Diabetes as a Cause of Clinically Significant Functional Cobalamin Deficiency

LAWRENCE R. SOLOMON, MD

OBJECTIVE—Functional cobalamin (Cbl) deficiency (i.e., high methylmalonic acid [MMA] values despite normal serum Cbl levels) is common in the elderly and associated with neuropathy and anemia. Because diabetes is also common in the elderly and diabetic neuropathy resembles that of Cbl deficiency, the role of diabetes in functional Cbl deficiency was explored.

RESEARCH DESIGN AND METHODS—A retrospective review was performed of all ambulatory community-dwelling adults with normal renal function evaluated for Cbl deficiency over a 12-year period in a primary care setting. Functional Cbl deficiency was defined as MMA values >250 nmol/L with Cbl levels >400 pg/mL.

RESULTS—In nondiabetic subjects, MMA values varied directly with age and inversely with serum Cbl. In diabetic subjects, MMA values also increased with age but did not fall as Cbl levels increased. Thus, when Cbl levels were >400 pg/mL, mean MMA values and the incidence of functional Cbl deficiency were both significantly greater in elderly diabetic subjects (at least 70 years old) than in elderly nondiabetic subjects. Moreover, neuropathy was present in 62% of diabetic subjects with high MMA values and in only 18% of diabetic subjects with normal MMA values. Finally, pharmacologic doses of Cbl improved MMA values and neuropathy in 88 and 86% of evaluable diabetic subjects, respectively.

CONCLUSIONS—These observations suggest that functional Cbl deficiency is common in elderly diabetic individuals, is associated with neuropathy, and is responsive to Cbl therapy. A role for oxidative stress in the pathogenesis of functional Cbl deficiency is proposed.

Diabetes Care 34:1077–1080, 2011

Cobalamin (Cbl) deficiency is common in elderly subjects who may have nonspecific neurologic complaints or appear clinically normal (1). Elevated levels of the Cbl-dependent metabolites, methylmalonic acid (MMA) and total homocysteine (tHcy), in asymptomatic patients with low or low-normal serum Cbl levels have been termed "subtle" or "preclinical" Cbl deficiency, suggesting a continuum of Cbl depletion beginning with decreased vitamin stores leading then to metabolite accumulation and, finally, to clinical disease (2). However, 7–30% of diverse elderly populations have "functional" Cbl deficiency, defined by elevated metabolite values despite Cbl levels well within the normal reference range, and this disorder has been linked to decreased cognitive function, anemia, and neuropathy (3–9).

Functional Cbl deficiency might also result from a disorder common in the elderly. Diabetes is a particularly attractive candidate in this regard because it is associated with sensory and autonomic neuropathies similar to those seen in Cbl deficiency. Moreover, increased tHcy levels have been linked to diabetic neuropathy, and pharmacologic doses of Cbl alone or combined with other vitamins may improve peripheral and autonomic nerve function in diabetic humans (3,10).

More recently, a 10-year retrospective review of symptomatic ambulatory subjects evaluated for Cbl deficiency found 9 of the 30 patients who had neurologic improvement with Cbl therapy had diabetes (30%) and 8 of these 9 individuals (88%) had functional Cbl deficiency (11). Thus, the roles of diabetes and patient age in functional Cbl deficiency were evaluated in the total population (both symptomatic and asymptomatic) screened during this period and in the 2 years following.

RESEARCH DESIGN AND METHODS

Laboratory methods

Because tHcy levels increase in many settings, MMA was used as a more specific index of Cbl deficiency (3). Serum Cbl and MMA were measured as previously described (normal reference ranges: 200–1,100 pg/mL and 90–250 nmol/L, respectively) (11). When MMA and Cbl were measured on more than one occasion within a 4-week period without a change in treatment, the average values were used for analysis. Functional Cbl deficiency was defined as MMA levels >250 nmol/L when serum Cbl values were >400 pg/mL.

Patients

A retrospective review of the medical records of all ambulatory community-dwelling patients evaluated for Cbl deficiency between 1 August 1993 and 30 June 2005 was conducted as previously described (11). In a preliminary analysis of nondiabetic subjects with Cbl values >400 pg/mL, the geometric mean MMA value in subjects aged <60 years (173 pg/mL) was similar to that in those aged 60–69 years (187 pg/mL) but was significantly lower than that in subjects at least 70 years old (225 pg/mL; $P < 0.002$). Thus, subjects were divided into those aged <70 years and those at least 70 years old. Because MMA values increase in renal insufficiency, individuals with serum creatinine values >1.4 mg/dL were excluded from analysis (3). For a brief period, normal MMA values were reported as "less than 400 nmol/L" and these patients were also excluded from analysis ($n = 19$).

Concurrent Cbl and MMA values were obtained in 370 individuals on 432

From the Section of Palliative Care, Department of Medicine, Yale University School of Medicine, New Haven, Connecticut.
Corresponding author: Lawrence R. Solomon, lawrence.solomon@yale.edu.
Received 3 January 2011 and accepted 16 February 2011.
DOI: 10.2337/dc11-0009

occasions, including 47 individuals with type 2 diabetes evaluated on 59 occasions. Fifty-four patients who were not receiving Cbl therapy were evaluated on more than one occasion at least 1 year apart. Patients were considered to have diabetes if they were receiving treatment with hypoglycemic agents or if they had a fasting blood glucose level >125 mg/dL or an HbA_{1c} level >6.4%.

Treatments of diabetic patients included diet alone in 14 (30%), insulin in 11 (23%), a sulfonylurea in 15 (32%), a thiazolidinedione in 4 (9%), and metformin in 9 (19%), with more than one agent used in 6 subjects (13%). This study conforms to the principles of the Declaration of Helsinki of 1975 as revised in 2008, and the institutional human investigation committee determined that further review was not required.

Cbl treatment

Patients were treated with cyanocobalamin 2 mg/day orally or 1 mg i.m. three times a week for 2 weeks, weekly for 8 weeks, and monthly thereafter, and were reevaluated within 1 to 3 months. Based on the intraindividual variability of metabolite levels in this population, decreases of MMA were considered significant if they were >116 nmol/L (i.e., >1 SD above the mean intraindividual variability of MMA) or between 64 and 116 nmol/L (i.e., between the mean and 1 SD above the mean intraindividual variability of MMA) to a value of <251 nmol/L (11). Neurologic improvement was considered to be significant if paresthesias completely resolved, observed ataxic gait became normal, and/or vibratory sensation that was completely absent at least in the ankles before therapy became easily detectable within 6 months of treatment.

Statistical methods

Population studies suggest skewed distributions for Cbl, MMA, and creatinine (12). Thus, geometric means, two-tailed Student *t* tests using log-transformed data, χ^2 values, and Spearman correlation coefficients were determined using StatPlus:mac 5.7 software (AnalystSoft, Vancouver, BC, Canada).

RESULTS

Effects of age and diabetes status on MMA values

Older individuals with Cbl values >400 pg/mL had mean MMA values that were 51 and 26% higher than their younger counterparts in diabetic and nondiabetic populations, respectively (Table 1). The incidence of functional Cbl deficiency was also greater in older than in younger individuals within the diabetic (8 of 11 [73%] vs. 6 of 17 subjects [35%]) and nondiabetic (19 of 47 [40%] vs. 17 of 73 subjects [23%]) populations ($P \leq 0.05$).

Diabetic subjects with Cbl levels >400 pg/mL had higher mean MMA values than nondiabetic subjects in both age-groups, and this difference was statistically significant in the older population (Table 1). However, mean values for Cbl and creatinine were not significantly different in diabetic and nondiabetic subjects of comparable age. Functional Cbl deficiency was present in 8 of 11 older diabetic subjects (73%) compared with 19 of 47 older nondiabetic subjects (40%; $P < 0.05$).

Table 1—*Effect of diabetes status relative to patient age on mean MMA values in subjects with Cbl levels >400 pg/mL*

	Age <70 years			Age at least 70 years		
Variable	Diabetic *n* = 17	Nondiabetic *n* = 73	*P*	Diabetic *n* = 11	Nondiabetic *n* = 47	*P*
MMA (nmol/L)	209	178	NS	315*	225†	0.03
Creatinine (mg/dL)	1.0	0.9	NS	1.1	1.0	NS
Cbl (pg/mL)	537	563	NS	622	648	NS
Age (years)	54	48	NS	76	77	NS

Values are geometric means for the number of subjects, and *P* values compare individuals with and without diabetes in the same age-group. NS, not significant. *$P < 0.05$ compared with diabetic subjects aged <70 years. †$P < 0.0025$ compared with nondiabetic subjects aged <70 years.

Determinants of MMA in diabetic and nondiabetic subjects

Age was a significant determinant of MMA in both diabetic and nondiabetic populations (Table 2). The relationship between age and MMA was particularly strong when Cbl levels exceeded 400 pg/mL (diabetes: $r = +0.52$, $P < 0.006$, $n = 28$; nondiabetes: $r = +0.30$, $P < 0.001$, $n = 120$). Cbl was a significant determinant of MMA only in nondiabetic subjects, but creatinine was not a significant determinant of MMA in either population.

Effect of Cbl therapy on MMA values

MMA responses to Cbl therapy were evaluable in 24 of 29 diabetic patients (83%) and in 110 of 173 nondiabetic patients (64%) with MMA values >250 nmol/L. Of these 134 patients, 77 received parenteral therapy (57%). Metabolic responses to Cbl therapy occurred in 115 of 134 patients (86%) overall (Table 3). MMA values fell into the normal range in 110 patients (81%). The response rate was not affected by diabetes status, patient age, or serum Cbl level (Table 3).

An additional eight diabetic subjects with renal insufficiency and high MMA values, despite normal serum Cbl levels, received Cbl therapy, including seven with creatinine values of 1.7 to 2.3 mg/dL and one individual receiving chronic hemodialysis with a creatinine value of 9.4 mg/dL. MMA values decreased significantly in five patients (63%) and returned to a normal value in three (38%; data not shown).

Neuropathy in diabetic subjects and the effects of Cbl therapy

Of the 47 diabetic subjects, 17 had no neurologic findings, 15 had neuropathy

Table 2—*Determinants of MMA in diabetic and nondiabetic populations*

	Diabetic (*n* = 57)*		Nondiabetic (*n* = 369)†	
Determinant	*r*	*P*	*r*	*P*
Cbl (pg/mL)	+0.03	NS	−0.31	<0.001
Age (years)	+0.38	<0.005	+0.15	<0.005
Creatinine (mg/dL)	+0.07	NS	+0.08	NS

Spearman correlation coefficients were determined as described in the RESEARCH DESIGN AND METHODS. *Omits two diabetes outliers with MMA values of 1,309 and 1,829 nmol/L. †Omits four nondiabetic outliers with MMA values of 1,319, 3,279, 1,928, and 4,911 nmol/L.

Table 3—*Metabolic responses to Cbl therapy in patients with elevated MMA values*

	Age <70 years		Age at least 70 years		All ages	
Cbl	Diabetic	Nondiabetic	Diabetic	Nondiabetic	Diabetic	Nondiabetic
<401 pg/mL	8/8 (100)	51/57 (89)	3/3 (100)	26/30 (87)	11/11 (100)	77/87 (89)
>400 pg/mL	4/6 (67)	10/14 (71)	6/7 (86)	7/9 (86)	10/13 (77)	17/23 (74)
All	12/14 (86)	61/71 (86)	9/10 (90)	33/39 (85)	21/24 (88)	94/110 (85)

Data are presented as *n* (%). Responses to treatment with cyanocobalamin are as defined in the RESEARCH DESIGN AND METHODS.

that was unexplained except for the presence of diabetes, and 15 had neuropathy that was possibly multifactorial. When the latter group was excluded from analysis, 21 of the remaining 32 patients had high MMA values (63%), and 13 of these patients had neuropathy (62%). In contrast, only 2 of the 11 patients (18%) with normal MMA values had neurologic signs or symptoms ($P < 0.02$).

At least 3 months of Cbl treatment was given to 14 diabetic subjects with neuropathy and increased MMA values, and significant responses were noted in 9 of 10 subjects (90%) with otherwise unexplained neuropathy and in 3 of 4 (75%) with multifactorial neuropathy, for an overall response rate of 86% (Table 4). Pretreatment Cbl levels were within the normal range in all 14 subjects and were >400 pg/mL in 9 subjects, including 8 of the 12 responders (75%). One evaluable subject with neuropathy received metformin (patient 13, Table 4).

CONCLUSIONS—Despite associations of high tHCy values with diabetic neuropathy and reports of clinical responses of diabetic neuropathy to Cbl therapy, the role of diabetes as a possible cause of functional Cbl deficiency has not been evaluated (3,13). Indeed, only three studies have reported MMA values in diabetic subjects, and these were limited by the lack of an age-matched control group, the presence of renal failure, or a focus on frank diet-related Cbl deficiency (3,13).

The current study confirms the association between age and functional Cbl deficiency in nondiabetic subjects and extends this observation to the diabetic population (Tables 1 and 2). Moreover, elderly diabetic patients with Cbl values >400 pg/mL had higher mean MMA values than both younger diabetic individuals and elderly nondiabetic individuals, suggesting an additive effect of diabetes and age on the risk of functional Cbl deficiency (Table 1). Differences between individuals with and without diabetes were not explained by differences in Cbl, age, or creatinine level, and pharmacologic doses of Cbl consistently decreased MMA values in all groups (Tables 1 and 3).

The clinical significance of functional Cbl deficiency is controversial. Associations with cognitive dysfunction, anemia, and neuropathy have been reported, and clinical responses to Cbl therapy have been noted (3,6,11,14). In contrast, clinical responses were not seen in six therapeutic trials (3,15–17). The current study indeed suggests that functional Cbl deficiency in diabetic subjects is clinically significant because neuropathy was more common when MMA values were high, and Cbl therapy improved neuropathy in 86% of evaluable patients (Table 4). Similar neurologic responses to Cbl therapy in diabetic subjects have been previously reported, but measures of Cbl and MMA were not obtained (3,10).

The mechanism of functional Cbl deficiency in diabetes and aging is unknown. Interestingly, functional Cbl deficiency in some inborn errors of Cbl metabolism results from the impaired reduction of Cbl from the Co^{III} transport form to the active Co^{I} or Co^{II} coenzyme states, and correction of metabolic and clinical abnormalities in these disorders requires extremely high doses of parenteral Cbl (18). Moreover, Cbl is particularly susceptible to oxidation, and enzymatic reduction is also required for the decyanation of therapeutic cyanoCo^{III}Cbl (19–21).

Because aging and diabetes are both associated with increased oxidative stress, a role for oxidative stress in the pathogenesis of functional Cbl deficiency and for the requirement of pharmacologic doses of Cbl in this setting is proposed (Fig. 1) (22–24). This hypothesis also implies that treatment with a reduced form of Cbl (e.g., methylcobalamin) may have an advantage over the oxidized forms of Cbl that are usually administered (i.e., cyanocobalamin and hydroxycobalamin). Indeed, high doses of methylcobalamin have been reported to improve diabetic neuropathy, but the relationship of these responses to functional Cbl deficiency has not been studied (3,25).

Table 4—*Neurologic responses to Cbl therapy in diabetic subjects with high MMA values*

				MMA		Neurologic responses			
Patient	Age	Sex	Cbl	Pre-Rx	Post-Rx	Any	Paresthesias	Ataxic gait	Vibration sense
1	71	F	916	306	222	Yes*	Resolves	ND	Returns†
2	68	M	706	318	199	Yes‡	Resolves	ND	Returns†
3	77	M	468	282	166	Yes§	Resolves	ND	ND
4	72	M	370	296	143	Yes	Resolves	ND	ND
5	82	M	422	360	253	Yes*	NA	ND	Returns†
6	65	F	444	406	313	Yes	Resolves	ND	Returns†
7	59	F	377	353	89	Yes‡	Resolves	Resolves	ND
8	56	F	587	277	179	Yes	Resolves	Improves	NA
9	58	F	322	354	126	Yes	NA	Resolves	Normal
10	53	M	800	342	189	No	No change	No change	ND
11	53	F	406	265	178	Yes	NA	Improves	Returns†
12	76	M	721	251	ND	Yes	Improves	Improves	Returns†
13	68	M	322	269	200	Yes	Resolves	ND	Returns†
14	53	M	400	450	200	No	No change	No change	ND

Diabetes was the only apparent cause for neuropathy in patients 1–10, whereas other causes for neuropathy were present in patients 11–14. Patients 8 and 13 were treated with cyanocobalamin orally, and the other 12 subjects received intramuscular cyanocobalamin as described in the RESEARCH DESIGN AND METHODS. F, female; M, male; NA, not applicable because of normal pretherapy findings; ND, not tested; Rx, pharmacologic therapy. *Orthostatic hypotension also resolved. †Vibration sensation returned to the ankles in patients 2, 6, 12, and 13; to both the knees and ankles in patients 5, 7, and 11; and to the hips, knees, and ankles in patient 1. ‡Restless legs also resolved. §Light touch sensation also returned to the legs.

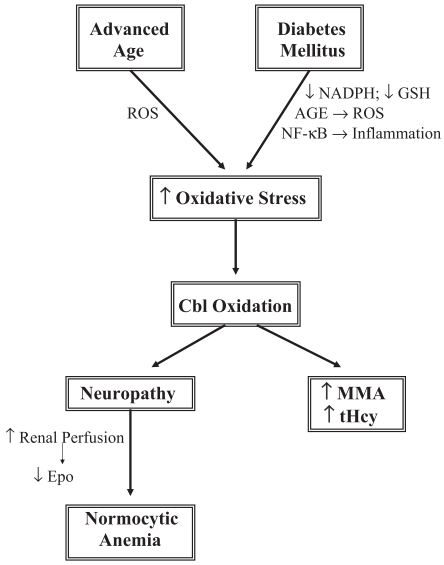

Figure 1—*Oxidative stress as a mediator of functional Cbl deficiency. AGE, advanced glycation end products; Epo, erythropoietin; GSH, reduced glutathione; NF-κB, nuclear factor-κB; ROS, reactive oxygen species.*

This study is the first to report MMA values in diabetic subjects with normal Cbl levels and provide information on the Cbl responsiveness of neuropathy in relation to the presence of functional Cbl deficiency. Future studies of larger numbers of subjects with randomized, placebo-controlled trials of Cbl therapy are required to confirm and extend these observations.

Acknowledgments—No potential conflicts of interest relevant to this article were reported.

L.R.S. designed the research, analyzed data, and prepared the manuscript.

This study was presented in abstract and poster form at the 47th Annual Meeting of the American Society of Hematology, Atlanta, Georgia, 12 December 2005.

The author thanks D. Bonke (Merck, Darmstadt, Germany) for his suggestions regarding the possible impact of oxidative pathways on Cbl, and R. Green (Department of Medical Pathology and Laboratory Medicine, University of California Davis Medical Center, Sacramento, CA) for his suggestion regarding the potential therapeutic use of methylcobalamin.

References

1. Stabler SP, Allen RH. Vitamin B12 deficiency as a worldwide problem. Annu Rev Nutr 2004;24:299–326
2. Carmel R. Current concepts in cobalamin deficiency. Annu Rev Med 2000;51:357–375
3. Solomon LR. Disorders of cobalamin (vitamin B12) metabolism: emerging concepts in pathophysiology, diagnosis and treatment. Blood Rev 2007;21:113–130
4. Tangney CC, Tang Y, Evans DA, Morris MC. Biochemical indicators of vitamin B12 and folate insufficiency and cognitive decline. Neurology 2009;72:361–367
5. Nardin RA, Amick ANH, Raynor EM. Vitamin B(12) and methylmalonic acid levels in patients presenting with polyneuropathy. Muscle Nerve 2007;36:532–535
6. Turner MR, Talbot K. Functional vitamin B12 deficiency. Pract Neurol 2009;9:37–41
7. Lorenzl S, Vogeser M, Müller-Schunk S, Pfister HW. Clinically and MRI documented funicular myelosis in a patient with metabolical vitamin B12 deficiency but normal vitamin B12 serum level. J Neurol 2003;250:1010–1011
8. Morris MS, Jacques PF, Rosenberg IH, Selhub J. Folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. Am J Clin Nutr 2007;85:193–200
9. Graber JJ, Sherman FT, Kaufmann H, Kolodny EH, Sathe S. Vitamin B12-responsive severe leukoencephalopathy and autonomic dysfunction in a patient with "normal" serum B12 levels. J Neurol Neurosurg Psychiatry 2010;81:1369–1371
10. Talaei A, Siavash M, Majidi H, Chehrei A. Vitamin B12 may be more effective than nortriptyline in improving painful diabetic neuropathy. Int J Food Sci Nutr 2009;60(Suppl. 5):71–76
11. Solomon LR. Cobalamin-responsive disorders in the ambulatory care setting: unreliability of cobalamin, methylmalonic acid, and homocysteine testing. Blood 2005;105:978–985
12. Vogiatzoglou A, Oulhaj A, Smith AD, et al. Determinants of plasma methylmalonic acid in a large population: implications for assessment of vitamin B12 status. Clin Chem 2009;55:2198–2206
13. Herrmann W, Schorr H, Obeid R, Makowski J, Fowler B, Kuhlmann MK. Disturbed homocysteine and methionine cycle intermediates S-adenosylhomocysteine and S-adenosylmethionine are related to degree of renal insufficiency in type 2 diabetes. Clin Chem 2005;51:891–897
14. Nilsson K, Gustafson L, Hultberg B. Improvement of cognitive functions after cobalamin/folate supplementation in elderly patients with dementia and elevated plasma homocysteine. Int J Geriatr Psychiatry 2001;16:609–614
15. Eussen SJ, de Groot LC, Joosten LW, et al. Effect of oral vitamin B-12 with or without folic acid on cognitive function in older people with mild vitamin B-12 deficiency: a randomized, placebo-controlled trial. Am J Clin Nutr 2006;84:361–370
16. Kwok T, Tang C, Woo J, Lai WK, Law LK, Pang CP. Randomized trial of the effect of supplementation on the cognitive function of older people with subnormal cobalamin levels. Int J Geriatr Psychiatry 1998;13:611–616
17. Seal EC, Metz J, Flicker L, Melny J. A randomized, double-blind, placebo-controlled study of oral vitamin B12 supplementation in older patients with subnormal or borderline serum vitamin B12 concentrations. J Am Geriatr Soc 2002;50:146–151
18. Rosenberg DS, Fenton WA. Inherited disorders of folate and cobalamin transport and metabolism. In *The Online Metabolic and Molecular Bases of Inherited Disease* [Internet]. Valle D, Beaudet A, Vogelstein B, Kinzler K, Antonarakis S, Ballabio A, Eds. Available from http://www.ommbid.com/. Accessed 6 May 2009
19. Wolthers KR, Scrutton NS. Cobalamin uptake and reactivation occurs through specific protein interactions in the methionine synthase-methionine synthase reductase complex. FEBS J 2009;276:1942–1951
20. Kim J, Gherasim C, Banerjee R. Decyanation of vitamin B12 by a trafficking chaperone. Proc Natl Acad Sci USA 2008;105:14551–14554
21. Birch CS, Brasch NE, McCaddon A, Williams JHH. A novel role for vitamin B(12): cobalamins are intracellular antioxidants in vitro. Free Radic Biol Med 2009;47:184–188
22. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 2005;54:1615–1625
23. Kregel KC, Zhang HJ. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. Am J Physiol Regul Integr Comp Physiol 2007;292:R18–R36
24. Anderson EJ, Kypson AP, Rodriguez E, Anderson CA, Lehr EJ, Neufer PD. Substrate-specific derangements in mitochondrial metabolism and redox balance in the atrium of the type 2 diabetic human heart. J Am Coll Cardiol 2009;54:1891–1898
25. Sun Y, Lai MS, Lu CJ. Effectiveness of vitamin B12 on diabetic neuropathy: systematic review of clinical controlled trials. Acta Neurol Taiwan 2005;14:48–54