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Vitamin B_{12} Status in Children with Cystic Fibrosis and Pancreatic Insufficiency

Asim Maqbool, MD[1,2], **Joan I. Schall, PhD**[1], **Maria R. Mascarenhas, MD**[1,2], **Kelly A. Dougherty, PhD**[1,2], and **Virginia A. Stallings, MD**[1,2]

[1]Division of Gastroenterology, Hepatology, and Nutrition, The Children's Hospital of Philadelphia

[2]Department of Pediatrics, University of Pennsylvania Perelman School of Medicine, Philadelphia PA

Abstract

Objective—Unexpectedly high serum B_{12} concentrations were noted in most study subjects with cystic fibrosis (CF) and pancreatic insufficiency (PI) participating in a nutrition intervention at the baseline evaluation. The objectives of this study were to determine dietary, supplement-based and enzyme-based B_{12} intake, serum B_{12} concentrations, and predictors of vitamin B_{12} status in children with CF and PI.

Study Design—Serum B_{12} status was assessed in subjects (5-18 yrs) and categorized as elevated (Hi-B_{12}) or within reference range (RR-B_{12}) for age and sex. Serum homocysteine, plasma $B_{6,}$ red blood cell folate, height, weight, and body mass index Z scores, pulmonary function, energy, dietary and supplement-based vitamin intake were assessed.

Results—106 subjects, mean age 10.4 ± 3.0 years participated. Median serum B_{12} was 1083 pg/ml, with 56% in the Hi-B_{12} group. Dietary and supplement-based B_{12} intake were both high representing 376% and 667% Recommended Dietary Allowance (RDA). The Hi-B_{12} group had significantly greater supplement-based B_{12} intake than the RR-B_{12} group (1000 vs. 583% RDA, $p<0.001$). By multiple logistic regression analysis, high supplement-based B_{12} intake and age >12 years increased risk for Hi-B_{12}, while higher FEV_1 decreased risk (Pseudo-R^2=0.18, $P<0.001$).

Conclusions—Serum B_{12} was elevated in the majority of children with CF and PI. Supplement-based B_{12} intake was 6 to 10 times the RDA, and strongly predicted elevated serum B_{12} status. The health consequences of lifelong high supplement-based B_{12} intake and high serum B_{12} are unknown and require further study, as does the inversed correlation between serum B_{12} and FEV_1.

Keywords

Supplement-based vitamin intake; cobalamin; B_{12}, B_6, folate, cyanide

Corresponding author and reprint requests: Asim Maqbool, MD Division of Gastroenterology, Hepatology, and Nutrition, The Children's Hospital of Philadelphia, 34th and Civic Center Boulevard, Room 8C09H, Philadelphia PA 19104, (215) 590-3247 (phone), (215) 590-5326 fax, maqbool@email.chop.edu.

Clinical Trial Registration: Study of LYM-X-ORB™ to Improve Fatty Acid and Choline Status in Children with Cystic Fibrosis and Pancreatic Insufficiency, NCT00406536.

INTRODUCTION

While fat soluble vitamin status is frequently described in patients with cystic fibrosis (CF) and pancreatic insufficiency (PI), less is known regarding B vitamins. Vitamin B_{12} (cobalamin) metabolism is a complex process that begins in the oropharynx and is completed in the terminal ileum[1]. This process requires an intact gastrointestinal tract, acidification of gastric contents, pancreatic function for alkalinization of duodenal fluid and the provision of trypsin to degrade the R-binder protein and allow B_{12} to bind to intrinsic factor (IF). The IF-B_{12} complex is actively absorbed at the terminal ileum[1]. There are possible CF and PI- related perturbations in B_{12} metabolism, specifically impaired trypsin and bicarbonate production[2, 3]. Approximately 85% of the CF population has PI[4] and are at risk for malabsorption. Pancreatic enzyme replacement therapy (PERT) partially corrects B_{12} malabsorption and improves B_{12} concentrations[5-7] .

Unexpectedly high serum B_{12} concentrations were noted in most of the study subjects participating in a nutrition intervention trial in children with CF and PI. The objectives of this current report were to determine dietary, vitamin supplement-based and pancreatic enzyme-based B_{12} intake, serum B_{12} concentrations, and predictors of vitamin B_{12} status in children with CF and PI, to try to identify etiology of elevated serum B_{12}.

METHODS

Children with CF and PI ages 5.0 to 17.9 were recruited from ten CF Centers from March 2007 to May 2010 to participate in a randomized placebo-controlled double-blind longitudinal trial to evaluate the impact of a nutritional supplement, LYM-X-SORB™, on nutritional and growth status. LYM-X-SORB™ is an lipid matrix is composed of lysophosphatidyl choline, triglycerides and essential fatty acids that has been previously been shown to be absorbable without pancreatic enzyme therapy (PERT) and to improve nutritional status and clinical outcomes for children with CF and PI[8]. Vitamin B_{12}, B_6 (pyridoxine) and folate status were assessed as part of this larger study due to their role as co-factors in the phospholipid and 1-methyl donor metabolism. Here we report on these B vitamins at the baseline pre-intervention visit. The diagnoses of CF and PI were made by the home CF Center based on clinical symptoms, and duplicate quantitative pilocarpine iontophoresis sweat test and/or by genotype. In all study subjects PI was confirmed by fecal elastase testing (Genova Diagnostics, Asheville, NC), with < 15μg/g stool required for enrollment. Children were excluded if they had an FEV_1 <40% predicted, significant liver disease, history of meconium ileus with significant ileal resection or known current ileal disease, insulin dependent diabetes, or other conditions known to affect growth. The study protocol was approved by the Committee for the Protection of Human Subjects of the Institutional Review Board at the Children's Hospital of Philadelphia (CHOP), and at the subject's home institution. Written informed consent and age-appropriate assent were obtained from the parent/legal guardian and each subject, respectively. Subjects with CF were seen at the CHOP Clinical Translational Research Center (CTRC) for overnight admissions while in their usual state of good health. Evaluations included measures of growth and nutritional status, serum vitamin status and other biomarkers, clinical status,

spirometry, medication use (including pancreatic enzyme replacement therapy, acid reduction agents as well as antibiotics), dietary and supplemental vitamin intake.

Energy and Nutritional Intake

Dietary intake was assessed with 3-day home-based weighed food records. Subjects and parents/guardians were provided with measuring cups, spoons, and digital food scales after they were given detailed verbal instructions to weigh and record each food/beverage consumed. Diet records were reviewed and analyzed (Nutrition Data System for Research, National Coordinating Center, University of Minnesota, Minneapolis, MN)[9]. Energy intake was assessed using the Dietary Reference Intakes (DRI)[10], and expressed as percent Estimated Energy Requirement (%EER) for active children, as previously determined for children with CF and PI[11]. Dietary intake of B_{12}, B_6, and folate are expressed as percent Recommended Dietary Allowance (%RDA)[12,13].

Vitamin Intake

Details of the specific brands of vitamin, mineral and caloric supplemental intake, frequency and dose were recorded from study participant report. The supplement-based vitamin product content was verified from the manufacturers' information. Supplemental intakes for vitamins B_{12}, B_6 and folic acid were expressed as both intake in μg/d or mg/d and as %RDA[12, 13]. PERT use, including brand, number of capsules and lipase units taken per day were determined by study participant report. The vitamin B_{12} content of selected PERT products, including Pancreaze™ (11,500 lipase units/capsule; Janssen Pharmaceuticals), Creon™ (11,500 lipase units/capsule; Abbott Laboratories), and Zenpep™ (10,000 lipase units/capsule; Aptalis Pharma) were determined as μg/100g product by the turbidimetric method (Eurofins, Inc, Des Moines, IA).

Vitamin B Status and Biochemistry

Fasting blood samples were collected at baseline and serum vitamin B_{12} was analyzed by quantitative chemoluminescent immunoassay (CHOP Clinical Laboratory, Philadelphia, PA). Plasma B_6 (pyridoxine-5-phosphate) was determined by high performance liquid chromatography (ARUP Laboratory, Salt Lake City, UT). Whole blood was collected for red blood cell folate concentrations by chemoluminescence (ARUP Laboratory, Salt Lake City, UT) and reported correcting for the hematocrit. For serum B_{12}, subjects were designated as having elevated B_{12} (Hi- B_{12}) if their B_{12} concentrations were above versus within the established age- and sex-specific clinical laboratory reference ranges (RR-B_{12})[14, 15]. Supplement-based B_{12} in CF specific and in over the counter vitamin products was in the form of cyanocobalamin (i.e., complexed to cyanide). When it became evident that specifically supplement- based B_{12} intake was very high, whole blood cyanide concentrations were determined by quantitative colorimetry on a limited subset of study subjects (ARUP Laboratory, Salt Lake City, UT) at the 12 month study visit. Potential cyanide toxicity is associated with blood concentrations > 100 μg/dL[16]. CBC with differential, hepatic function panels, and comprehensive metabolic panels were assessed by standard methods; homocysteine, methionine and cysteine were assessed by high performance liquid chromatography (CHOP Clinical Laboratory). High sensitivity C-

reactive protein (hsCRP) was assessed by quantitative immunoturbidimetry (ARUP Laboratories, Salt Lake City, UT).

Body Composition, Growth and Clinical Status

Height and weight were measured using standard techniques[17] with a stadiometer accurate to 0.1 cm (Holtain, Crymych, UK), and a digital electronic scale accurate to 0.1 kg (Scaletronix, White Plains, NY). Height was adjusted for genetic potential[18] from measured or reported biological parent heights. Z scores for height (HAZ), mid-parent adjusted height (adjHAZ), weight (WAZ), and body mass index (BMI; kg/m^2, BMIZ) were computed[19]. Pulmonary function was evaluated by standard methods[20] by spirometry (Medical Graphics Corporation, Minneapolis, MN). FEV_1 percent predicted (FEV_1%) was calculated using Wang[21] and Hankinson[22] equations recommended for use in children and adolescents with CF and used as the measure of pulmonary function.

Statistical Methods

Measures of central tendency and variability were calculated for each outcome. Descriptive statistics were calculated for continuous variables using means and standard deviations or medians and ranges as appropriate for normally distributed or skewed data. Frequency distributions were used for categorical variables. Significant differences between Hi-B_{12} versus RR-B_{12} groups were assessed for continuous variables using unpaired student's t tests for normally distributed and Mann-Whitney tests for skewed data, and for categorical variables using chi-squared tests. Four categories for B_{12} supplemental intake were constructed representing low to high B_{12} supplemental intake as %RDA (Category 1=0-499, 2=500-999, 3=100-1499, and 4= ≥1500 %RDA). Children were also divided into younger (≤12 yrs) and older (>12 yrs) groups. Potential predictors of elevated B_{12} status were identified as age group, sex, genotype, BMIZ, FEV_1 % predicted, serum B_6 and B_{12} supplemental intake categories (%RDA). These potential predictors were then tested as both individual predictors in logistic models and also using multiple logistic regression analyses, with Hi-B_{12} vs. RR-B_{12} serum status as the dichotomous outcome. Best predictor multiple logistic models for B_{12} status included only those variables that entered the model at $p<0.10$ level of significance. All statistical analyses were performed using STATA 12.0 (College Station, TX), and results were considered significant at $p<0.05$.

RESULTS

Serum B_{12} was assessed for 106 of the 110 subjects (96%) enrolled in the study; median B_{12} was 1083 pg/ml, and 56% and 44% of the subjects had Hi-B_{12} and RR-B_{12}, respectively. There were no children with serum B_{12} below the age- and sex-specific reference ranges. Baseline demographic, growth and clinical status are presented in Table 1.Serum vitamin status, dietary and supplemental intake for energy, B_{12}, B_6 and folic acid are presented in Table 2 for the entire sample and for subjects in the Hi- B_{12} and RR-B_{12} groups. Overall, children were 10.4±3.0 years of age, 72% were <12 yrs and 57% were male. CF genotype was available for 104 subjects, with 59% classified as ΔF508 homozygous, 34% heterozygous (ΔF508/other) and 7% subjects with other CF genotypes. Growth status was suboptimal in these children with mild lung disease. In contrast to serum B_{12} status, serum

B_6 and RBC folate concentrations were within age-and sex-specific reference ranges for the majority of the sample (85% and 94%, respectively). No children were below reference ranges for B_6 and 4% were below for RBC folate concentrations. With respect to hepatic enzyme assessments, AST was mildly above reference range in 28%, ALT in 43%, and GGT in 34% of subjects, and were not associated with B_{12} status. Dietary and supplemental intake of energy and the B vitamins are also presented in Table 2 for 95 children who completed the 3-day weighed food. Overall, energy intake was higher in subjects with CF (115% EER) than the estimated requirement for healthy active children. Dietary and vitamin supplement-based B_{12} intake were both high representing 376% and 667% RDA, respectively. Total intake of B_{12} was 18.4 μg/d, corresponding to 1175 %RDA, or nearly 12 times the recommended intake, primarily due to the high CF specific vitamin supplemental B_{12} intake. B_6 intake was high, 5 to 6 times the RDA, with majority from vitamin supplement-based intake. Folic acid intake was 2 to 3 times the RDA, and was evenly distributed between dietary and vitamin supplement-based intake.

PERT (porcine pancreatic extract) also contributed to B_{12} intake. Subjects reported a median intake of 18 PERT capsules per day (range 9 to 42), representing a median of 270,000 lipase units per day. There are between 10 and 15 μg B_{12} in every 100 g of enzymes. One PERT capsule contains approximately 0.2 g of enzyme, depending upon the specific product. Therefore 18 capsules per day contain ~3.6 g enzymes, or 0.36 to 0.54 μg B_{12}, contributing another ~20 to 30% RDA for a typical 9 year old child with CF and PI. The contribution of B_{12} from PERT was not included in the total B_{12} intake calculations for this report. Acid reduction medications (Histamine H_2 receptor antagonists and/or proton pump inhibitors) were used by 65% of study subjects. Inhaled tobramycin was used by 29% of study subjects, and oral antibiotics by 36% of study subjects. Both inhaled and oral antibiotics were used by 49% of study subjects. There were no differences in acid reduction medication, inhaled, oral or total antibiotic use between B_{12} status groups.

Children in the Hi-B_{12} group were significantly older, had poorer BMIZ and lung function than those with RR-B_{12} (Table 1). Homocysteine was lower and cysteine was higher in Hi-B_{12} group, and methionine did not differ by group. Total intake of B_{12} was significantly higher in the Hi-B_{12} compared to RR-B_{12} group, 13 versus 10 times higher than the RDA, respectively. This was driven by the increased supplement-based B_{12} intake (10 times the RDA in the Hi-B_{12} group versus 6 times the RDA in the RR-B_{12} group). Furthermore, there were no group differences in ietary B_{12} intake between B_{12} status groups. Study subjects with Hi-B_{12} were significantly more likely to be taking > 1000 %RDA supplemental B_{12} than those with RR-B_{12}. Supplemental intakes of both B_6 and folic acid were also significantly higher in the Hi- B_{12} group. B_{12} groups did not differ by sex, genotype or PERT use (data not shown).

Significant predictors of B_{12} status were tested using multiple logistic regression analysis and the best model is shown in Table 3. From the multiple logistic regression model, higher supplemental B_{12} intake and older age significantly increased risk for Hi-B_{12} status, while higher FEV_1% predicted reduced risk, with these predictors combined explaining 18% of the variance in serum B_{12} status ($P<0.001$). Compared to subjects receiving <500 %RDA of supplemental B_{12}, there is a 2.3 fold increased risk for Hi-B_{12} for subjects for every

additional 500 %RDA, reaching a 6.9 fold increase for those receiving ≥1500 %RDA B_{12} from supplement-based B_{12} intake. BMIZ was inversely associated with B_{12} status in the simple model, but was no longer significant in the multivariable model. Other potential predictors were tested (for example, the use of acid reduction medications and antibiotic use) and did not add significantly to the model.

For the assessment of possible cyanide-related toxicity with sustained high cyanocobalamin supplement intake, whole blood cyanide was analyzed in seven subjects. Cyanide was below detectible concentrations (< 5μg/dL) for six subjects, and 8 μg/dL in one subject.

DISCUSSION

Serum B_{12} concentrations were greater than the age and sex reference ranges for more than half of the subjects, and was mostly driven by supplement-based B_{12} intake. Total B_{12}, B_6, and folic acid intakes were generally greater in subjects with CF than those observed in children of similar age and sex from the general population[23]. These high B_{12} intake and serum values were unexpected in this population of patients.

In healthy individuals approximately 50% B_{12} intake is absorbed, with approximately 98% by active transport at the terminal ileum and 2% via passive intestinal diffusion. Absorbed B_{12} is bound to transcobalamin (TC) II in the circulation and at the tissue level, and to TC-I in the serum and in the enterohepatic circulation[1]. Perturbations in B_{12} digestion and absorption have been reported in CF, including altered glycosylation of intrinsic factor (IF)[2], hyperacidity, IF hypersecretion[24], and loss of pancreatic function (poor duodenal alkalinization and protease insufficiency with incomplete R binder degradation)[2]. Patients with meconium resections may have impaired B_{12} absorption and enterohepatic circulation[25]. However, despite these potential perturbations in B_{12} digestion and absorption, alkalinization of the duodenal fluid[5], and providing PERT [5] or trypsin[26], normalized B_{12} absorption in subjects with CF and PI. B_{12} concentrations measured after pancreatin treatment (pancreatic extract predecessor to modern PERT) in subjects with CF and PI were normal or elevated[27]. Early studies documented abnormal absorption of crystalline B_{12} and impaired Schilling's tests. With modern PERT and B_{12} intake from food and B_{12} as cobalamin rather than the crystalline form, B_{12} metabolism is likely similar to healthy children and younger adults. B_{12} deficiency has not been described in the CF literature since 1973[6]. It is unclear if B_{12} supplementation is required in patients with CF and PI under current standards of care.

In the current report, we determined B_{12} intake from the diet and from vitamin supplements to understand the contributions of sources to intake and serum concentrations. Dietary intake of B_{12} were similar to that reported in healthy children, and both had dietary intake of about three times the RDA[23]. CF specific vitamins contain approximately the B_{12} dose found in multivitamins designed for older adults who have increased risk of B_{12} deficiency and pernicious anemia. In addition to B_{12} in the diet and vitamin supplements, PERT products contain $B_{12.}$ There was a relationship of age with serum $B_{12,}$ and CF vitamin supplement-based intake increases with age. Often between 8 and 12 years of age, the vitamin pill intake is increased from one CF specific vitamin per day to two per day in order to increase the fat

soluble vitamin intake[28]. The amount of B_{12} and other vitamins in the product also increases. Age associations are observed with other clinical and nutritional outcomes in CF, specifically, negative associations with FEV_1% and BMIZ[4]. In part this age-based intake multivitamin intake increase may contribute to the inverse relationship of both FEV_1 and BMIZ with serum B_{12} observed in this cohort.

The finding that B_{12} concentrations were inversely related to homocysteine concentrations likely reflects the 1-methyl metabolic pathway (homocysteine is converted to methionine) or to the transulfuration pathway (homocysteine is converted to cysteine). Homocysteine and methylmalonic acid are used for diagnosing subclinical B_{12} deficiency[29, 30].

Serum B_{12} may be elevated with inflammation and was postulated as a possible etiology for elevated concentrations in subjects with CF in early studies[27]. Elevated serum B_{12} was noted in subjects with CF and was associated with both elevated serum transcobalamin II and normal unsaturated B_{12} binding capacity. These investigators postulated the findings were related to hepatic dysfunction, recurrent pulmonary infection or increased turnover of myeloid cells. Elevated B_{12} levels can occur in chronic myelogenous (leukemic) conditions, renal failure and liver disease. We evaluated blood markers for inflammation, renal function, hepatic function and complete blood counts with differentials. High sensitivity-CRP concentration and white blood cell count (WBC) did not differ between B_{12} status groups. There were no subjects with a myeloproliferative disorder or renal disease. Liver enzymes were mildly elevated as expected in a group of subjects with CF and PI and were not associated with B_{12} status. There were no associations with hemoglobin, hematocrit, platelet count, liver enzymes, total protein, albumin, BUN or creatinine with serum B_{12} (data not shown). In this study there was no indication that serum B_{12} was acting as an acute phase reactant, or that it was related to other diagnoses.

The consequences of lifelong high dose supplement-based B_{12} and sustained elevated serum concentrations are unknown. In the non-CF medical setting, elevated serum B_{12} is an indication for a diagnostic work up to rule out serious disease[16, 31]. Our data demonstrate that with current CF care, patients take CF-specific vitamins containing high doses of B_{12} and many patients will likely have elevated serum B_{12}. Thus, the elevated B_{12} cannot serve as a sign of potential serious disease. Supplement-based B_{12} is in the form of cobalamin (cyanocobalamin). Cyanide is released intracellularly and is rapidly cleared and cyanide toxicity is not expected. While elevated pulmonary secretion cyanide levels have been reported in CF, these likely result from *Pseudomonas* and *Burkholderia* secretions[32]. In the current study, only one of seven subjects had a detectable cyanide level, and this was within the laboratory reference range. From these limited data, the risk of systemic cyanide exposure is likely low.

Of interest, FEV_1 differed between B_{12} status groups and persisted in the multiple logistic regression models after adjusting for age. In CF, FEV_1 declines with age; B_{12} dose increases with age, and the upper limit of serum B_{12} reference range declines with age. Other factors that may be related to the inversed relationship between B_{12} and FEV_1 include the observation that sicker patients may take more medication including supplements (more prescriptions and/or better adherence to prescribed treatment). Further study is required to

better understand mechanisms and clinical significance of the B_{12} and FEV_1 inversed correlation.

It is unclear if there are clinical benefits of B_{12} intake beyond that to sustain a normal B_{12} status. Scambi et al[33] reported improvements in phospholipid docosahexaenoic acid (DHA) status in young children with CF with daily 5-methyltetrahydrofolate (7.5 mg) and B_{12} (0.5 mg) supplementation in a 24 week intervention. The phospholipid DHA improvement was thought to be a result of the interaction of folate, B_{12}, phospholipid DHA and 1-methyl metabolic pathway[33]. The intakes of B_{12} and B_6 were orders of magnitude greater than recommended for healthy people. There are currently no specific CF-specific B-vitamin intake recommendations different than those for the general population. There is no known toxicity of vitamin B_{12}[12,13]. Chronic supplement based B_6 intake >1000 μg/d may increase the risk for peripheral neuropathy[13]. An adverse effect of high folic acid intake has the potential to mask co-incident B_{12} deficiency and associated pernicious anemia and progressive neurological damage[13]. Folic acid intakes were not unusually high in our sample of children.

In summary, total B_{12} intake (diet, supplement, PERT) was high, and largely due to high supplement-based B_{12} intake. The B_{12} and B_6 vitamin intake was higher than recommended, and provided no known benefit. Lifelong high B_{12} intake will result in sustained elevated serum B_{12}. The associated risks or benefits of prolonged high B_{12} intake and elevated serum B_{12} in people with CF is unknown. Studies are needed to determine the B_{12} dose that supports concentrations and status within the reference ranges and to evaluate possible B_{12}-related clinical outcomes across the increasing life span of people with CF. The inverse correlation between B_{12} and FEV_1 merits further investigation.

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ABBREVIATIONS AND ACRONYMS

Adj HAZ	Height-for-age Z score after height adjustment for mid-parent height
AI	Adequate Intake
BMI	Body mass index

BMIZ	BMI-for-age Z score
CF	Cystic fibrosis
CHOP	Children's Hospital of Philadelphia
CTRC	Clinical Translational Research Center
DHA	Docosahexaenoic acid
DRI	Dietary Reference Intake
EER	Estimated Energy Requirement
FEV_1	Forced expiratory volume at one second
Hi-B_{12}	Serum B_{12} above reference range for age and sex
HAZ	Height-for-age Z score
RR-B_{12}	Serum B_{12} within reference range for age and sex
hsCRP	High sensitivity C-reactive protein
PERT	Pancreatic enzyme replacement therapy
PI	Pancreatic insufficiency
RDA	Recommended Dietary Allowance
WAZ	Weight-for-age Z score

References

1. Carmel, R. Cobalamin (B12). In: Shils, ME.; Moshe, S.; Ross, AC., et al., editors. Modern Nutrition in Health and Disease. 10 th ed.. Lippincott Williams & Wilkins; Baltimore, MD: 2006. p. 482-97.
2. Gueant JL, Champigneulle B, Gaucher P, et al. Malabsorption of vitamin B12 in pancreatic insufficiency of the adult and of the child. Pancreas. 1990; 5:559–67. [PubMed: 2235967]
3. Harms HK, Kennel O, Bertele RM, et al. Vitamin B12 absorption and exocrine pancreatic insufficiency in childhood. Eur J Pediatr. 1981; 136:75–9. [PubMed: 7215391]
4. Cystic Fibrosis Foundation Patient Registry 2011 Annual Report. Bethesda, MD: 2012. http://www.cff.org/UploadedFiles/research/ClinicalResearch/2011-Patient-Registry.pdf
5. Carmel R, Hollander D, Gergely HM, et al. Pure human pancreatic juice directly enhances uptake of cobalamin by guinea pig ileum in vivo. Proc Soc Exp Biol Med. 1985; 178:143–50. [PubMed: 3966072]
6. Deren JJ, Arora B, Toskes PP, et al. Malabsorption of crystalline vitamin B12 in cystic fibrosis. N Engl J Med. 1973; 288:949–50. [PubMed: 4693247]
7. Veeger W, Abels J, Hellemans N, et al. Effect of sodium bicarbonate and pancreatin on the absorption of vitamin B12 and fat in pancreatic insufficiency. N Engl J Med. 1962; 267:1341–4. [PubMed: 13996533]
8. Lepage G, Yesair DW, Ronco N, et al. Effect of an organized lipid matrix on lipid absorption and clinical outcomes in patients with cystic fibrosis. J Pediatr. 2002; 141:178–85. [PubMed: 12183711]
9. Feskanich D, Sielaff BH, Chong K, et al. Computerized collection and analysis of dietary intake inflammation. Comput Methods Programs Biomed. 1989; 1:47–57. [PubMed: 2582746]
10. Institute of Medicine (U.S.). Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. National Academies Press; Washington, D.C.: 2005.

11. Trabulsi J, Ittenbach RF, Schall JI, et al. Evaluation of formulas for calculating total energy requirements of preadolescent children with cystic fibrosis. Am J Clin Nutr. 2007; 85:144–51. [PubMed: 17209190]
12. Otten, JJ.; Hellwig, JP.; Meyers, LD. DRI, dietary reference intakes : the essential guide to nutrient requirements. National Academies Press; Washington, D.C.: 2006.
13. Institute of Medicine (U.S.). Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. National Academy Press; Washington, D.C.: 1998.
14. Hicks JM, Cook J, Goodwin ID, et al. Vitamin B12 and Folate. Pediatric Reference Ranges. Arch Pathol Lab Med. 1993; 117:704–6. [PubMed: 8323433]
15. Soldin, SJ.; Wong, EC.; Brugnara, C., et al. Pediatric Reference Intervals. 7th ed.. ACC Press; Washington DC: 2011.
16. Wu, A. Tietz Clinical Guide to Laboratory Tests. 4th Edition ed.. W.B. Saunders Company; Elsevier Press; St. Louis, MO: 2006.
17. Lohman, TG.; Roche, AF.; Martorell, R. Anthropometric standardization reference manual. Human Kinetics Books; Champaign, IL: 1988.
18. Himes JH, Roche AF, Thissen D, et al. Parent-specific adjustments for evaluation of recumbent length and stature of children. Pediatrics. 1985; 75:304–13. [PubMed: 3969331]
19. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, et al. CDC growth charts: United States. Adv Data. 2000:1–27. [PubMed: 11183293]
20. Zapletal, A.; Šamánek, M.; Paul, T. Lung function in children and adolescents: methods, reference values. Karger; New York: 1987.
21. Wang X, Dockery DW, Wypij D Jr. et al. Pulmonary function between 6 and 18 years of age. Pediatr Pulmonol. 1993; 15:75–88. [PubMed: 8474788]
22. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. Am J Respir Crit Care Med. 1999; 159:179–87. [PubMed: 9872837]
23. Rhodes, DC.; Clemens, J.; Goldman, J., et al. What We Eat in America 2009-2010: United States Department of Agriculture. Agricultural Research Service; 2012.
24. Naimi D, Gueant JL, Hambaba L, et al. Gastric intrinsic factor hypersecretion stimulated by pentagastrin in cystic fibrosis. J Pediatr Gastroenterol Nutr. 1987; 6:899–903. [PubMed: 3681575]
25. Simpson RM, Lloyd DJ, Gvozdanovic D, et al. Vitamin B12 deficiency in cystic fibrosis. Acta Paediatr Scand. 1985; 74:794–6. [PubMed: 4050427]
26. Lindemans J, Neijens HJ, Kerrebijn KF, et al. Vitamin B12 absorption in cystic fibrosis. Acta Paediatr Scand. 1984; 73:537–40. [PubMed: 6464742]
27. Lindemans J, Abels J, Neijens HJ, et al. Elevated serum vitamin B12 in cystic fibrosis. Acta Paediatr Scand. 1984; 73:768–71. [PubMed: 6524365]
28. Borowitz D, Baker RD, Stallings V. Consensus report on nutrition for pediatric patients with cystic fibrosis. J Pediatr Gastroenterol Nutr. 2002; 35:246–59. [PubMed: 12352509]
29. Carmel R. Biomarkers of cobalamin (vitamin B-12) status in the epidemiologic setting: a critical overview of context, applications, and performance characteristics of cobalamin, methylmalonic acid, and holotranscobalamin II. Am J Clin Nutr. 2011; 94:348S–58S. [PubMed: 21593511]
30. Stabler SP. Clinical practice. Vitamin B12 deficiency. N Engl J Med. 2013; 368:149–60. [PubMed: 23301732]
31. Ermens AA, Vlasveld LT, Lindemans J. Significance of elevated cobalamin (vitamin B12) levels in blood. Clin Biochem. 2003; 36:585–90. [PubMed: 14636871]
32. Anderson RD, Roddam LF, Bettiol S, et al. Biosignificance of bacterial cyanogenesis in the CF lung. J Cyst Fibros. 2010; 9:158–64. [PubMed: 20156704]
33. Scambi C, De Franceschi L, Guarini P, et al. Preliminary evidence for cell membrane amelioration in children with cystic fibrosis by 5-MTHF and vitamin B12 supplementation: a single arm trial. PLoS One. 2009; 4:e4782. [PubMed: 19277125]

Table 1
Characteristics of Children with CF and PI by B_{12} Status Groups

	Serum B_{12} Groups			
	All	**Hi-B_{12}**	**RR-B_{12}**	**P-value**[1]
Number	106	59	47	
Age, yr	10.4 ± 3.0	11.0 ± 3.3	9.6 ± 2.6	0.02
Age > 12y, %	28	39	15	0.01
Sex, % male	57	51	64	0.18
WAZ	−0.38 ± 0.78	−0.48 ± 0.68	−0.24 ± 0.89	0.11
HAZ	−0.39 ± 0.92	−0.44 ±0.97	−0.32 ± 0.86	0.51
Adj HAZ [2]	−0.68 ± 0.89	−0.73 ± 0.92	−0.61 ± 0.85	0.49
BMIZ	−0.19 ± 0.77	−0.32 ± 0.68	−0.03 ± 0.85	0.05
FEV_1, % predicted [2]	96 ± 23	90 ± 23	103 ± 20	0.005

HAZ, height-for-age Z score; adjHAZ, height-for age Z score adjusted for mid-parental height; WAZ, weight-for-age Z score; BMIZ, body mass index-for-age Z score;

[1] Means ± SD and t-tests for normally distributed variables.

[2] n=103 (n=58 for Hi-B_{12}, n=45 for RR - B_{12}).

Table 2
Blood Biomarkers and Dietary Intake by B_{12} Status Group

	Serum B_{12} Groups			
	All	**Hi-B_{12}**	**RR-B_{12}**	**P-value[1]**
Blood Biomarker				
B_{12}, pg/mL[A]	1083 (371, 3455)	1414 (823, 3455)	853 (371, 1284)	<0.001
B_6, ng/mL[2,A]	16.9 (5.0, 91.7)	17.6 (5.0, 91.7)	15.4 (5.4, 39.4)	0.05
RBC folate, ng/mL[3,A]	496 (231, 1045)	509 (231, 857)	488 (278, 1045)	0.95
Homocysteine, μmol/L[A]	4.8 (2.2, 9.0)	4.7 (2.2, 8.9)	5.1 (2.8, 9.0)	0.01
Methionine, nmol/mL[A]	24.9 (11.1, 65.7)	23.5 (16.1, 65.7)	25.9 (11.1, 56.3)	0.46
Cysteine, nmol/mL	43.7 (12.4, 79.0)	46.5 (12.4, 66.3)	41.4 (28, 79)	0.01
hsCRP, mg/L [4]	0.4 (0.1, 48.4)	0.4 (0.1, 48.4)	0.4 (0.1, 5.5)	0.61
WBC, thou/μL [4]	7.1 (4.1, 17.8)	7.7 (4.5, 17.8)	6.9 (4.1, 16.7)	0.24
Dietary Intake				
Number	95	54	41	
Energy, kcal	2325 (865, 4909)	2359 (865, 4909)	2320 (1413, 4014)	0.94
%EER	115 (41, 228)	114 (41, 223)	117 (64, 228)	0.49
Total B_{12}, μg	18.4 (5.5,158.9)	21.9 (8.0, 158.9)	15.1 (5.3, 33.3)	<0.001
Total B_{12}, %RDA	1175 (310, 8826)	1391 (479, 8826)	1000 (310, 1872)	<0.001
Diet , μg	6.0 (1.5, 18.1)	6.4 (2.6, 18.1)	5.6 (1.5, 14.0)	0.17
Diet, %RDA	376 (79, 1085)	379 (79, 1085)	373 (83, 780)	0.80
Supplement, %RDA	667 (0, 8333)	1000 (250, 8333)	583 (0,1375)	<0.001
0 - 499 %RDA	15	11	20	
500 - 999 %RDA	47	35	63	
1000 - 1499 RDA	29	39	17	
1500+ %RDA	8	15	0	0.002
Total B_6, mg	4.6 (1.9, 78.2)	5.5 (1.9, 78.3)	3.9 (2.4, 8.3)	<0.001
Total B_6, %RDA	545 (243, 7827)	591 (243, 7827)	502 (247, 1131)	0.01
Diet, mg	1.9 (0.5, 8.8)	1.9 (0.7, 8.8)	1.8 (0.5, 5.6)	0.13
Diet, %RDA	217 (51, 931)	219 (59, 881)	214 (51, 931)	0.50
Supplement, mg/d	2.3 (0.0, 75.0)	3.0 (0.6, 75.0)	1.9 (0.0, 4.0)	0.01
Supplement, %RDA	317 (0, 7500)	317 (105, 7500)	300 (0, 425)	0.09
Total Folate, μg	755 (335, 1872)	933 (376, 1872)	616 (335, 1518)	<0.001
Total Folate, %RDA	281 (113, 840)	332 (128, 840)	227 (113, 506)	0.003
Diet, μg	413 (140, 1472)	451 (156, 1473)	385 (140, 1251)	0.03
Diet, %RDA	159 (39, 491)	167 (39, 491)	143 (47, 417)	0.14
Supplement, μg	400 (0, 1200)	400 (66, 1200)	200 (0, 800)	0.004
Supplement, %RDA	100 (0, 400)	133 (33, 400)	100 (0, 267)	0.02

hsCRP = high sensitivity C-reactive protein (<3 mg/L normal); WBC, white blood cell count; %EER, percent Estimated Energy Requirement; RDA = Recommended Dietary Allowance

[1]Means ± SD and t-tests for normally distributed variables; median (range) and Mann-Whitney for non-normally distributed variables.

[2]n=103 (n=58 for Hi-B_{12}, n=45 for RR - B_{12}).

[3]n=97(n=54 for Hi-B_{12}, n=43 for RR - B_{12}).

[4]n=102 (n=57 for Hi-B_{12}, n=45 for RR - B_{12}).

[A]**Biomarker Reference Ranges:** B_{12}, pg/mL: Males: 4-6.9 years: 245-1078; 7-9.9 years 271-1170; 10-12.9 years 183-1088; 13-18 years 214-865; >18 year old 199-732. Females: 4-6.9 years 313-1407; 7-9.9 years 247-1174; 10-12.9 years 197-1019; 13-18 years 182-820; >18 year old 199-732. (CHOP Clinical Laboratory, Philadelphia, PA). B_6, ng/mL: 5-30 (ARUP Laboratories, Salt Lake City, UT). Folate, ng/mL: 280-903 ng/mL (ARUP Laboratories, Salt Lake City, UT). Homocysteine , µmol/L: 6-10.9 years: 0.8-6.5. 11-16.9 years 5.7-11.7. >17 years 10.5-16.7 (ARUP Laboratories, Salt Lake City, UT). Methionine, nmol/mL: 8-49 nmol/mL (ARUP Laboratories, Salt Lake City, UT).

Table 3
Multiple Logistic Regression Model Predicting High Serum B_{12} Status Group

	Hi-B_{12} Status Group					
n=103	**Odds ratio**	**SE**	**Z**	**P**	**95% CI**	**Pseudo-R^2**
				<0.001		0.18
B_{12} supplement intake group, %RDA[1]	2.26	0.67	2.78	0.005	1.27-4.03	
Age, > 12 years	3.38	1.85	2.23	0.026	1.16-9.87	
Sex, female	2.34	1.11	1.80	0.072	0.93-5.93	
FEV_1, % predicted	0.98	0.01	−2.18	0.029	0.96-0.99	
Constant	0.30	0.47	−0.77	0.441	0.01-6.55	

[1] B_{12} supplementation intake categories, %RDA: category 1 = 0-499; category 2 = 500-999; category 3 = 1000-1499; category 4 = ≥1500