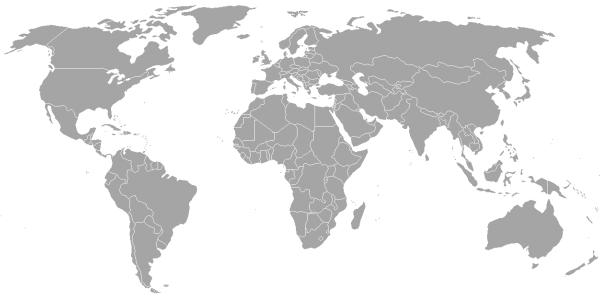
Eur J Gen Med 2015; 12(3):261 -266
DOI : 10.15197/ ejgm.01427

Case Report



Vitamin B12 Deficiency

Some Observations, Some Misconceptions

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ABSTRACT

Functional vitamin B_{12} deficiency is a syndrome where a wide variety of symptoms in the presence of "normal" serum levels of the vitamin respond to vitamin B_{12} therapy. A series of patients with functional vitamin B_{12} deficiency are described whose presenting features were drenching night sweats and fatigue. Reliance on serum vitamin B_{12} levels as a diagnostic test would have obscured the cause of their symptoms. Serum homocysteine and/or methlymalonic acid levels should be done in all patients with suspected B12 deficiency. Normal levels of these metabolites do not exclude diagnosis and empirical treatment may be justifiable in certain cases. Author also argues that oral vitamin B12 treatment results in suboptimal clinical response in a vast majority of patients and intramuscular route should be preferred in most patients.

Key words: *Vitamin B12, night sweats, homocysteine, mathylmalonic acid, autonomic dysfunction*

B12 Vitamini Eksikliği

Bazı Gözlemler, Bazı Yanılgılar

ÖZET

Fonksiyonel vitamin B_{12} eksikliği B_{12} tedaviye yanıtta vitaminin normal serum seviyelerinin mevcudiyetinde semptomlarının çok çeşitli olduğu bir sendromdur. Fonksiyonel B_{12} vitamini eksikliği olan bir dizi hastaların olan başvuru özellikleri gece terlemeleri ve yorgunluk derceleri burada açıklanmıştır. Bir tanı testi olarak serum vitamin B_{12} düzeylerine güven belirtilerin nedenini gizlemektedir. Serum homosistein ve/veya methlymalonic asit düzeyleri şüpheli B12 eksikliği olan tüm hastalarda yapılmalıdır. Bu metabolitlerin normal düzeyleri tanıyı dışlamaz ve ampirik tedavi bazı olgularda gerekli olabilir. Yazar hastaların çoğunluğunda vitamin B12 tedavisinin suboptimal klinik cevap ile sonuçlanabileceğini çoğu hastada intramuskuler yolun tercih edilmesi gerektiğini savunuyor.

Anahtar kelimeler: *Vitamin B12, gece terlemesi, homosistein, metilmalonik asit, otonomik disfonksiyon*

INTRODUCTION

Vitamin B_{12} is a water-soluble vitamin, made up of a central cobalt atom and a planar corrin ring. Two forms of vitamin B_{12} are biologically active as cofactors in enzyme reactions: adenosylcobalamin and methylcobalamin. Cobalamin is a required cofactor for two enzymes: the cytoplasmic methionine synthase and the mitochondrial methylmalonyl-CoA mutase. By converting homocysteine to methionine, methionine synthase prevents accumulation of homocysteine in tissues and serum. Generation of methionine allows for several methylation reactions necessary for nucleotide, DNA, RNA and protein synthesis (1). Vitamin B_{12} in food is released by the action of gastric acid and proteolytic enzymes in the stomach. Pancreatic enzymes and an alkaline pH in the upper small intestine degrade the haptocorrin-cobalamin complex. Released B_{12} is captured by another protein, intrinsic factor. Specific receptors in terminal ileum recognize this complex and internalize it. 1% of vitamin is also absorbed by passive diffusion. The complex is degraded in the entrocytes and attaches to another protein, transcobalamin II, termed holotranscobalamin (holo-TC). This complex is than released into the portal circulation and is the only form capable of entering different cells. Only 6-20% of total plasma vitamin B_{12} is

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Received: 24.08.2014, Accepted: 05.10.2014

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present in the "active" form bound to transcobalamin II. The remaining major portion is bound to transcobalamin I and III (2).

CASE 1

A 57-year old man was referred for assessment of 3-4 years history of drenching night sweats needing replacement of bed-sheets almost on a nightly basis. The sweating involved only the upper portion of his body from top of the head to mid chest around the level of the nipples and seemed to be worse after drinking alcohol. He denied any bowel symptoms, weight loss, fever, cough or skin rash. His past medical history was significant for hypertension treated with hydrochlorothiazide. He had a 27-pack year history of smoking, drank occasionally and did not use illicit drugs. Prior to his referral he was found to have normal serum levels of fasting glucose, urea, creatinin and electrolytes, liver enzymes and thyroid stimulating hormone (TSH) and T4. 24-hour urine catecholamines were measured in 2009 on 2 occasions and showed modest elevation of norepinephrine at 653 and 681nmol/d (0.0-505) and total catecholamines at 739 and 768nmol/d (0.0-630). Other indices of catecholamine metabolism including 5-hydroxyindolacetic-acid (5-HIAA) were normal. CT scan of the adrenal glands was reported normal. A repeat 24-hour urinary catecholamine measurement in 2011 was normal.

His BMI was 25, pulse rate 68/min and blood pressure 150/90mmHg without any postural hypotension. Examination of the cardiovascular, respiratory and abdominal systems was unremarkable. Vibration sense was reduced in both feet up to ankles. Other sensations were intact. Rest of the neurological examination was normal. Vitamin-B_{12} injections 1000mcg daily for 7 days followed by monthly injections were prescribed on the basis of elevated homocysteine levels. Celiac antibodies, antibodies to parietal cells and intrinsic factor were negative. Patient reported a dramatic response of his sweating after the second injection of vitamin-B_{12} and remained asymptomatic at 3 months follows up.

CASE 2

A 74-year old man was referred for episodic sweating, intense enough to require change of clothing or bed sheets, of 10 years duration. These episodes occurred one to three times a week and lasted 10 to 30 minutes. There was no diurnal variation. At times coffee or brandy would precipitate these episodes. He also had long-standing fatigue, muscle cramps and diarrhea; opening his bowels 3-5 times daily without any abdominal pain and had not noticed any blood or mucus in his stools. Two colonoscopies and a barium follow-through examination in the past had been reported normal. He denied palpitations, dizziness, heat-intolerance, fevers, dyspnea, cough or weight loss. His past medical history was significant for hypertension and an acute myocardial infarction several years ago that was treated medically. He was not a vegetarian. He did not smoke, drank occasionally and denied using illicit drugs. His medication included rosuvastatin, ramipril, ranitidine, allopurinol, atenolol, aspirin and vitamin B complex.

Blood pressure was 140/80mmHg, pulse 68/min and respiratory rate of 16/min. Examination of the cardiovascular, abdominal, respiratory and musculoskeletal system was unremarkable. Thyroid was not enlarged and there was no lymphadenopathy. Vibration sense was reduced in his feet but other sensations as well as motor system and cranial nerves were intact. Serum urea, creatinin, electrolytes, liver enzymes, calcium profile, thyroid function tests, and HbA1c results were normal. Measurement of 24-hour urine catecholamines showed modest elevation in metanephrine at 5.72µmol/d (0.80-

***Table**. Biochemical characteristics of patients with vitamin B12 deficincy*

	Hb (120-160 g/L)	MCV (82.0-97.0 fL)	WBC (4.1-10.0 ×109/L)	Platelets (150-400×109)	TSH (0.49-4.67 mIU/L)	Vitamin B12 (170-880 pmol/L)	Homocysteine (5.0-12.0 µmol/L)	Methylmalonic Acid (0.06-0.36 µM)
Case 1	*144*	*99.3*	*6.8*	*290*		*152*	*14.9*	
Case 2	*161*	*100.5*	*8.6*	*122*		*293*	*72.3*	
Case 3	*156*	*87.4*	*7.4*	*280*		*262*	*13.4*	*0.49*
Case 4	*156*	*88.6*	*7.4*	*319*	*1.66*	*361*	*7.1*	*0.23*
Case 5	*156*	*94.5*	*10.6*	*218*	*2.4*	*323*	*16.7*	

5.40) and normetanephrine at 4.87µmol/d (0.80-4.40). Urine 5-HIAA was normal. 24 hour Holtor monitor recording showed large number of premature ventricular complexes (PVCs) and a few short salvos of asymptomatic idioventricular rhythm. Dipyridamole tetrofosmin cardiac perfusion study showed no active ischemia.

Vitamin-B_{12} deficiency was diagnosed on the basis of elevated homocysteine levels. Vitamin-B_{12} injections 1000mcg daily were given for 7 days. He noticed marked improvement in his symptoms after the sixth injection. Diarrhea, fatigue and muscle cramps vanished altogether and sweating improved markedly. Treatment was continued with monthly B_{12} injections.

CASE 3

A 71-year-old woman was referred for assessment of extreme tiredness, dizziness and drenching night sweats of several years duration. The sweating episodes were mainly nocturnal and would at times require changing sheets. She denied palpitations, fevers, or weight loss. Her past medical history was significant for coronary artery disease, congestive heart failure, gastro-esophageal reflux disease, hypertension and hypothyroidism. She was taking sucralfate, nitroglycerine patch, amitriptyline, clopidogrel, spironolactone, furosemide, quinine sulphate, pantoprazole, levothyroxine, verapamil, atenolol and lorazepam. She did not smoke and drank alcohol socially. She was moderately obese. Blood pressure was 135/80mmHg whilst lying with a postural drop of 25/10mmHg. Rest of the physical examination including that of cardiovascular and nervous systems, was unremarkable.

Serum urea, creatinine, electrolytes, fasting glucose, liver enzymes and TSH were normal. Cosyntropin stimulation test showed adequate cortisol reserves. Echocardiogram showed normal left ventricular size and function. Dipyridamole tetrofosmin cardiac perfusion study did not show any evidence of active ischemia. Quinine sulphate, amitiptyline and spironolactone were discontinued with no improvement in her symptoms. Intramuscular vitamin-B_{12} at a dose of 1000mcg daily for 7 days was commenced and than maintained at monthly injections. She reported no dizziness or night sweats when reviewed several weeks later.

CASE 4

A 45-year-old man was diagnosed with multiple sclerosis and vitamin B_{12} deficiency in 2009. He had been on vitamin B_{12} injections for 2 years but than switched to oral vitamin B_{12} 1000µg daily. He presented with 4-month history of drenching night sweats primarily affecting head and face. He denied cough, fevers, or weight loss. He drank very little alcohol and did not smoke. Examination revealed pulse rate of 74/min and blood pressure of 140/90mmHg. Rest of the physical examination, including neurological examination was normal.

Urea, creatinine and electrolytes were in the normal range. Gastric parietal cell antibodies and intrinsic factor antibodies were negative. Oral vitamin B_{12} was replaced with intramuscular vitamin B_{12} 1000mcg monthly. When reviewed 4 months later, his symptoms had resolved completely.

CASE 5

A 47-year-old man presented with 6-month history of extreme fatigue. The family physician had found him to have vitamin D deficiency. He was commenced on vitamin D 2000 units daily but his symptoms did not resolve. He had no other significant past medical history, drank alcohol socially and smoked half a pack a day. He was moderately obese. Blood pressure was 152/92mmHg but had been normal a few days before his appointment when he was tested for obstructive sleep apnea. He had mild sleep apnea. Vitamin D was replaced with ergocalciferol 50,000 units per week and further tests were ordered. Urea, creatinine, electrolytes, and liver enzymes were normal. 25 hydroxy vitamin D levels were 66nmol/l (>75). Anti-tissue transglutaminase IgA, anti-deamidated gliadin protein IgA and anti-deamidated gliadin protein IgG were in the reference range. Vitamin B12 levels were in the normal range but homocysteine levels were elevated

1 mg of vitamin B_{12} was given via intramuscular route daily for 10 days followed by monthly injections. He was seen 3 weeks later and reported dramatic improvement in his symptoms. Over the next few months however, the frequency of injections had to be increased to every 2 weeks because of residual symptoms with satisfactory response.

DISCUSSION

There is widespread global prevalence of vitamin B_{12} deficiency. In addition to anemia, it can also present with a variety of neurologic and psychiatric manifestations in the absence of anemia (3). These include peripheral neuropathy, myelopathy, cognitive, and autonomic dysfunction. Sensory neuropathy manifesting as paraesthesias in the extremities and ataxia of gait is the commonest of all the neurological manifestations of vitamin-B12 deficiency. In advanced cases corticospinal tract involvement may lead to spastic paraparesis, termed sub acute combined degeneration of spinal cord. Dementia and amnesia are the most common cerebral syndromes associated with vitamin-B_{12} deficiency but psychiatric syndromes like depression, hypomania, agitation and psychosis have also been described. Urinary incontinence, impotence and orthostatic hypotension are well-recognized autonomic manifestations (4).

Although autonomic dysfunction due to vitamin-B_{12} deficiency is well recognized (5), vitamin-B_{12} deficiency has not been reported as a cause of drenching night sweats previously. This is surprising since changes in the peripheral autonomic nervous system may be the earliest manifestations of small-fiber neuropathy and hyperhidrosis frequently accompanies small-fiber peripheral neuropathy (6). Episodic hyperhidrosis also occurs commonly in patients with familial dysautonomia, a hereditary sensory and autonomic neuropathy (7). The sympathetic and parasympathetic components of heart rate variability have been found to be significantly lower in B12 deficient patients (8,9). Beitzke et al found major hemodynamic and autonomic impairment in patients with vitamin-B_{12} deficiency (10). Defective sympathetic activation and decreased catecholamine release has been postulated as pathogenic mechanisms. Reduction of sudomotor sympathetic unmyelinated fibers has been described in patients with vitamin-B_{12} deficiency and orthostatic hypotension (11). Similarly in patients with spinal cord injury at or above the level of T_6, spinal preganglionic sympathetic neurons disconnected from supra-spinal regulation, have been shown to exhibit episodic unchecked reflexes (12).

The exact mechanism of excessive sweating in vitamin-B_{12} deficiency is a matter of speculation and will require further studies. Spinal sympathetic overactivity is one plausible explanation and involvement of only the upper trunk in the first and fourth case case in our series would suggest segmental hyperactivity of sympathetic preganglionic neurons involving T1 to T12 segments. Sparing of proximal nerve segments in the dying-back neuropathy may be argued to cause sweating in these patient but will not explain the exclusive involvement of upper trunk only and would more likely cause sweating abnormalities in the proximal parts of both upper and lower limbs. Modest rise in urinary catecholamines in cases 2 and 3 would be consistent with sympathetic over-activity.

"Functional" vitamin B_{12} deficiency is a syndrome where a wide variety of symptoms in the presence of "normal" serum levels of the vitamin respond to vitamin B_{12} therapy. Megaloblastic anemia and sub-acute combined degeneration of spinal cord are only the extreme manifestations observed at the far end of the spectrum with severe deficiency. On the other hand, non-specific symptoms like fatigue are the earliest manifestations. Autonomic dysfunction, it seems, is a common manifestation of functional vitamin B_{12} deficiency and seems to occur early in the course of disease process. This spectrum of disease usually presents in the absence of any changes in red blood cell indices and is easily misdiagnosed since serum levels of vitamin are in the "normal" range. Indeed, short-term fluctuations in HCy and MMA levels may also result in normal levels of these metabolites (case 4 in our series), thus obscuring the deficiency. This has been described previously in a patient who had total absence of vibratory sensation in the iliac crest, knees and ankles and normal levels of vitamin and metabolites, which resolved completely after 2 months of vitamin B_{12} therapy (13). In this study, only 16% of patients with clinical response to cobalamin therapy had low serum levels of the vitamin and values were above 300pg/mL in 54% of cases. In addition, HCy and MMA values were in the normal range in 49% and 23% of cases respectively. Both metabolite levels were normal in 21% of cases. It has been suggested that measurement of holo-TC may be a more sensitive marker of functional vitamin deficiency since it is the only fraction that is internalized by cells via specific receptors. But the half-life of holo-TC is only 90 min and levels at any one time may be misleading (14).

In our patients, vitamin B_{12} deficiency was diagnosed on the basis of low vitamin levels in only one patient (case 1), low normal vitamin levels were found in the rest. Deficiency was diagnosed on the basis of elevated Hcy levels in them except one (case 4), in whom both vita-

min and Hcy levels were in the normal range. But despite normal levels and on oral replacement treatment, this patient responded only to intramuscular treatment. Case 3 had elevated levels of both Hcy and MMA but normal vitamin levels.

Despite widespread prevalence of vitamin B_{12} deficiency, its diagnosis is fraught with problems. Vitamin B_{12} status can be assessed by directly measuring the vitamin in the blood or by measuring the metabolites that accumulate as a result of deficiency. Vitamin B_{12} assay is usually the first step but both false positive and false negative results are common. It has been shown that 10% of patients with clinical or metabolic evidence of vitamin B_{12} deficiency have plasma or serum vitamin B_{12} levels of 150 to 221 nmol/L (15). Measurement of metabolites like total homocysteine (Hcy) and/or methylmalonic acid (MMA) provides an alternative method of diagnosing vitamin B12 deficiency. Levels of both Hcy and MMA are elevated in >98% of patients with clinical vitamin B_{12} deficiency (16). This study, however, used low vitamin B_{12} levels (<200pg/mL) as the diagnostic criteria for vitamin B_{12} deficiency and therefore would have included only patients with extreme deficiency.

Measurement of the transcobalamin fraction of vitamin B12 (holo-TC) approaches sensitivity and specificity about equivalent to total serum vitamin B_{12} and measurement of holo-TC in conjunction with vitamin B_{12} improves the predictive value for identifying vitamin B_{12} deficiency (17). Low serum holo-TC may be the earliest biochemical finding in functional vitamin B12 deficiency. These assays are, however, not yet widely available.

The preferred route of administration of vitamin B12 has been debated for a long time. Majority of cobalamin in the circulation is bound to haptocorrin and is unavailable for cellular uptake. Only cobalamin bound to transcobalamin is taken up by endocytosis mediated by the cell surface transcobalamin receptor. Only 6-20% of total plasma vitamin B_{12} is in the active form, bound to transcobalamin II (18). In our experience (case 4), oral cobalamin therapy did not improve health outcome. It improved only after oral route was switched to intramuscular route. Most of the studies of oral vitamin B_{12} therapy used serum levels of vitamin and its metabolites as the markers of response to therapy. However, correction of an abnormal laboratory value does not mean successful outcome. An objective improvement in health outcome is only meaningful if accompanied by a clinical response. For example, in a study of 80 patients over a 3-month period, although 80-90% of patients achieved normal serum cobalamin levels on an oral dose of 650 to 1000mcg daily, clinical improvement was observed only in 20 - 30% of patients (19). Similarly, in an open study of vitamin B_{12} deficiency related to food-cobalamin malabsorption in 10 patients, oral crystalline cobalamin was prescribed at a dose of 650mcg per day for at least 3 months. Normalization of vitamin levels was seen in 80% of patients, along with significant increase in hemoglobin levels and decrease in mean corpuscular volume but clinical improvement occurred only in 20% of patients (20). We can only speculate the reasons behind lack of response to oral vitamin B_{12} and it may be that cobalamin somehow undergoes a transformation in the portal circulation so is made less able to be internalized by the cells and only when cobalamin is able to bypass portal circulation, the cells internalize it. This seems to be a saturable process since a minority of patients does respond clinically to oral therapy.

National Health and Nutrition Evaluation Survey (NHANES) recommend using at least two markers, at least one biomarker of circulating concentrations of vitamin B_{12} (vitamin B_{12} or holo-TC) and one biomarker of functional vitamin B_{12} status (MMA or Hcy) (21). The panel advised that vitamin B_{12} deficiency should be diagnosed only when both markers are abnormal. This approach however, will result in under-diagnosis of vitamin B_{12} deficiency and since untreated deficiency can result in serious morbidity and even permanent neurological damage, it would therefore, be prudent to treat empirically all those with suggestive clinical findings and “normal” biochemistry with a close follow up for clinical response.

Functional vitamin B_{12} deficiency is common and a major cause of morbidity. It can manifest with a wide variety of symptoms including fatigue and drenching night sweats. Low threshold should be kept to measure markers of vitamin B_{12} deficiency in patients presenting with these symptoms. In some patients empirical vitamin treatment will be justified if no other cause of symptoms is found and levels of metabolites are in the normal range.

Low vitamin B_{12} levels and increased levels of HCy and MMA are not sensitive markers of functional vitamin B_{12} deficiency. Further research is needed to identify true markers of early vitamin B_{12} deficiency. Holo-TC may be one such candidate. Oral vitamin B_{12} treatment results

in suboptimal clinical response in a vast majority of patients and intramuscular route should be preferred in most patients. Future research of vitamin B12 treatment should compare clinical rather than biochemical responses of oral and intramuscular therapy vitamin therapy.

REFERENCES

1. *Krautler B. Biochemistry of B12-cofactors in human metabolism. Subcell Biochem 2012;56:323-46.*
2. *Herrmann W, Obeid R, Schorr H, Geisel J. Functional vitamin B12 deficiency and determination of holotranscobalamin in populations at risk. Clin Chem Lab Med 2003;41:1478-88.*
3. *Stabler SP. Vitamin B12 deficiency. N Eng J Med 2013;368:149-60.*
4. *Kumar N. Handbook Clin Neurol 2014;120:915-26.*
5. *Beitzke M, Pfister P, Fortin J, Skrabal F. Automic dysfunction and hemodynamics of vitamin B12 deficiency. Autonomic Neurosci Basic Clin 2002;97:45-54.*
6. *Low VA, Sandroni P, Fealey RD, Low PA. Detection of small-fiber neuropathy by sudomotor testing. Muscle Nerve 2006;34:57-61.*
7. *Slaugenhaupt SA, Blumenfeld A, Gill SP, et al. Tissue-specific expression of a splicing mutation in the IKBKAP gene causes familial dysautonomia. Am J Hum Genet 2001;68:598-605.*
8. *Sözen AB, Demirel S, Akkaya V, et al. Autonomic dysfunction in vitamin B12 deficiency: a heart rate variability study. J Auton Nerv Syst 1998;71:25-7.*
9. *Aytemir K, Aksoyek S, Buyukasik Y, et al. Assessment of autonomic nervous system functions in patients with vitamin B12 deficiency by power spectral analysis of heart rate variability. Pacing Clin Electrophysiol 2000;23:975-8.*
10. *Beitzke M, Pfister P, Fortin J, Skrabal F. Autonomic dysfunction and hemodynamics in vitamin B12 deficiency. Autonomic Neurosci: Basic and Clin 2002;97:45-54.*
11. *Toru S, Yokota T, Inaba A, et al. Autonomic dysfunction and orthostatic hypotension caused by vitamin B12 deficiency. J Neurol Neurosurg Psychiatry 1999;66:804-5.*
12. *Weaver LC. What causes autonomic dysreflexia after spinal cord injury? Clin Auton Res 2002;12:424-6.*
13. *Solomon LR. Cobalamin-responsive disorders in the ambulatory care setting: unreliability of cobalamin, methylmalonic acid, and homocysteine testing. Blood 2005;105:978-85.*
14. *Carmel R. Diagnosis and management of clinical and subclinical cobalamin deficiencies: Why controversies persist in the age of sensitive metabolic testing. Biochimie 2013;95:1047-55.*
15. *Stabler SP, Allen RH, Savage DG, Lindenbaum J. Clinical spectrum and diagnosis of cobalamin deficiency. Blood 1990;76:871-81.*
16. *Savage DG, Lindenbaum J, Stabler SP, Allen RH. Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin and folate deficiencies. Am J Med 1994;96:239-46.*
17. *Miller JW, Garrod MG, Rockwood AL, et al. Measurement of total vitamin B12 and holotranscobalamin, singly and in combination, in screening for metabolic vitamin B12 deficiency. Clin Chem 2006;52:278-85.*
18. *Hannibal L. DiBello PM. Jacobsen DW. Clin Chem Lab Med 2013;51:477-88.*
19. *Andrès E, Perrin AE, Demangeat C, et al. The syndrome of food-cobalamin malabsorption revisited in a department of internal medicine. A monocentric cohort study of 80 patients. Eur J Intern Med 2003;14:221-6.*
20. *Andrès E, Kurtz JE, Perrin AE, et al. Oral cobalamin therapy for the treatment of patients with food-cobalamin malabsorption. Am J Med 2001;111:126-9.*
21. *Yetley EA, Pfeiffer CM, Phinney KW, et al. Biomarkers of vitamin B-12 status in NHANES: a roundtable summary. Am J Clin Nutr 2011;94 (Suppl):313S-321S.*