Published in final edited form as:
*J Nutr*. 2009 November ; 139(11): 2119–2123. doi:10.3945/jn.109.107359.

# Increases in Plasma Holotranscobalamin Can Be Used to Assess Vitamin B-12 Absorption in Individuals with Low Plasma Vitamin B-12[1,2,3]

**Dattatray S. Bhat**[4], **Nileema V. Thuse**[4], **Himangi G. Lubree**[4], **Charudatta V. Joglekar**[4], **Sadanand S. Naik**[4], **Lalita V. Ramdas**[4], **Carole Johnston**[4], **Helga Refsum**[5,6], **Caroline H. Fall**[7], and **Chittaranjan S. Yajnik**[4,*]

[4]Kamalnayan Bajaj Diabetology Research Centre, King Edward Memorial Hospital Research Centre, Pune, 411011, India

[5]Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, OQ13QX, UK

[6]Institute of Basic Medical Sciences, Department of Nutrition, University of Oslo, Oslo, 0316, Norway

[7]MRC Epidemiology Resource Centre, University of Southampton, Southampton, So16 6YD, UK

## Abstract

Low plasma concentrations of vitamin B-12 are common in Indians, possibly due to low dietary intakes of animal-source foods. Weather malabosrption of the vitamin contributes to this has not been investigated. A rise in plasma holotranscobalamin (holo-TC) concentration after a standard dose of oral vitamin B-12 has been proposed as a measure of gastrointestinal absorption in people with normal plasma vitamin B-12 concentrations. We studied 313 individuals (children and parents, 109 families) in the Pune Maternal Nutrition Study. They received 3 doses of 10 μg (n=191) or 2 μg (n=122) of cyanocobalamin at 6 h intervals. A rise in plasma holo-TC of ≥15% and >15 pmol/L above baseline was considered normal vitamin B-12 absorption. The baseline plasma vitamin B-12 was <150 pmol/L in 48% of participants; holo-TC was <35 pmol/L in 98%, and total homocysteine was high in 50% (>10 μmol/L in children and >15 μmol/L in adults). In 10 μg group plasma holo-TC concentration increased by 4.8 -fold from (mean ± SD) 9.3 ± 7.0 pmol/L to 53.8 ± 25.9 pmol/L, and in 2 μg group by 2.2 -fold from 11.1 ± 8.5 pmol/L to 35.7 ± 19.3 pmol/L. Only 10% of participants, mostly fathers, had an increase less than the suggested cut-points. Our results suggest that an increase in plasma holo-TC may be used to assess vitamin B-12 absorption in individuals with low vitamin B-12 status. Because malabsorption is unlikely to be a major reason for the low plasma vitamin B-12 concentrations in this population, increasing dietary vitamin B-12 should improve their status.

### Keywords

Vitamin B-12; cyanocobalamin; holotranscobalamin; absorption; India

[1]Study is supported by: Wellcome Trust, London, U.K. (Grant Number: 079877/Z/06/Z)

[2]Authors Disclosures: D. S. Bhat, N. V. Thuse, H. G. Lubree, C. V. Joglekar, S. S. Naik, L. V. Ramdas, C. Johnston, H. Refsum, C. H. Fall, and C. S. Yajnik, no conflicts of interest.

[3]Supplimental figure 1 is available with the online posting of this paper jn.nutrition.org.

[*]To whom correspondence should be addressed: Diabetes Unit, KEM Hospital, Rasta peth, Pune, India 411011. Tele/fax no. +91-20-26111958. diabetes@vsnl.com.


Europe PMC Funders Author Manuscripts

## Introduction

Low vitamin B-12 concentrations are common in Indians (1) and contribute to hyperhomocysteinemia despite normal folate status (2, 3). This is unlike the situation in Europeans (4), where low folate status is the predominant determinant of hyperhomocysteinemia. We have demonstrated an association between low maternal vitamin B-12 concentrations in pregnancy and insulin resistance (5) and reduced neurocognitive performance in the children (6). Vitamin B-12 deficiency could also contribute to the etiology of neural tube defects in Indians (7). Prevention of these conditions in India may require supplementation with vitamin B-12.

Low vitamin B-12 status among Indians is usually attributed to low dietary intake due to a mainly vegetarian diet (1, 8, 9) but it is not uncommon even in those who eat non-vegetarian foods (9). In our studies, even though vegetarian diet was a significant predictor of low vitamin B-12 status, one half to two thirds of participants who eat non-vegetarian food more than 3 d/wk also had low plasma vitamin B-12 concentrations (C. S. Yajnik, D. S. Bhat, H. G. Lubree, C. V. Jojalekar, unpublished data). We also found an inverse association of plasma vitamin B-12 concentrations with levels of education, income and hygiene, suggesting that factors other than low dietary content could contribute to vitamin B-12 status (3). Tropical sprue, and gastrointestinal infestations (10, 11) and infections (including *Helicobacter pylori*) (12, 13) also contribute to low vitamin B-12 concentrations, possibly due to malabsorption. This possibility is rarely investigated because of the complexity of methods for investigating vitamin B-12 absorption. For example, the Schilling (14) test requires radioactive isotope. It is essential to determine whether the low vitamin B-12 status among Indians only reflects dietary inadequacy or if malabsorption is also a contributing factor, becasuse this will have implications for treatment.

Recently, Bor et al (15) demonstrated an increase in plasma holotranscobalamin (holo-TC) concentration after a small oral dose of vitamin B-12 (9 μg × 3 doses), and suggested that this approach can be used to test vitamin B-12 absorption in a clinical setting. Another recent study by van Castel-Roberts et al (16) investigated circulating concentrations of holo-TC using Bor's protocol in a sample of healthy US men and women. Blood samples taken 24 h after the first dose provided the optimal results. Both these studies were performed in participants with normal vitamin B-12 concentrations. We have used the same protocol with slight modification (10 μg × 3 doses) as a test of vitamin B-12 absorption in a population with low vitamin B-12 status. In addition, we also investigated the response, using a much lower dose (2 μg × 3 doses).

## Participants and methods

The participants were families from an extended cohort of the Pune Maternal Nutrition Study (PMNS). The design and methods have been reported elsewhere (17). In short, the PMNS is a prospective community-based study to investigate the relationship of maternal nutrition to fetal growth; the participants have been followed up to assess future risk of type 2 diabetes and cardiovascular disease. The study was established in 6 villages 40-50 km from Pune city, between June 1994 and April 1996 and covered a population of >35,000. We screened all married non-pregnant women who were < 35 y and not sterilized, were screened. Of 2,675 eligible women, 2,466 (92%) agreed to participate. During the study period 814 women became pregnant and participated in the main PMNS cohort. After the main study, we enrolled an additional 153 pregnant women from the same initial sample of eligible women, to study early fetal growth. Of these, 129 delivered in the study and the babies and parents have been followed up with the original cohort; 119 children remained in follow-up at 9 y of age. These children and their parents were invited to take part in the

current study, which took place from May–November 2006. The women, their husbands and the children, were comparable to the main PMNS cohort in body size measurements and socioeconomic status. Children as well as adults were included in the study because our earlier work suggested that vitamin B-12 status varies with age.

The King Edward Memorial (KEM) Hospital Ethics Committee approved the study, and we obtained informed written consent from the parents, and assent from the children.

The participants reported to the Diabetes Unit, KEM Hospital, Pune, the evening before the study. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (CMS Instruments), and body weight to the nearest 5 g (Conveigh, Electronic Instruments). A standard vegetarian dinner was provided between 20-21 h, after which they rested overnight in the Diabetes Unit. The only food of animal origin during period was a small amount of milk in tea. All participants were examined for gross clinical signs of protein-energy under-nutrition and vitamin deficiencies (vitamins A, B complex, C, D). In children, a FFQ was administered to record the portion size and frequency of consumption of different foods, over a period of 1 y. Vitamin B-12 intakes were calculated from the FFQ data using published tables of the nutrient content of Indian foods (18). A fasting blood sample was collected in the morning (baseline), and a 10 μg vitamin B-12 (cyanocobalamin) capsule was administered under supervision every 6 h thereafter (8, 14 and 20 h). A fasting blood sample was collected the following morning, ~ 12 h after the last dose (post-dose sample).

After completing sampling for approximately one-half of the families, it was clear from our data that absorption was satisfactory in most cases. We then reasoned that we could improve the utility of the study, including for policy makers, by investigating a smaller dose, nearer to the Recommended Dietary Allowance (12) of 2.4 to 2.8 μg for adults and 0.9 to 2.4 μg for children. The remaining families received 2 μg capsules, rather than 10 μg capsules. In total, 65 families received 10 μg × 3 doses (10 μg group) and 44 received 2 μg × 3 doses (2 μg group ).

## Laboratory analysis

Blood samples from the ante-cubital vein were collected in the sitting position, in an EDTA vacutainer. Hemoglobin was measured on a Beckman Coulter Analyzer (A$^{C.T}$ diff$^{TM}$Analyzer). The remaining blood was centrifuged at 2500 x *g* for 15 min at 4°C within 1h of collection, and plasma was stored at −70°C until further analysis. Plasma cobalamin (B-12) was measured by microbiological assay using a colistin sulfate-resistant strain of *Lactobalillus. leichmanii* (19, 20). Plasma holo-TC was measured using magnetic beads (microspheres) with immobilized monoclonal antibody specific for human TC (21) followed by the conventional microbiological assay developed for cobalamin estimation (22). Plasma folate was measured by microbiological assay using a chloramphenicol-resistant strain of *Lactobalillus. casei* (23, 24). Plasma total homocysteine (tHcy) was measured by fluorescence polarization immunoassay (Abbott) (25). In our laboratory, for vitamin B-12, holo-TC, folate, and tHcy analysis, between-day CV were < 8%, <9%, <7%, and <3%, respectively.

## Definitions

We defined anemia as a hemoglobin concentration below 120 g/L in children and mothers, and below 130 g/L in fathers (26). We defined microcytosis as mean corpuscular volume as <80 fL and macrocytosis and >100 fL. Hyperhomocysteinemia was defined as plasma tHcy concentration >15 μmol/L in adults, and >10 μmol/L in children (27). Low folate concentrations were defined as plasma folate concentration <7.0 nmol/L (28). Low vitamin B-12 concentrations were defined as plasma vitamin B-12 concentration <150 pmol/L (2).

Low holo-TC concentration was defined as <35 pmol/L (29). Poor vitamin B-12 absorption was defined by the Bor et al. (16) criteria as a rise in plasma holo-TC <15%, and <15 pmol/L after 3 doses of 10 μg oral vitamin B-12.

### Statistical methods

The data are presented as mean ± SD. Differences between baseline and post-dose measurements (vitamin B-12, tHcy, and holo-TC) were tested using paired t-tests. Differences between groups were tested using unpaired t-tests. Results in children and parents are analyzed separately. The results in children and parents for 2 μg and 10 μg groups were combined in the multiple linear regression analysis of factors associated with rise in plasma holo-TC. SPSS version 11.0 for windows was used for statistical analysis.

## Results

A total of 313 individuals (109 children, 96 fathers, and 108 mothers) participated in the study. None were taking vitamin supplements or drugs known to influence vitamin B-12 absorption such as proton pump inhibitors and metformin. One child from 10 μg group could not swallow the capsules, and one child from 2 μg group vomited during the test; these were excluded from the analysis.

None of the children, fathers, and mothers had clinical signs of protein-energy under-nutrition or vitamin deficiencies; some were anemic (Table 1). Sixty four percent of anemic participants had microcytic erythrocytes, none had macrocytosis. Five participants (3 fathers and 2 mothers) had macrocytic erythrocytes but were not anemic. FFQ data were available for children only. None of them were vegan. One-third were lacto-vegetarian (milk but no other non-vegetarian foods) and two-thirds ate non-vegetarian foods (eggs, meat, and fish as well as milk). Less than one-half of the later group (44%) consumed non-vegetarian foods more than twice a week, but the average portion size of non-vegetarian foods was small. The median calculated daily dietary intake of vitamin B-12 was <0.1 μg in the lacto-vegetarian children and 0.2 μg in the non-vegetarian children.

Baseline plasma vitamin B-12 concentrations were low, folate concentrations were normal, and tHcy concentrations were high (Table 2). Twenty seven percent of children, 70% of fathers, and 49% of mothers had low vitamin B-12 concentrations. Ninety eight percent of participants had low plasma holo-TC concentrations. In contrast, only 2% of children, 15% of fathers, and 9% of mothers had low folate concentrations. Hyperhomocysteinemia was observed in 48% of children, 74% of fathers, and 34% of mothers.

Plasma holo-TC concentrations increased after oral cynocobalamin (Table 2, Supplemental Figure 1). In 10 μg group the rise was 4.8-fold and in 2 μg group the rise was 2.2-fold. Plasma vitamin B-12 concentrations also increased, and the two were related ($r=0.57$; $P<0.001$, adjusted for age and sex). The increases in holo-TC and vitamin B-12 were greater in children than in the parents ($P<0.001$), and comparable in fathers and mothers. This may have resulted from a greater dose of vitamin B-12 per kg body weight in children (10 μg group: 1.39 ± 0.17 μg/kg; 2 μg group: 0.28 ± 0.03 μg/kg) compared with fathers (10 μg group: 0.53 ± 0.09; 2 μg group: 0.10 ± 0.02) and mothers (10 μg group: 0.65 ± 0.10; 2 μg group: 0.13 ± 0.02).

According to the criteria listed by Bor et al (15) (used only for 10 μg group), four children (6%), 10 fathers (17%), and 5 mothers (8%) were classified as poor vitamin B-12 absorbers. The poor absorbers and normally absorbing counterparts had similar baseline plasma vitamin B-12, holo-TC, tHcy and blood hemoglobin concentrations; none of poor absorbers had macrocytic erythrocytes.

In a multivariate analysis, combining children and parents from both the groups, and including age, sex, dose/kg of cyanocobalamin, and baseline vitamin B-12 concentration, the rise in plasma holo-TC concentration was directly proportional to the dose/kg of cyanocobalamin (standardized β=0.446; P<0.001) and inversely related to age (standardized β= −0.147; P=0.01). The rise in holo-TC was higher in participants with a baseline plasma vitamin B-12 concentration >150 pmol/L at baseline than in those with concentrations <150 pmol/L (unadjusted (mean ± SEM) 42.3 ± 1.8 pmol/L vs 30.6 ± 1.9 pmol/L; p=0.005; and 42.0 ± 1.6 pmol/L vs 30.9 ± 1.6 pmol/L, p=0.06, adjusted for age, sex, dose/kg of vitamin B-12 and body weight).

After oral cyanocobalamin, plasma folate concentrations did not change, but those of tHcy decreased from 18.3 ± 14.4 to 17.1 ± 13.5 μmol/L in 10 μg group and from 19.0 ± 18.9 to 17.0 ± 17.5 μmol/L in 2 μg group ($p$<0.001).

## Discussion

We have demonstrated the usefulness of the Bor et al. (15) protocol to investigate gastrointestinal absorption of vitamin B-12 in participants with low circulating vitamin B-12 concentrations. The absolute rise in holo-TC in our participants (44 pmol/L) was comparable to that in vitamin B-12-sufficient Europeans (46 pmol/L) (15); however, the percentage rise was much higher (~550% compared with ~50%) because of lower baseline concentrations (median 7 pmol/L compared with 72 pmol/L). Our findings provide experimental evidence that vitamin B-12 absorption is adequate in the majority of Indians and argue against malabsorption as a prominent cause of their low vitamin B-12 concentrations. Furthermore, it was reassuring that a physiological dose of cyanocobalamin (2 μg × 3) was absorbed satisfactorily, suggesting that a modest increase in vitamin B-12 intake should improve their status.

Vitamin B-12 in food is protein-bound. It is released in the stomach by the action of pepsin and gastric acid, and then combines with salivary R-binder. This complex is broken down by pancreatic proteases in the duodenum, to release free B-12, which combines with intrinsic factor, secreted by gastric parietal cells (30). The intrinsic factor-vitamin B-12 complex interacts with a specific receptor in terminal ileal enterocytes, and is absorbed by endocytosis (31, 32). During transport across the enterocyte, vitamin B-12 is complexed with transcobalamin to form holo-TC, which is released into the blood (31). Unlike protein-bound vitamin B-12 in food (33), the free vitamin B-12 (cyanocobalamin) used in our study binds directly to R-binder, bypassing the stage of gastric degradation. From this stage onwards, vitamin B-12 from food and free B-12 follow the same absorption pathway. Thus, our study does not test gastric degradation but suggests normal handling beyond this stage. Holo-TC has a short half-life (34, 35) and is therefore proposed to reflect recent vitamin B-12 absorption. However, circulating holo-TC concentrations are also influenced by hepatic and renal uptake, production, and release from the ileum and kidney, tissue requirements, and other unknown factors (36). There is a debate about whether basal holo-TC concentrations represent ‘an early general cobalamin insufficiency or specifically decreased cobalamin absorption’ (36). A dose-dependent rise in holo-TC during the 24 hours following vitamin B-12 administration, suggests that the levels are influenced by recent absorption.

The rise in holo-TC was ~ 28% lower (P=0.005) in those with low vitamin B-12 concentrations compared to that in those with normal vitamin B-12 concentration. This raises the possibility that malabsorption may play a contributory role in the low vitamin B-12 concentrations in this population. It would be interesting to measure the rise in holo-

TC before and after vitamin B-12 repletion, to confirm whether adequate vitamin B-12 status changes vitamin B-12 absorption.

Fewer than 10% of the participants in our study had a lower than expected rise in holo-TC based on the Bor et al. protocol (15). This may be evidence of poor vitamin B-12 absorption, in these individuals, but because the Bor protocol was developed in populations with normal vitamin B-12 status, weather our findings in some participants were due to malabsorption needs further investigation (37). However, the original test using a dose of 9 μg × 3 has been validated in inherited syndromes of vitamin B-12 malabsorption (38). In our population, more parents (12%) than children (6%) had a lower than expected rise in holo-TC, which may be due to the relatively lower dose of vitamin B-12 per kilogram body weight they received. Vitamin B-12 absorption also decreases with age (39, 40), and deficiency is common in the elderly, even in Western non-vegetarian populations (41). One of the proposed mechanisms is an increasing prevalence of ‘chronic gastritis’ with age, which results in hypochlorhydria and impaired synthesis of intrinsic factor and pepsin. The etiology of chronic gastritis includes autoimmune damage (pernicious anemia) and *H. pylori* infection (12, 13, 31). Further studies are required to identify the cause(s) of the low plasma vitamin B-12 in these participants.

Our study has many strengths. It was community-based, had a large sample-size, high participation rates, and low drop-out rates. We enrolled children as well as adults, and included both genders. None of the participants were taking vitamin supplements, and care was taken to ensure no interference from diet during the test period by requiring that vegetarian diets be consumed. Participants were admitted to our research unit to ensure that the test was carried out under strict supervision. In addition to the diagnostic dose we also studied absorption of vitamin B-12 at a dose close to the physiological level, which may be important in guiding public health policy. Our study cannot be strictly compared to the Bor et al (15), because we used 10 μg × 3 doses instead of 9 μg × 3 doses because of availability. We do not think that the slightly higher dose is likely to change our conclusions because absorption was similarly impressive for the 2 μg × 3 doses. A definite limitation of the study is that because we used free cyanocobalamin, we could not test the gastric degradation step which is essential for absorption of protein-bound dietary vitamin B-12 (33). However, the test would probably be able to detect the malabsorption related to tropical sprue or gastrointestinal infections, which are thought to contribute to subclinical vitamin B-12 status in tropical areas. The holo-TC test is simple, relatively inexpensive, and has the potential to be used in population-based studies, but further work regarding dosing, and validation against a gold standard test, would be desirable.

Thus, we have demonstrated adequate intestinal absorption of free cyanocobalamin in a community-based study in a South Asian Indian population with high prevalence of low vitamin B-12 concentrations. The health implications of low vitamin B-12 concentrations are not fully understood, because there is poor association with macrocytosis or severe anemia, or neurological symptoms, and also the fact that this population has perhaps lived with such low concentrations for generations. This issue needs to be further investigated. This study is a small part of such a systematic programme of research. Our results suggest that the gastro-intestinal malabsorption of the vitamin is unlikely to be a prominent cause of the high prevalence of low vitamin B-12 concentrations, and the consequent hyperhomocysteinemia in children and young adults, though this could be a contributing factor in middle-aged and elderly adults. The marked increase in plasma concentrations following even low doses of vitamin B-12 suggests that fortification of food items or regular use of supplements would be useful approaches. The results of such long-term supplementation in this population are described in a separate paper.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

We thank Dr K. J. Coyaji, Director of the KEM Hospital, Pune, for providing required facilities. We thank P. S. Hardikar, D. A. Raut, T. B. Konde, K. N. Raut, P. C. Yajnik, A. Nanivadekar, T.M. Deokar, S.D. Chougule, A. B. Bhalerao, V. A. Solat, and A. Gaikwad for their invaluable contribution to the study. We thank Prof. Elaine Rush for her valuable comments. We are grateful to Rajendra Jha, and Chandrashekhar Mainde for providing vitamin capsules for the study.

The authors responsibilities were as follows: C.S.Y. and C.H.F. planned the study, participated in the analysis, interpretation and writing the manuscript. D.S.B. supervised the study, blood collection and laboratory analysis and wrote the original manuscript. S.S.N. helped in quality control of the analysis. N.V.T., H.G.L., L.V.R. participated in subject recruitment and data collection. C.V.J. did the statistical analysis. C.J. helped in laboratory analysis of holo-TC and tHcy. H.R . supervised the laboratory analysis, interpretation of the data and writing the manuscript. All authors read and approved the final manuscript.

## ABBREVATIONS USED

| | |
|---|---|
| **holo-TC** | holotranscobalamin |
| **KEM** | King Edward Memorial |
| **PMNS** | Pune Maternal Nutrition Study |
| **tHcy** | total homocysteine |

## Literature Cited

1. Chanarin I, Malkowska V, O'Hea AM, Rinsler MG, Price AB. Megaloblastic anaemia in a vegetarian Hindu community. Lancet. 1985; 2:1168–72. [PubMed: 2865624]
2. Refsum H, Yajnik CS, Gadkari M, Schneede J, Vollset SE, Orning L, Guttormsen AB, Joglekar A, Sayyad MG, Ulvik A, Ueland PM. Hyperhomocysteinemia and elevated methylmalonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians. Am J Clin Nutr. 2001; 74:233–41. [PubMed: 11470726]
3. Yajnik CS, Deshpande SS, Lubree HG, Naik SS, Bhat DS, Uradey BS, Deshpande JA, Rege SS, Refsum H, Yudkin JS. Vitamin B12 deficiency and hyperhomocysteinemia in rural and urban Indians. J Assoc Physicians India. 2006; 54:775–82. [PubMed: 17214273]
4. Selhub J, Jacques PF, Bostom AG, Wilson PW, Rosenberg IH. Relationship between plasma homocysteine and vitamin status in the Framingham study population. Impact of folic acid fortification. Public Health Rev. 2000; 28:117–45. [PubMed: 11411265]
5. Yajnik CS, Joglekar CV, Lubree HG, Rege SS, Naik SS, Bhat DS, Uradey B, Raut KN, Shetty P, Yudkin JS. Adiposity, inflammation, insulin resistance and hyperglycaemia in rural and urban Indian men. Diabetologia. 2008; 51:39–46. [PubMed: 17972060]
6. Bhate V, Deshpande S, Bhat D, Joshi N, Ladkat R, Watve S, Fall C, de Jager CA, Refsum H, Yajnik C. Vitamin B12 status of pregnant Indian women and cognitive function in their 9-year-old children. Food Nutr.Bull. 2008; 29:249–254. [PubMed: 19227049]
7. Godbole K, Deshmukh U, Yajnik C. Nutro-genetic determinants of neural tube defects in India. Indian Pediatrics. 2009; 46:1–9.
8. Antony AC. Vegetarianism and vitamin B-12 (cobalamin) deficiency. Am J Clin Nutr. 2003; 78:3–6. [PubMed: 12816765]
9. Price SR. Observations on dietary practices in India. Hum Nutr Appl Nutr. 1984; 38:383–9. [PubMed: 6526685]
10. Mathan VI. Tropical sprue in southern India. Trans R Soc Trop Med Hyg. 1988; 82:10–14. [PubMed: 3051540]

11. Herbert V. Megaloblastic anemias. Lab Invest. 1985; 52:3–19. [PubMed: 3880840]
12. Lindsay HA. Causes of vitamin B12 and folate deficiency. Food Nutr.Bull. 2008; 29(sup.2):S20–S34. [PubMed: 18709879]
13. Dali-Youcef N, Andres E. An update on cobalamin deficiency in adults. QJM. 2009; 102:17–28. [PubMed: 18990719]
14. Schilling RF. Intrinsic factor studies II. The effect of gastric juice on the urinary excretion of radioactivity after the oral administration of radioactive vitamin B12. J Lab Clin Med. 1953; 42:860–6. [PubMed: 13109347]
15. Bor MV, Nexo E, Hvas AM. Holo-transcobalamin concentration and transcobalamin saturation reflect recent vitamin B12 absorption better than does serum vitamin B12. Clin Chem. 2004; 50:1043–9. [PubMed: 15044317]
16. van Castel-Roberts KM, Morkbak AL, Nexo E, Edgemon CA, Maneval DR, Shuster JJ, Valentine JF, Kauwell GP, Bailey LB. Holo-transcobalamin is an indicator of vitamin B-12 absorption in healthy adults with adequate vitamin B-12 status. Am J Clin Nutr. 2007; 85:1057–61. [PubMed: 17413105]
17. Rao S, Yajnik CS, Kanade A, Fall CH, Margetts BM, Jackson AA, Shier R, Joshi S, Rege S, Lubree H, Desai B. Intake of micronutrient-rich foods in rural Indian mothers is associated with the size of their babies at birth: Pune Maternal Nutrition Study. J Nutr. 2001; 131:1217–24. [PubMed: 11285330]
18. Gopalan, C.; Rama Sastri, BV.; Balasubramanian, SC. Nutritivevalue of Indian foods. Revised ed. NIN, ICMR; Hyderabad: 1989.
19. Kelleher BP, Walshe KG, Scott JM, O'Broin SD. Microbiological assay for vitamin B12 with use of a colistin-sulfate-resistant organism. Clin Chem. 1987; 33:52–4. [PubMed: 3542297]
20. Kelleher BP, Broin SD. Microbiological assay for vitamin B12 performed in 96-well microtitre plates. J Clin Pathol. 1991; 44:592–5. [PubMed: 1856292]
21. Ulleland M, Eilertsen I, Quadros EV, Rothenberg SP, Fedosov SN, Sundrehagen E, Orning L. Direct assay for cobalamin bound to transcobalamin (holo-transcobalamin) in serum. Clin Chem. 2002; 48:526–32. [PubMed: 11861443]
22. Refsum H, Johnston C, Guttormsen AB, Nexo E. Holotranscobalamin and total transcobalamin in human plasma: determination, determinants, and reference values in healthy adults. Clin Chem. 2006; 52:129–37. [PubMed: 16239338]
23. Horne DW, Patterson D. Lactobacillus casei microbiological assay of folic acid derivatives in 96-well microtiter plates. Clin Chem. 1988; 34:2357–9. [PubMed: 3141087]
24. Tamura T, Freeberg LE, Cornwell PE. Inhibition of EDTA of growth of Lactobacillus casei in the folate microbiological assay and its reversal by added manganese or iron. Clin Chem. 1990; 36:1993. [PubMed: 2122927]
25. Shipchandler MT, Moore EG. Rapid, fully automated measurement of plasma homocyst(e)ine with the Abbott IMx analyzer. Clin Chem. 1995; 41:991–4. [PubMed: 7600701]
26. Nutritional anaemias. Report of a WHO scientific group. World Health Organ Tech Rep Ser. 1968; 405:5–37. [PubMed: 4975372]
27. Refsum H, Smith AD, Ueland PM, Nexo E, Clarke R, McPartlin J, Johnston C, Engbaek F, Schneede J, McPartlin C, Scott JM. Facts and recommendations about total homocysteine determinations: an expert opinion. Clin Chem. 2004; 50:3–32. [PubMed: 14709635]
28. Clarke R, Grimley EJ, Schneede J, Nexo E, Bates C, Fletcher A, Prentice A, Johnston C, Ueland PM, Refsum H, Sherliker P, Birks J, Whitlock G, Breeze E, Scott JM. Vitamin B12 and folate deficiency in later life. Age Ageing. 2004; 33:34–41. [PubMed: 14695861]
29. Miller JW, Garrod MG, Rockwood AL, Kushnir MM, Allen LH, Haan MN, Green R. Measurement of total vitamin B12 and holotranscobalamin, singly and in combination, in screening for metabolic vitamin B12 deficiency. Clin Chem. 2006; 52:278–85. [PubMed: 16384886]
30. Cuskelly GJ, Mooney KM, Young IS. Folate and vitamin B12: friendly or enemy nutrients for the elderly. Proc Nutr Soc. 2007; 66:548–58. [PubMed: 17961276]
31. He Q, Madsen M, Kilkenney A, Gregory B, Christensen EI, Vorum H, Hojrup P, Schaffer AA, Kirkness EF, Tanner SM, de la CA, Giger U, Moestrup SK, Fyfe JC. Amnionless function is

required for cubilin brush-border expression and intrinsic factor-cobalamin (vitamin B12) absorption in vivo. Blood. 2005; 106:1447–53. [PubMed: 15845892]

32. Moestrup SK, Verroust PJ. Megalin- and cubilin-mediated endocytosis of protein-bound vitamins, lipids, and hormones in polarized epithelia. Ann Rev Nutr. 2001; 21:407–28. [PubMed: 11375443]
33. Carmel R. Malabsorption of food cobalamin. Baillieres Clin Haematol. 1995; 8:639–55. [PubMed: 8534965]
34. Hom BL, Olesen HA. Plasma clearance of 57cobalt-labelled vitamin B12 bound in vitro and in vivo to transcobalamin I and II. Scand J Clin Lab Invest. 1969; 23:201–11. [PubMed: 5383307]
35. Schneider RJ, Burger RL, Mehlman CS, Allen RH. The role and fate of rabbit and human transcobalamin II in the plasma transport of vitamin B12 in the rabbit. J Clin Invest. 1976; 57:27–38. [PubMed: 1245601]
36. Carmel R. Measuring and interpreting Holo-Transcobalamin (Holo-Transcobamin II). Clin chem. 2002; 48(no.3):407–9. [PubMed: 11861432]
37. Carmel R. the disappearance of cobalamin absorption testing: A critica diagnostic loss. J. Nutr. 2007; 137:2481–84. [PubMed: 17951489]
38. Bor MV, Cetin M, Aytac S, Altay C, Nexo E. Nonradioactive vitamin B12 absorption test evaluated in controls and in patients with inherited malabsorption of vitamin B12. Clin Chem. 2005; 51:2151–5. [PubMed: 16166166]
39. Scarlett JD, Read H, O'Dea K. Protein-bound cobalamin absorption declines in the elderly. Am J Hematol. 1992; 39:79–83. [PubMed: 1550110]
40. Carmel R. Cobalamin, the stomach, and aging. Am J Clin Nutr. 1997; 66:750–59. [PubMed: 9322548]
41. McLean E, de Benoist B, Allen LH. Review of the magnitude of folate and vitamin B12 deficiencies worldwide. Food Nutr Bull. 2008; 29:S38–51. [PubMed: 18709880]



Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

## TABLE 1

Anthropometric characteristics and hemoglobin concentrations of children, fathers and mothers[1].

| | Children | Fathers | Mothers |
|---|---|---|---|
| n | 109 | 96 | 108 |
| Age, *y* | 9.0 ± 0.2 | 36.9 ± 3.8 | 30.2 ± 3.0 |
| Height, *cm* | 126.5 ± 5.6 | 165.7 ± 7.1 | 153.2 ± 5.3 |
| Weight, *kg* | 21.9 ± 2.9 | 59.3 ± 10.0 | 47.7 ± 8.2 |
| Hemoglobin, *g/L* | 125.0 ± 1.0 | 143.0 ± 1.4 | 120.0 ± 1.6 |
| Anemic[2], % | 24 | 13 | 46 |

[1] Values are mean ±SD, unless specified

[2] Hemoglobin <120 g/L for children, and mother, and <130 g/L for father (26)

Europe PMC Funders Author Manuscripts





**TABLE 2**

Plasma vitamin B-12, holo-TC, tHcy and folate concentrations in Indian children and their parents before and after receiving 3 × 10 μg doses or 3 × 2 μg doses of cyanocobalamin[1].

| | 10 μg group | | | 2 μg group | | |
|---|---|---|---|---|---|---|
| | **Children** | **Fathers** | **Mothers** | **Children** | **Fathers** | **Mothers** |
| n | 64 | 61 | 65 | 43 | 35 | 43 |
| Plasma vitamin B-12, *pmol/L* | | | | | | |
| Baseline | 191 ± 60 | 136 ± 62 | 180 ± 176 | 222 ± 107 | 126 ± 68 | 161 ± 95 |
| Post-dose | 302 ± 95 | 193 ± 74 | 233 ± 121 | 293 ± 123 | 158 ± 71 | 194 ±103 |
| Change % | 63.1 ± 39.6* | 47.7 ± 31.1* | 40.1 ± 24.0* | 38.9 ± 27.4* | 31.3 ± 21.1* | 23.5 ± 14.9* |
| Plasma Holo-TC[2], *pmol/L* | | | | | | |
| Baseline | 10.3 ± 5.6 | 7.7 ± 4.2 | 9.7 ± 9.7 | 11.9 ± 7.3 | 10.5 ± 8.1 | 10.7 ± 10.0 |
| Post-dose | 67.4 ± 29.5 | 45.7 ± 22.2 | 47.9 ± 19.4 | 49.5 ± 16.3 | 24.9 ± 15.7 | 30.5 ± 17.0 |
| Change % | 655.6 ± 406.8* | 577.5 ± 406.3* | 498.6 ± 280.5* | 399.6 ± 207.0* | 150.6 ± 99.9* | 229.2 ± 143.1* |
| Plasma tHcy, *μmol/L* | | | | | | |
| Baseline | 11.0 ± 3.7 | 29.2 ± 19.2 | 15.3 ± 8.3 | 10.3 ± 4.0 | 35.9 ± 27.5 | 14.1 ± 7.1 |
| Post-dose | 10.0 ± 3.4 | 27.8 ± 18.2 | 14.5 ± 7.7 | 9.6 ± 3.9 | 32.9 ± 25.7 | 12.1 ± 6.1 |
| Change % | −8.2 ± 12.5* | −4.1 ± 11.8* | −3.7 ± 21.6* | −6.1 ± 11.8* | −8.2 ± 11.5* | −12.5 ± 16.3* |
| Plasma folate, *nmol/L* | | | | | | |
| Baseline | 15.9 ± 5.0 | 10.4 ± 3.7 | 13.9 ± 7.0 | 15.7 ± 4.5 | 11.1 ± 4.2 | 12.8 ± 7.1 |
| Post-dose | 16.4 ± 5.5 | 10.6 ± 3.6 | 13.0 ± 6.9 | 14.8 ± 4.2 | 10.9 ± 4.2 | 11.5 ± 6.9 |
| Change % | 9.8 ± 42.5 | 3.4 ± 17.0 | −6.2 ± 20.3* | −3.0 ± 24.5 | −1.1 ± 16.6 | −8.6 ± 20.6* |

[1] Values are mean ±SD,

* Significant change. P< 0.05.

[2] Individual data in supplemental Figure 1