the protozoan *Trypanosoma cruzi*. Currently 18 million individuals in Latin America alone suffer from Chagas disease.⁵⁸ Leprosy is caused by the bacterium *Mycobacterium leprae*, with approximately 250 000 new cases each year.⁵⁹

In an animal study from Buenos Aires, five groups of mice (five mice per group) were infected with *T. cruzi* and the number of parasites found in circulation (parasites/mL blood) was measured every 2 d after infection.⁵³ Mice were randomized to the following treatment groups: vitamin B₁₂; vitamin B₁₂ and ascorbic acid; benznidazole (Bnz); vitamin B₁₂, ascorbic acid and Bnz; and control (0.1 M phosphate buffer solution). Treatments were administered daily by intraperitoneal injection from days 5 to 9 and days 12 to 16 after infection. At the peak of parasitemia for the control group (i.e., day 13), the concentrations of circulating parasites were significantly lower in all intervention groups compared with controls (group 1: $2.23 \pm 0.28 \times 10^6$ parasites/mL, group 2: $1.26 \pm 0.17 \times 10^6$ parasites/mL, group 3: $1.43 \pm 0.12 \times 10^6$ parasites/mL and group 4: $1.33 \pm 0.25 \times 10^6$ parasites/mL vs the control group: $4.18 \pm 0.02 \times 10^6$ parasites/mL; $p<0.01$ for each group vs control group). Additionally, the group receiving vitamin B₁₂, ascorbic acid and Bnz (group 4) had a significantly higher survival rate after 100 d compared with the control group (83.3% vs 0%; $p<0.05$).⁵³ No other significant differences were noted for the vitamin B₁₂-only group compared with the control group.

In the same study,⁵³ *in vitro* experiments using *T. cruzi* epimastigotes from 3-d cultures, bloodstream *T. cruzi* trypomastigotes and *T. cruzi* amastigotes cultured in a Roswell Park Memorial Institute medium without a phenol red murine macrophage cell line were treated with different doses of cyanocobalamin to determine the effects of vitamin B₁₂ on parasite growth inhibition compared with Bnz treatment. Growth inhibition was measured as the change in cell density (cell count or light absorbance) from pretreatment to posttreatment. With increasing vitamin B₁₂ concentrations, the *T. cruzi* epimastigote growth rate decreased. The half maximal inhibitory concentration (IC₅₀) values for trypomastigotes and epimastigotes were 2.6- to 4-fold higher (9.46 ± 1.2 vs 30.26 ± 2.85) and 1.7- to 3.6-fold higher (2.42 ± 0.54 vs 5.86 ± 0.93) for vitamin B₁₂ compared with Bnz.

In a cross-sectional study in India, Karat and Rao⁵⁰ characterized the hematological status of leprosy patients ($n=904$), categorized into four groups based on clinical presentation: lepromatous, tuberculoid, borderline and indeterminate. The mean serum vitamin B₁₂ concentrations were significantly higher in leprosy patients in the lepromatous group compared with patients in the other clinical groups (i.e., tuberculoid, borderline and indeterminate groups) ($p<0.05$). In men, serum vitamin B₁₂ concentrations were lower in patients in the lepromatous group

Table 3. Evidence of vitamin B₁₂ and NTDs from intervention studies

Reference	Sample size (n)	Methods	Treatment	Outcome	Main findings
Olivares et al. ⁵² ; Aragon, Spain	86 children: group 1: 26 <i>Giardia lamblia</i> infections; group 2: 40 <i>Enterobius vermicularis</i> infections; group 3: 20 <i>Cryptosporidium parvum</i> infections	Stool samples collected and stained to identify <i>G. lamblia</i> , <i>C. parvum</i> , <i>E. vermicularis</i> at baseline and 2–3 weeks posttreatment; groups with <i>G. lamblia</i> and <i>E. vermicularis</i> received treatment for infection; blood collected at baseline and 3 months posttreatment when patients were asymptomatic	Group 1: tinidazole (50 mg/kg/day, 2 doses for 2 weeks) and metronidazole (25 mg/kg/day for 7 days if first treatment failed); group 2: no treatment; group 3: pyrantel pamoate (10 mg/kg/day, 2 doses over 2 weeks)	Serum vitamin B ₁₂ <200 ng/mL	There were no significant differences in the three groups for vitamin B ₁₂ status at baseline or posttreatment ($p>0.05$); mean vitamin B ₁₂ concentrations significantly increased among individuals with <i>E. vermicularis</i> at baseline vs posttreatment (615.95±193.10 pg/mL vs 665.00±186.54; $p=0.004$); mean vitamin B ₁₂ significantly increased in all groups from baseline to posttreatment (630.57±200.97 pg/mL vs 667.97±181.55 pg/mL)

(236.4 pg/100 mL [standard error {SE} 7.6]), tuberculoid group (226.9 pg/100 mL [SE 13.2]), borderline group (208.1 pg/100 mL [SE 16.0]) and indeterminate group (250.6 pg/100 mL [SE 24.5]) compared with the general population (260.9 pg/100 mL [SE 9.7]). In women, serum vitamin B₁₂ concentrations were lower in the patients in the tuberculoid group (228.5 pg/100 mL [SE 15.9]), borderline group (250.0 pg/100 mL [SE 30.4]) and indeterminate group (167.8 pg/100 mL [SE 13.7]) compared with the general population (274.0 pg/100 mL [SE 11.2]), although vitamin B₁₂ concentrations in the lepromatous group (279.0 pg/100 mL [SE 19.1]) were similar to the general population (274.0 pg/100 mL [SE 11.2]).

In the following year, additional analyses of the previous cross-sectional population-based survey were conducted among individuals with lepromatous leprosy ($n=321$).⁵¹ Investigators examined the associations between the bacterial load (i.e., bacterial index) and serum vitamin B₁₂ concentrations (pg/100 mL). A higher leprosy bacterial load was significantly associated with higher serum vitamin B₁₂ concentrations ($p<0.05$). Mean serum vitamin B₁₂ concentrations were also higher among patients with late disease (>1 y) compared with early disease (<1 y; 226.8 pg/100 mL [SE 22.4] vs 209.8 [SE 15.21]; $p<0.05$). Mean serum vitamin B₁₂ concentrations were significantly lower in individuals undergoing long-term treatment (i.e., with a specific antileprosy drug administered for >1 y) compared with patients with late disease (>1 y; 200.8 pg/100 mL [SE 11.26] vs 226.8 [SE 22.4], $p<0.05$).

Discussion

There is limited evidence to date on the relationship between vitamin B₁₂ status and NTD infection. In this review, 8 of the 10 included studies provided support for a potential role of vitamin B₁₂ in the occurrence or severity of NTD infections, including helminths, *T. cruzi* and leprosy. Two human population studies demonstrated that individuals with an NTD infection had lower vitamin B₁₂ status or impaired vitamin B₁₂ absorption compared with uninfected individuals, and two studies demonstrated that serum vitamin B₁₂ concentrations increased after infection treatment. In contrast, one observational study noted higher vitamin B₁₂ concentrations in individuals with an NTD infection. Overall, there is limited evidence of the effects of vitamin B₁₂ on NTD pathogenesis. Only one experimental study has been conducted to date, using animal and cell models, to examine the effects of vitamin B₁₂ supplementation on NTD severity and no randomized trials have been conducted in at-risk human populations.

Research on vitamin B₁₂ and NTDs to date has primarily focused on helminthic infections. Cross-sectional and observational cohort studies have demonstrated that helminthic infections are associated with lower vitamin B₁₂ concentrations, even after adjusting for potential confounders, including socioeconomic status and food security,⁴⁶ and intestinal damage due to helminthic infection impaired vitamin B₁₂ absorption.⁴⁹

Table 4. Evidence of vitamin B₁₂ and NTDs from animal and cell culture studies

Reference	Methods	Treatment	Outcome	Main findings
Ciccarelli et al. ⁵³ ; Buenos Aires, Argentina	Animal: 6- to 8-week-old mice were infected with 5×10 ³ bloodstream <i>T. cruzi</i> trypomastigotes intraperitoneally. Mice were administered treatment intraperitoneally on days 5–9 and days 12–16 postinfection. Parasitemia measured every 2 days using a Neubauer chamber. <i>In vitro</i> : Anti- <i>T. cruzi</i> epimastigote activity: <i>T. cruzi</i> epimastigotes inoculated in fresh medium to reach a concentration of (1.5×10 ⁷ –2.5×10 ⁷ cells/mL). were cultured with vitamin B ₁₂ or Bnz for 3 days and cell count measured with a Neubauer chamber. Anti- <i>T. cruzi</i> trypomastigote activity: <i>T. cruzi</i> trypomastigotes (1.5×10 ⁶ trypomastigotes/mL) were seeded on a microplate with vitamin B ₁₂ or Bnz. Cells counted after 24 h. Amastigote growth inhibition: Murine macrophages (J774 cell line), at 5×10 ³ /100 µL RPMI medium, were infected with trypomastigotes expressing β-galactosidase at a parasite:cell ratio of 10:1. After 1 d of coculture, plates were washed. At 7 days postinfection, galactosidase activity was measured. Cytotoxicity assay: Vero cells (9×10 ⁵ cells/mL) seeded to 24-well plate. After 48 h, vitamin B ₁₂ and/or Bnz was added and incubated for 1 d. PBS/MTT solution added to plates and trypan blue precipitate produced and absorbance measured.	Animal: group 1: vitamin B ₁₂ (1.5 mg/kg of body weight/day), group 2: vitamin B ₁₂ and ascorbic acid (1.5 mg/kg/day), group 3: Bnz (0.75 mg/kg/day), group 4: Bnz +ascorbic acid+vitamin B ₁₂ , group 5: controls. <i>In vitro</i> : anti- <i>T. cruzi</i> epimastigotes: group 1: vitamin B ₁₂ 0.125–15 µM, group 2: Bnz 0.75–25 µM. Anti- <i>T. cruzi</i> trypomastigotes activity: group 1: vitamin B ₁₂ 0.37–72 µM, group 2: Bnz 0.38–38 µM. Amastigote growth inhibition: vitamin B ₁₂ or Bnz drugs. Cytotoxicity assay: group 1: vitamin B ₁₂ (6–2400 µM), group 2: Bnz (3–3000 µM). Intracellular oxidative activity: 15, 30 and 60 µM of vitamin B ₁₂ at 3, 7 or 24 h of treatment	Animal: parasitemia levels determined by Neubauer count. <i>In vitro</i> : Anti- <i>T. cruzi</i> epimastigote activity; cellular density (CD): cells/culture measured by Neubauer count; % inhibition: CD day 3–CD day 0. Anti- <i>T. cruzi</i> trypomastigote activity: % lysed parasites. Amastigote growth inhibition: % inhibition: 100–(absorbance of treated cells–absorbance of untreated cells). Cytotoxicity: selectivity index (SI): 50% cytotoxicity concentration on Vero cells/IC50 of compound for <i>T. cruzi</i> cells. Intracellular oxidative activity: flow cytometry expressed ratio Gmt:Gmc (treated:untreated cells)	Animal: All treatment groups: decrease in circulating parasites compared with control group (group 1: 2.23±0.28×10 ⁶ parasites/mL, group 2: 1.26±0.17×10 ⁶ parasites/mL, group 3: 1.43±0.12×10 ⁶ parasites/mL and group 4: 1.33±0.25×10 ⁶ parasites/mL vs controls (1.33±0.25×10 ⁶ parasites/mL; p<0.05 for all group vs control comparisons). Group 4 vs controls had a significantly higher survival rate after 100 days (83.3% vs 0%; p<0.05). <i>In vitro</i> : anti- <i>T. cruzi</i> epimastigotes: vitamin B ₁₂ at 0.45 µM concentration caused a decrease in parasitemia from day 3 onward. Anti- <i>T. cruzi</i> growth inhibition: vitamin B ₁₂ IC50s compared with Bnz treatment for trypomastigotes and epimastigotes were 2.6–4 times greater (9.46±1.2 vs 30.26±2.85) and 1.7–3.6 times greater (2.42±0.54 vs 5.86±0.93); no difference was reported for amastigotes. Cytotoxicity assay: vitamin B ₁₂ had no cytotoxic effect SIs for vitamin B ₁₂ on epimastigotes, trypomastigotes, and amastigotes were >991.7, >253.7, and >224.5 µM, respectively.

Vitamin B₁₂ concentrations also improved after anthelmintic treatment, suggesting indirect evidence of the impact of helminthic infections on host vitamin B₁₂ status.⁵²

Lower circulating vitamin B₁₂ concentrations may be associated with helminth infections due to malabsorption. Evidence from a breadth of studies has established the link between helminth infections and micronutrient deficiencies resulting from malabsorption due to intestinal obstruction and damage to the mucosal lining, which likely compromises absorption of vitamin B₁₂.^{60,61} Vitamin B₁₂ can only be absorbed in the ileum when bound to intrinsic factor.⁶² Malabsorption of vitamin B₁₂ may occur if there is destruction of the receptor of intrinsic factor-bound vitamin B₁₂, cubulin, such as in chronic intestinal infections, or impaired production of intrinsic factor that occurs with disrupted intestinal pH. Lower gastric pH due to *Helicobacter pylori* infection causes atrophy of the gastric glands, which also produce intrinsic factor necessary for vitamin B₁₂ absorption.⁶³ Helminthic infections may also decrease the absorption of vitamin B₁₂ due to hyporexia and increased gastric motility from diarrhea, two common symptoms of intestinal helminths.^{60,61}

Vitamin B₁₂ deficiency may predispose an individual to helminth infections by disrupting gastrointestinal epithelial growth. Given the role of vitamin B₁₂ in DNA synthesis and methylation,⁶⁴ insufficient vitamin B₁₂ status may diminish the host's ability to regenerate gastrointestinal epithelial cells, which require high rates of turnover in the body.⁶⁵ Gut epithelial cells play a critical role in protection against pathogens (e.g., helminths), providing a barrier to entry as part of the host innate immunity, and through production of mucous and immunoglobulin A.⁶⁶ Additionally, gastrointestinal epithelial tuft cells promote a type 2 T helper cell immune response to pathogens, such as helminths.⁶⁷

Other mechanisms may explain the potential links between vitamin B₁₂ and NTDs, though direct evidence is limited. Parasites utilize the host's energy and micronutrients for survival and reproduction. For example, *Plasmodium falciparum*, the malaria-causing protozoan, utilizes host folate and is often associated with a high prevalence of folate deficiencies in malaria-endemic areas.^{68,69} The potential requirements of NTD agents for vitamin B₁₂ are not known.

Vitamin B₁₂ status may increase the likelihood of an NTD infection by affecting host immunity and cell susceptibility to oxidative damage and inflammation. In one-carbon metabolism, vitamin B₁₂ is required as a cofactor for methionine synthase to remethylate homocysteine to methionine. Insufficient vitamin B₁₂ results in elevated homocysteine concentrations,^{30,70} which have been associated with oxidative stress and increased release of inflammatory cytokines.⁷¹ Moreover, the one-carbon cycle is essential for nucleotide synthesis and DNA formation; both processes are critical when high rates of cell synthesis are needed, such as in childhood development and in response to infection.⁷⁰ As such, inadequate vitamin B₁₂ status may impair rapid immune cell proliferation of monocytes, natural killer cells or B and T cells normally required for innate and adaptive immune response to infection.

Research gaps and future directions

This review has several limitations that constrain the interpretation of findings, including the limited number and design of the

studies, small number of participants, lack of intervention studies and appropriate control groups and overall low quality of evidence. Several of the research studies were conducted among high-risk populations, including incarcerated individuals,⁴⁴ pregnant women⁴⁶ and young children,^{45,47,48} which may be subject to selection bias and limits the generalizability of findings.

To date, most studies on NTDs and vitamin B₁₂ have been observational in nature and were limited by inadequate adjustment for potential confounders, including concurrent infections, socioeconomic status and general malnutrition. In resource-limited settings, vitamin B₁₂ deficiency is often concomitant with inadequate consumption of animal-source foods, protein intake and other micronutrient deficiencies, such as folate and iron.⁷² These nutritional deficiencies are also associated with impaired immune response, cell growth and epithelial integrity and an increased risk or severity of NTD infections. Several poverty-related factors are potential confounders of the association between vitamin B₁₂ deficiency and increased risk of NTDs. For example, lower income or socioeconomic status may be associated with both malnutrition (e.g., inadequate vitamin B₁₂ intake or bioavailability) and poorer sanitation and living conditions (i.e., toilets, potable water, fecal contamination, open defecation), which are associated with increased exposure to NTDs. Although some of the observational studies included in this review adjusted for potential confounders in multivariate analyses, residual confounding of the associations between vitamin B₁₂ and NTDs by these covariates may lead to biased results and constrain the interpretation of findings.

Few rigorous prospective observational studies or randomized clinical trials have been conducted to date in which the primary objective was to examine the role of vitamin B₁₂ in the etiology of NTDs. Most of the studies included in this review were cross-sectional in design and assessed the prevalence of vitamin B₁₂ status and NTD infection at a single time point. This constrains the ability to establish temporal associations between vitamin B₁₂ and NTD infections and infer causality. The limited temporal assessment also constrains our ability to examine changes in vitamin B₁₂ status during the course of infection to determine if or when nutritional supplementation may be beneficial.

No randomized clinical trials have been conducted to date to determine the effects of vitamin B₁₂ on the risk and severity of NTDs. Only one study has been conducted to examine the effects of vitamin B₁₂ interventions in Chagas disease, but this study was conducted in animal and cell models. This study included vitamin B₁₂ in combination with Bnz and/or other micronutrients, and similar effects were not observed with vitamin B₁₂ supplementation alone. Further experimental studies in cell and animal models are required to determine the effects of vitamin B₁₂ on NTD risk and morbidity. In particular, future studies should include more detailed biochemical analysis, including measurement of inflammatory markers, to evaluate the effect of vitamin B₁₂ on host inflammation in the context of infection.

The studies included in this review all assessed total serum or plasma vitamin B₁₂ levels as the only biomarker of vitamin B₁₂ status and few studies used standardized cut-offs for vitamin B₁₂ deficiency or insufficiency. Total vitamin B₁₂ is a circulating biomarker of vitamin B₁₂ status that does not reflect the metabolic uptake of vitamin B₁₂. Studies that measure both

circulating and functional vitamin B₁₂ biomarkers, such as HoloTC and MMA,^{30,73–76} would improve the assessment of vitamin B₁₂ status.^{30,73–76} Additionally, although research to date suggests that helminthic infections impair vitamin B₁₂ absorption, measurements of small intestine epithelial integrity and circulating and functional vitamin B₁₂ biomarkers at multiple time points are needed to elucidate the directions of associations, how these temporal associations vary over time and potential mechanisms.

Conclusions

The lack of sufficient literature on the role of vitamin B₁₂ in NTDs provides limited evidence to inform specific recommendations. Although findings from some experimental animal and observational human studies suggest that there may be a potential benefit for vitamin B₁₂ on the risk and severity of some NTDs, the limited number and design of studies, lack of intervention studies and overall low quality of evidence constrain the interpretation of findings. Future prospective studies are needed to establish the role of vitamin B₁₂ in the etiology of NTDs and potential clinical significance, including how vitamin B₁₂ status and NTD-related morbidities change over the course of infection. Laboratory studies in animal and cell models are needed to elucidate the underlying mechanisms linking vitamin B₁₂ status and NTD infections. Further research is needed to inform the development of appropriate interventions for NTD prevention and control.

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