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## REVIEW

# Disorders of cobalamin (Vitamin B12) metabolism: Emerging concepts in pathophysiology, diagnosis and treatment

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**KEYWORDS**

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Homocysteine;  
Vitamin B12;  
Diabetes mellitus

**Summary** Although cobalamin (vitamin B12) was isolated almost 60 years ago, its biochemical, physiologic and neurologic effects remain incompletely defined. New observations suggest renal regulation of cobalamin metabolism; actions of cobalamin on nucleic acid and protein function; and a role for cobalamin in cytokine and growth factor regulation. Clinically, no gold standard has emerged for the diagnosis of cobalamin deficiency. Moreover, cobalamin resistance may occur in diabetes, renal insufficiency and advanced age, leading to functional cobalamin deficiency despite adequate cobalamin nutrition. Finally, high-dose cobalamin therapy may have salutary pharmacologic effects on neurologic function in a variety of disorders. Many studies lacked appropriate control groups. However, at this time, therapeutic trials with pharmacologic doses of cobalamin are suggested when findings consistent with cobalamin deficiency are present regardless of the results of diagnostic tests. While oral cobalamin immediate-release is adequate for many patients, its effectiveness in reversing neurologic abnormalities has yet to be established.

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## Introduction

Past reviews of cobalamin (Cbl)(Vitamin B12) deficiency highlight diagnostic approaches to this disorder; the role of food Cbl malabsorption in Cbl depletion; and the presence of a “subtle” preclinical deficiency state.<sup>1–4</sup> Recent studies suggest unique biochemical and physiologic actions of Cbl; limitations in diagnostic testing; possible Cbl resistance; a role for Cbl as a pharmacologic agent; and

*Abbreviations:* Cbl, Cobalamin; MMA, Methylmalonic acid; HCys, Homocysteine; THF, Tetrahydrofolate; RDI, Recommended dietary intake; HIV, Human immunodeficiency virus-1; TNF, Tumor necrosis factor  $\alpha$ ; EGF, Epidermal growth factor; IL-6, Interleukin 6; OCS, Oral contraceptives; DM, Diabetes mellitus; TCA, Tricarboxylic acid.

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increasing use of oral Cbl therapy. These latter issues form the focus of this review.

### “Classic” cobalamin biochemistry

Cbl participates in two enzymatic processes in mammalian cells. In the methionine synthase reaction, homocysteine (HCys) is converted to methionine allowing for the “recycling” of 5-methyl-tetrahydrofolate (THF) to N5,10-methylene-THF which is needed for the *de novo* synthesis of thymidyllic acid and ultimately, for DNA formation (Fig. 1). Since conversion of N5,10-methylene-THF to N5-methyl-THF is irreversible, Cbl deficiency “traps” folic acid as N5-methyl-THF. Concurrently, HCys accumulates while methionine decreases, leading to a decrease in S-adenosylmethionine which further limits N5,10-methylene-THF formation by decreasing the synthesis of formyl-THF (“formate starvation”). Decreased methionine and S-adenosylmethionine may limit many methylation reactions including those involving DNA and myelin basic protein.

In the methylmalonylCoA mutase reaction, methylmalonylCoA, derived from propionic acid synthesized by intestinal bacteria, is converted to succinylCoA, a precursor for fatty acid and heme synthesis (Fig. 2). Thus, Cbl deficiency results in methylmalonic acid (MMA) accumulation.

Impaired thymidylate synthesis, detected by the deoxyuridine suppression test, is the most sensitive marker of Cbl depletion, followed by elevation of MMA and HCys in peripheral blood.<sup>5</sup> Similarly, decreased methylation of colonic DNA, increased uracil incorporation into DNA, and increased serum MMA and HCys levels antedate clinical changes in Cbl-depleted rats.<sup>6</sup>

### Recommended dietary intake (RDI) of cobalamin

Based on studies of food Cbl absorption in normal subjects and of parenteral Cbl requirements in pernicious anemia, the RDI of Cbl was set at 2–3 µg/day.<sup>7,8</sup> However, micronucleus formation in peripheral blood lymphocytes, an index of chromosome breakage and loss, is minimized at plasma Cbl levels above 300 pmol/l (410 pg/ml), requiring a Cbl intake of 7 µg/day.<sup>9,10</sup> Similarly, uracil misincorporation into leukocyte DNA in normal young women receiving folate supplements is lower at serum Cbl levels above 400 pg/ml (i.e. double the accepted lower limit of normal).<sup>11</sup> Thus, the current RDI for Cbl may be inadequate to ensure genomic stability.

## Novel biochemical actions of cobalamin

### Effects on nucleic acid metabolism and genetic regulation

Cbl coenzymes directly affect nucleic acid metabolism *in vitro*. Specifically, methylcobalamin serves as a direct methyl donor in the DNA methylase reaction<sup>12</sup> and Cbl binding by RNA receptors has been identified.<sup>13–16</sup> Whether these reactions occur *in vivo* remain to be determined.

Similarly, growth of mammalian cells in Cbl-enriched media induces methionine synthase formation by modifying mRNA.<sup>17–19</sup> MethylCbl also stabilizes the methionine synthase enzyme protein in Cbl-deficient rats.<sup>20</sup> While enzyme induction by Cbl *in vivo* has not been studied, pharmacologic doses of B<sub>6</sub> vitamins do induce synthesis of vitamin B<sub>6</sub>-related enzymes in red cells of normal and anemic human subjects.<sup>21–23</sup>

### Effects on nitric oxide and human immunodeficiency virus-1 (HIV) metabolism

Cbl is a nitric oxide scavenger and inhibits nitric oxide synthase activity.<sup>24–27</sup> Since Cbl cream is effective treatment for atopic dermatitis, a disorder which responds to nitric oxide synthase inhibition, these observations may have clinical relevance.<sup>28</sup> Similarly, Cbl decreases HIV infectivity by inhibiting integrase activity and low serum Cbl levels are associated with HIV progression in clinical studies.<sup>29–31</sup>

### Pathogenesis of clinical manifestations in Cbl deficiency

Cbl deficiency causes megaloblastic anemia and neurocognitive abnormalities but effects on immune function and bone formation have also been described.

### Pathogenesis of megaloblastic anemia

Megaloblastic anemia likely reflects impaired thymidyllic acid synthesis and misincorporation of uracil into DNA in hematopoietic precursors.<sup>32</sup> Whether this results from “trapping” of N5-methyl THF and/or “formate starvation” remains uncertain.<sup>33</sup> The roles of impaired DNA methylation and accelerated apoptosis are also unclear.<sup>34</sup> Thus decreased methylation of colonic mucosa DNA occurs in Cbl-depleted rats, but methylation of DNA from

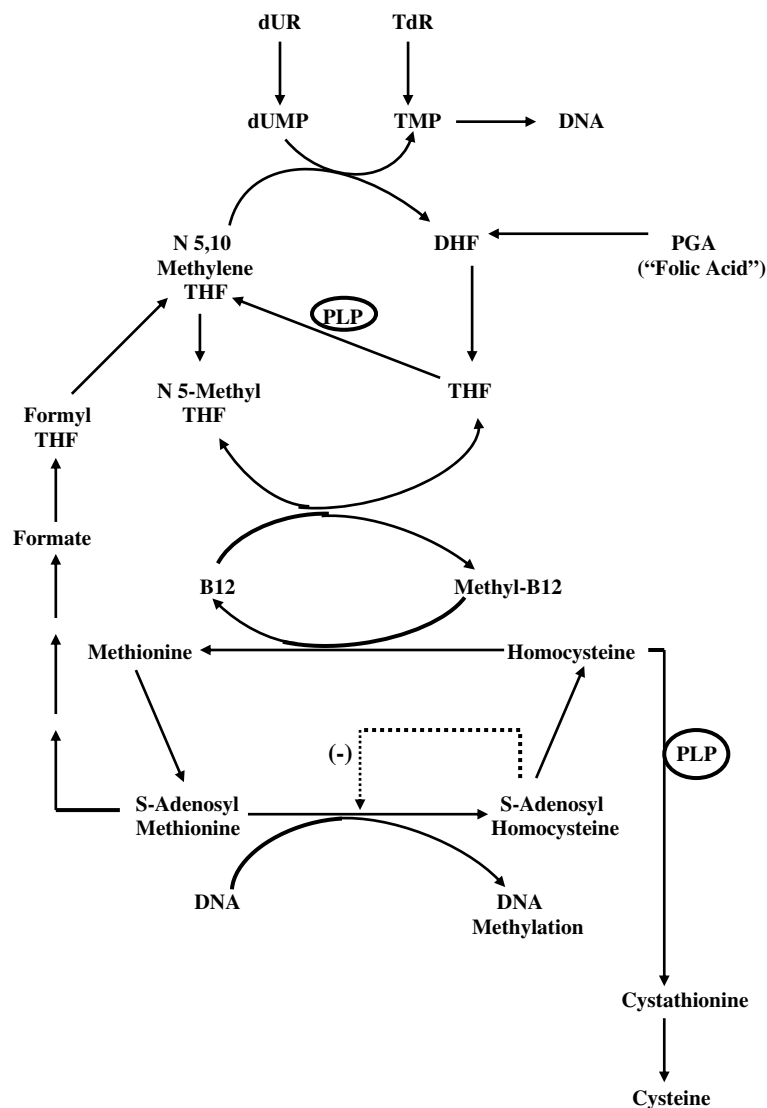


Figure 1 Methionine synthase pathway.

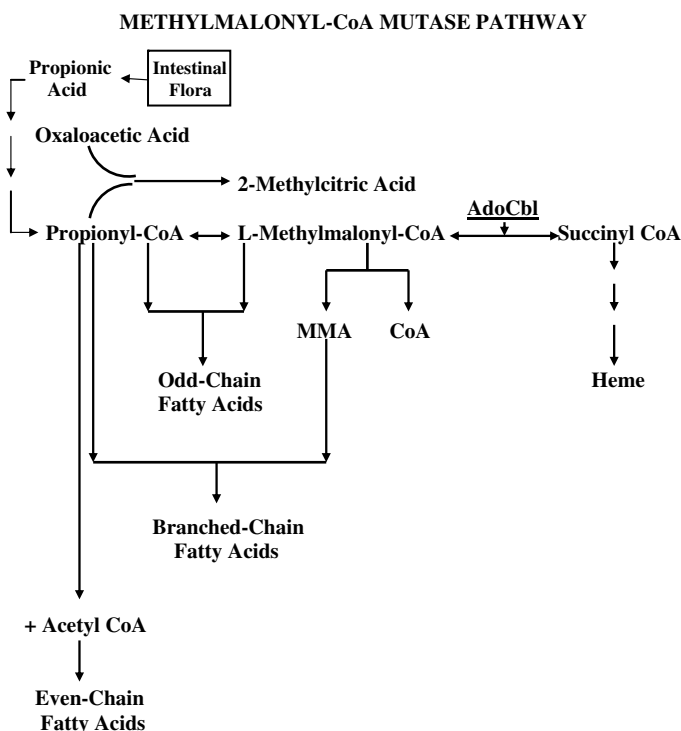
peripheral blood leukocytes of patients with pernicious anemia is normal.<sup>6,35</sup>

### Pathogenesis of neurocognitive defects

The spectrum of neurocognitive abnormalities in Cbl deficiency is broad and the findings on MRI and electrophysiologic examinations are diverse.<sup>36–45</sup> Moreover, neurologic changes often occur in the absence of hematologic abnormalities.<sup>37,41,45,46</sup> Thus, establishing the biochemical basis for these clinical manifestations has proven elusive and suggested mechanisms include: impaired activity of methionine synthase and/or methylmalonylCoA mutase; accumulation of Cbl analogues; concurrent abnormalities in folate metabolism or nutriture; and alterations in cytokine and growth factor regulation.

While a role for both the methylmalonylCoA mutase and the methionine synthase pathways in the pathogenesis of neurocognitive dysfunction in Cbl deficiency has been suggested, observations in experimental animals and in human subjects with inborn errors of Cbl metabolism do not consistently support either hypothesis.<sup>47,48</sup> Similarly, a role for Cbl analogues, which inhibit Cbl-dependent enzymes, has not been confirmed in animal models.<sup>49–52</sup>

Relatively increased serum folate, S-adenosylmethionine, cysteine and cysteine-glycine levels in patients with pernicious anemia and neurologic abnormalities suggest a role for folate-mediated inhibition of glycine N-methyltransferase in the pathogenesis of neuropathy.<sup>53</sup> Thus, folate nutriture and genetic variations in folate or thiol metabolism may interact with impairment of Cbl-dependent



**Figure 2** Methylmalonyl-CoA mutase pathway.

enzymes to produce nerve injury. In contrast, genetic polymorphisms of N-5,10-methylene tetrahydrofolate reductase do not correlate with the clinical manifestations of Cbl deficiency.<sup>54</sup> Polyamines are also decreased in the brains of Cbl-depleted rats but the significance of this finding is unknown.<sup>55</sup>

Observations in Cbl-deficient gastrectomized rats implicate cytokines and growth factors as mediators of neurologic damage.<sup>56,57</sup> The myelinolytic cytokine, tumor necrosis factor  $\alpha$  (TNF), is increased in the spinal fluid of these animals while the neurotrophic cytokines, epidermal growth factor (EGF) and interleukin 6 (IL-6), are decreased. EGF mRNA is also absent in neurons and glial cells. Moreover, neurologic lesions are prevented in Cbl-deficient rats by intraventricular injections of TNF antibodies, EGF or IL-6 and induced in normal rats by intraventricular injections of either EGF antibodies or TNF. Similarly, Cbl-deficient humans have increased TNF and decreased EGF levels in serum which improve with Cbl therapy.<sup>58</sup> Regulation of TNF synthesis by S-adenosylmethionine links the classic Cbl biochemical pathways with the role of cytokines in neurologic dysfunction.<sup>57</sup>

### Pathogenesis of immune dysfunction

An increased incidence of tuberculosis in vegetarians, impaired antibody responses to pneumococcal vaccine in elderly patients with low Cbl levels, and

abnormal lymphocyte subpopulations in Cbl-deficient subjects with megaloblastic anemia suggest a role for Cbl in immune function.<sup>59–64</sup> Cbl-deficient subjects have decreased total lymphocyte counts, decreased CD8<sup>+</sup> cells and impaired natural killer cell activity which correct with high-dose methylCbl therapy.<sup>65,66</sup> However, this regimen also increases total lymphocyte counts and CD8<sup>+</sup> cell counts in normal subjects, suggesting a pharmacologic effect of Cbl.<sup>66</sup>

### Pathogenesis of osteoporosis

Low serum Cbl levels increase the risk of osteoporosis.<sup>67,68</sup> In vitro studies and the finding of low serum skeletal alkaline phosphatase and osteocalcin levels in Cbl-deficient patients which correct with vitamin therapy suggest that Cbl is necessary for normal osteoblast activity.<sup>69,70</sup> Significantly, hip fractures are reduced in elderly subjects receiving 1500  $\mu\text{g}$ /day of mecoCbl in addition to 5 mg/day of folic acid orally.<sup>71</sup> TNF may cause osteoporosis by stimulating osteoclast activity.<sup>72</sup> Thus, the cytokine-related actions of Cbl may have a role in this setting as well.

### Diagnosis of cobalamin deficiency

Tests for Cbl deficiency include measurements of 1) total Cbl; 2) MMA and HCys, as indices of func-

tional Cbl deficiency; and 3) holotranscobalamin as a measure of the metabolically active fraction of circulating Cbl. Each approach has significant limitations. Moreover, since the pathogenesis of neurologic dysfunction in Cbl deficiency remains unclear, these tests may not be reliable markers of neurocognitive impairment.<sup>73</sup>

### Total Cbl

Measurement of serum Cbl is commonly used to screen for Cbl deficiency, but many patients with low Cbl levels are not Cbl-deficient (i.e. "false" low values) while significant clinical impairment may occur despite normal Cbl values (i.e. "false" high values)(Table 1).<sup>41,73–75</sup>

Cbl levels may reflect variations in the 2 major Cbl transport proteins, transcobalamin (formerly transcobalamin II) and haptocorrin (formerly transcobalamin I). Transcobalamin-bound Cbl represents less than 20% of circulating Cbl and readily enters tissues via specific receptors. Haptocorrin-bound Cbl represents more than 80% of circulating Cbl but is metabolically inert.

### High Cbl levels

Myeloproliferative disorders and renal failure increase both serum Cbl and haptocorrin levels.<sup>76,77</sup> Although serum Cbl was not measured, the presence of Cbl-responsive elevations of MMA in 20% of patients with myeloproliferative disorders suggests that high Cbl values in these conditions can mask functional Cbl deficiency.<sup>78</sup> Hepatic disorders

also increase serum Cbl either by increasing release of hepatic Cbl stores or decreasing clearance of holohaptocorrin.<sup>79</sup> In alcoholic liver disease, holohaptocorrin is increased but holotranscobalamin values are low and plasma levels of MMA and HCys are elevated, suggesting that tissue Cbl depletion may also occur in this setting.<sup>79,80</sup>

### Low Cbl levels

Haptocorrin values are lower in Caucasians than in African-Americans and genetic determinants of haptocorrin may explain 15% of "false" low serum Cbl values.<sup>81,82</sup>

Both serum Cbl and holohaptocorrin decrease progressively during pregnancy.<sup>83</sup> Normal hematocrit, MCV and urine MMA values were noted in one study of 32 pregnant women with low serum Cbl values. However, other studies report increased urine MMA values; a significant inverse relationship between serum Cbl and both hemoglobin and serum MMA levels; and a relationship between maternal serum Cbl and impaired Cbl status in the newborn.<sup>83–87</sup> Moreover, high serum MMA values were present in one-third of pregnant women regardless of the serum Cbl level and an association between high MMA values and neural tube defects has been reported.<sup>88,89</sup> Thus, low serum Cbl levels in pregnant women warrant further evaluation. Oral contraceptives (OCS) also decrease Cbl and haptocorrin levels without increasing urine or serum MMA values.<sup>90–94</sup> However, many of these subjects were evaluated after only 3–4 months of OCS use which may have been insufficient time for tissue Cbl depletion.

**Table 1** Determinants of serum cobalamin levels.

"FALSE" LOW VALUES <sup>a</sup>	"FALSE" HIGH/NORMAL VALUES <sup>b</sup>
Folate deficiency	High Haptocorrin Levels:
Pregnancy <sup>c</sup>	Myeloproliferative disorders
Oral contraceptives <sup>c</sup>	Renal disease
Multiple myeloma <sup>c</sup>	Increased tissue release of Cbl
HIV infection <sup>c</sup>	Liver disease
Low haptocorrin levels	Low/absent transcobalamin
	Low affinity transcobalamin polymorphisms
	Inherited Disorders of Cbl metabolism
	Recent Cbl therapy
	Cobalamin analogues <sup>d</sup> :
	Nitrous oxide therapy
	High dose Vitamin C
	Intestinal bacterial overgrowth
	Assay methodological errors

Abbreviations: Cbl, cobalamin; HIV, human immunodeficiency virus-1.

<sup>a</sup> Indicates low Cbl levels in the absence of impaired Cbl supply to tissues.

<sup>b</sup> Indicates high or normal Cbl levels despite impaired Cbl supply to tissues.

<sup>c</sup> See text.

<sup>d</sup> Dependent on assay method.

Moreover, women on OCS face different metabolic demands than those imposed by pregnancy. In contrast, hormone replacement therapy does not increase the risk of either low serum Cbl or elevated metabolite values.<sup>93,95</sup>

Multiple myeloma also decreases serum Cbl and haptocorrin levels. However, since increased uptake of Cbl by marrow myeloma cells has been suggested, further tests for functional Cbl deficiency are warranted.<sup>96,97</sup>

Similarly, HIV infection decreases both Cbl and haptocorrin levels, but holotranscobalamin is also decreased and clinical abnormalities of gastrointestinal function associated with abnormal Schilling tests provide a mechanism for Cbl depletion in this patient population.<sup>98–100</sup> Nonetheless, metabolic abnormalities consistent with frank Cbl deficiency are infrequent and Cbl-responsive hematologic abnormalities in HIV-infected patients are rare.<sup>98</sup> While HCys was increased in 9 of 40 patients with serum Cbl levels <200 pmol/l, red cell folate values were also low and HCys values returned to normal after treatment with *both* Cbl and folic acid.<sup>100</sup> Since MMA was not measured, the relative contributions of folate and Cbl deficiencies to hyper-homocysteinemia is uncertain. However, an association between low serum Cbl levels and both peripheral neuropathy and myelopathy has been noted. Moreover, Cbl therapy improved the neuropathy but not the myelopathy in this uncontrolled study.<sup>101</sup> Since the treatment regimen was not described and since improvement in myelopathy may require prolonged therapy, a role for Cbl deficiency cannot be excluded.<sup>102</sup>

### Normal Cbl levels

A common transcobalamin polymorphism (775G > C) with decreased affinity for Cbl and low holotranscobalamin levels has recently been identified.<sup>103</sup> Increased MMA and HCys values, present in some but not all studies, suggest that homozygotes may be at risk for functional Cbl deficiency at higher serum Cbl levels.<sup>103–108</sup> Similarly, partial transcobalamin deficiency with autosomal dominant inheritance is associated with both megaloblastic anemia and neurologic abnormalities.<sup>109</sup> Serum Cbl levels were normal in 12 of these 23 subjects. In contrast to familial recessive transcobalamin deficiency which becomes clinically manifest in infancy, these patients may not become symptomatic until adulthood.

Finally, current chemiluminescence and radioisotope dilution Cbl assay kits may give misleadingly normal values and the intraindividual variation in Cbl is greater than generally appreciated.<sup>73,110–112</sup>

### MMA and HCys

Because of the limitations in serum Cbl, MMA and HCys are often measured as confirmatory indices of functional Cbl depletion.<sup>75,113,114</sup> While increased levels of these metabolites were initially reported in almost all patients with clinically overt Cbl deficiency, only those subjects with low serum Cbl levels were included in these studies.<sup>114–116</sup> Indeed when treatment was not restricted to patients with low serum Cbl levels, clinical responses to Cbl therapy were commonly seen even when Cbl, MMA and HCys values were normal, particularly in patients with neurologic impairment.<sup>73</sup> Moreover, not all patients with significant hematologic or neurologic abnormalities and elevated MMA and/or HCys values improve with Cbl therapy even when Non-Responders with confounding clinical disorders are excluded from analysis.<sup>73,75,114–116</sup>

The intraindividual variation in MMA is small in normal subjects but much greater in patients with higher baseline MMA values and these fluctuations may obscure the recognition of Cbl deficiency.<sup>73,117</sup> Renal dysfunction also increases serum MMA values, with a positive correlation noted between MMA and serum creatinine even for creatinine values within the normal range.<sup>118,119</sup> However, an inverse relationship between MMA and Cbl persists in uremic subjects and MMA values decrease with Cbl therapy.<sup>120</sup> Thus, high MMA values in renal failure may represent true tissue Cbl deficiency (see Cbl Resistance). Conversely, MMA values may be normal in Cbl deficient patients receiving antibiotics which can eradicate the intestinal flora needed to synthesize propionic acid (Fig. 2).<sup>113</sup>

Intraindividual variation in HCys is also low in healthy subjects but far greater in patients with high HCys values.<sup>73,120–123</sup> Moreover, many genetic, acquired and environmental factors elevate HCys levels.<sup>120</sup> Thus, HCys is less specific than MMA for the diagnosis of Cbl deficiency.

### Holotranscobalamin

Holotranscobalamin levels are decreased in patients with elevated metabolite values as well as in subjects with clinically overt Cbl deficiency.<sup>124–127</sup> However, 38% of patients with low holotranscobalamin values have normal deoxyuridine suppression tests, suggesting that this measurement is not specific for Cbl depletion.<sup>128</sup> Moreover, increases in transcobalamin associated with inflammation may also limit the diagnostic utility of this test.<sup>129,130</sup>



## Subtle cobalamin deficiency

Subtle Cbl deficiency, defined as elevated metabolite levels usually in asymptomatic patients with low or normal serum Cbl values, is prevalent in the elderly and has been associated with food Cbl malabsorption.<sup>4,131</sup> The prognostic and clinical significance of subtle Cbl deficiency is uncertain. Anemia, neuropathy and impaired cognitive performance, as well as abnormalities in evoked potentials, somatosensory potentials and EEG recordings have all been reported in this disorder.<sup>132–135</sup> Moreover, in uncontrolled studies, Cbl therapy led to improvement in some, but not all, subjects.<sup>132,134</sup> In contrast, other studies suggest that this disorder is not progressive and that treatment is without clinical benefit.<sup>136–138</sup> However, since only 4 weekly Cbl injections were given with follow-up performed 2 months later and since MMA decreased to normal in only 69% of patients, therapy may have been inadequate.<sup>137,138</sup>

Importantly, subtle Cbl deficiency may increase the risk of adverse events in other clinical settings. Thus, nitrous oxide anaesthesia, which oxidizes Cbl, can precipitate acute neurologic decompensation postoperatively in patients with unrecognized Cbl deficiency while hematologic and gastrointestinal toxicity induced by the chemotherapeutic agent premetrexed is associated with high MMA and HCys values and ameliorated by folate and Cbl therapy.<sup>139–141</sup>

## “Cobalamin resistance”

Increased MMA levels despite normal serum Cbl values may be explained by insensitivity of serum Cbl to early Cbl depletion; abnormal plasma Cbl-binding proteins; or lack of specificity of MMA elevations for Cbl deficiency (albeit *not* by clinically silent inborn errors of Cbl metabolism).<sup>142–144</sup> Alternatively, this picture may reflect “Cbl resistance”, defined by the correction of abnormal metabolite levels in subjects with normal serum Cbl values by treatment with pharmacologic doses of Cbl. A role for the kidney in regulating Cbl metabolism has recently been proposed.<sup>145</sup> Thus, cobalamin resistance in diabetes, renal insufficiency and advanced age may reflect the alteration in renal function common to these disorders.

## Cobalamin resistance in the elderly

While decreased holotranscobalamin in elderly subjects has been reported, normal Cbl binding to serum transport proteins has recently been ob-

served.<sup>146,147</sup> Yet 7–30% of elderly subjects with normal serum Cbl levels have high metabolite values even when serum creatinine is normal.<sup>148–162</sup> High-dose Cbl therapy reduces MMA levels in most but not all elderly subjects with high MMA values.<sup>135,158,159,162</sup> Moreover, maximum reduction of both mean MMA values and the incidence of elevated MMA levels requires oral Cbl doses of 500–1000 µg/day which are 167–333 times higher than the RDI.<sup>163,164</sup>

## Cobalamin resistance in renal disease

Increased MMA and HCys levels are present in hemodialysis patients despite high serum Cbl and holotranscobalamin levels. Moreover, pharmacologic doses of Cbl decrease both metabolites albeit usually not to normal values.<sup>165–173</sup> Significantly, Cbl uptake by mononuclear cells from renal patients is decreased despite normal expression of holotranscobalamin receptors and elevated metabolite levels in these patients are inversely related to Cbl uptake.<sup>174</sup> Since diminished vibration thresholds in hemodialysis patients improve with methylcobalamin therapy, these findings may have clinical significance.<sup>175</sup>

## Cobalamin resistance in diabetes mellitus

Urinary MMA excretion increases in streptozotocin-diabetic rats despite increased serum Cbl levels and high doses of methylcobalamin decrease urinary MMA to normal.<sup>176</sup> While this study was not confirmed with a different MMA assay method, high-dose Cbl also improves nerve conduction and structure in these animals.<sup>177–179</sup>

In human diabetics: MMA values are increased even when serum Cbl values are greater than 600 pg/ml;<sup>180</sup> high metabolite values and neurologic abnormalities respond to Cbl therapy despite pre-treatment Cbl levels >300 pg/ml;<sup>73</sup> hyperhomocysteinemia is associated both with serum Cbl levels <350 pmol/ml (lower limit of normal = 150 pmol/l) and with the presence of diabetic neuropathy;<sup>181,182</sup> Cbl therapy improves objective measures of cardiac autonomic dysfunction;<sup>183–186</sup> Cbl therapy improves symptoms of both peripheral neuropathy and autonomic dysfunction (randomized double-blind placebo-controlled study);<sup>187</sup> and intrathecal high-dose methylcobalamin can relieve symptoms of neuropathy.<sup>188</sup> Nerve conduction velocities also improved in human diabetics treated with a high-dose combination of cyanocobalamin, lipid-soluble thiamine and pyridoxine.<sup>189</sup> However, improvement also occurred in streptozotocin-diabetic rats treated only with lipid-soluble thiamine.<sup>190</sup> Similarly, high-dose

Cbl in combination with high-dose folate improved endothelial function and insulin resistance in subjects with the metabolic syndrome.<sup>191</sup> Since high-dose Cbl therapy improves neurologic function in non-diabetic settings as well, these effects may result from nonspecific pharmacologic actions of Cbl rather than Cbl resistance (see Cbl AS A PHARMACOLOGIC AGENT).<sup>192–195</sup>

### Genetically determined causes of cobalamin resistance

Inborn errors of metabolism involving Cbl transport, intracellular metabolism, or interaction with the methionine synthase or methylmalonylCoA mutase apoenzymes are also characterized by elevation of MMA and/or HCys with normal serum Cbl levels.<sup>196</sup> High dose Cbl therapy can improve both the metabolic and clinical manifestations of these rare disorders. Similar roles for other vitamins have been suggested in the setting of genetic polymorphisms which decrease coenzyme binding affinity.<sup>197</sup>

### “Local” cobalamin resistance in other neurocognitive disorders

Functional impairment in Cbl activity limited to the central nervous system has been proposed in multiple sclerosis, Alzheimer’s disease and AIDS-associated myelopathy.<sup>198–200</sup> Since serum Cbl, MMA and HCys levels would likely be normal in these settings, “local” Cbl resistance cannot be distinguished from nonspecific pharmacologic actions of Cbl on the nervous system.

### Multiple sclerosis

In multiple sclerosis, high-dose Cbl therapy improved some objective and subjective measures of disease activity but these studies lacked adequate control groups.<sup>201,202</sup> However, the combination of Cbl and interferon has significantly more activity in a mouse model of demyelinating disease than either agent alone.<sup>203</sup>

### Alzheimer’s disease

An increased risk of Alzheimer’s disease is associated with low serum Cbl levels in general and low holotranscobalamin levels in particular.<sup>204–208</sup> Moreover, the suggestion that increased Cbl oxidation in the central nervous system may play a role in this disorder is supported by the presence of Cbl analogues in human brain tissue; the relative increase in Cbl analogues in serum from patients with Alzheimer’s

disease; and the observation that some analogues may have a direct inhibitory effect on methionine synthase and methylmalonylCoA mutase activities.<sup>198,205,209,210</sup> However, Cbl analogues are not associated with neurologic dysfunction in Cbl-deficient bats and adequate trials of Cbl therapy in cognitive dysfunction have yet to be performed.<sup>50,52,211</sup>

### AIDS-associated myelopathy

Decreased serum methionine and cerebrospinal fluid S-adenosylmethionine levels have been reported in AIDS-associated myelopathy despite normal blood levels of Cbl, MMA and HCys.<sup>200</sup> Moreover, central nerve conduction studies improved in this population after high-dose methionine therapy.<sup>212</sup> However, high-dose Cbl therapy has yet to be evaluated.

### Cobalamin as a pharmacologic agent

Since loading doses of Cbl far exceed physiologic requirements, clinical responses may result from *pharmacologic* effects on either Cbl-related processes or on cellular functions completely unrelated to the known biochemical actions of Cbl. Thus, neurologic responses to Cbl therapy were noted in 29 human subjects, 6 (21%) of whom had normal MMA and HCys values.<sup>73</sup> Similarly, pharmacologic doses of methylcobalamin accelerate nerve regeneration in rats with acrylamide neuropathy;<sup>192</sup> improve electrophysiologic parameters in the carpal tunnel syndrome;<sup>195</sup> protect retinal cells from glutamate-induced injury in vitro;<sup>193</sup> improve compound muscle action potentials in amyotrophic lateral sclerosis;<sup>194</sup> and alter lymphocyte populations in normal human subjects.<sup>66</sup> Pharmacologic doses of Cbl also improved neurologic function in hemodialysis patients as well as in diabetic rats and humans and transiently improved signs and symptoms of progressive vacuolar myelopathy following treatment of childhood leukemia.<sup>175,183–189,213</sup> The ability of Cbl to enter cells by a pathway independent of transcobalamin receptors provides a mechanism for the generation of high intracellular Cbl concentrations<sup>214</sup> while the novel biochemical effects of Cbl and the pharmacologic actions of Cbl on cytokines and immune function provide possible explanations for these clinical observations.<sup>9–19,29,30,56–59,66</sup>

### Treatment of cobalamin deficiency

Cbl therapy usually involves loading doses of daily to weekly parenteral injections over a period of



**Table 2** Studies of oral cyanocobalamin therapy.

Ref	Oral regimen	N	Time (Mo)	Prior schilling	Inclusion criteria				Responses				
					Cbl	MMA	HCys	Clinical	MMA	MCV	Hgb	Neuro	M.S.
226	2 mg/day	18	4	No	<160 (x2)	>376	>17.9	None	17/17	6/7	2/4	3/3	1/1 <sup>a</sup>
228	0.5–1.0 mg/d	30	1	Yes (N = 10)	<200 (x2)	ND	>13.0	None	ND	8/8	7/13	ND	ND
229	3–5 mg/wk	10	3	Yes (N = 10)	<160 (x2)	ND	>13.0	None	ND	5/7	6/10	2/4	?/2
227	1 mg/d × 10 1 mg/wk × 10 1 mg/mo <sup>g</sup>	26	3	No	<160	ND	ND	Hgb < 12 & MCV > 94	ND	112 ± 11 to 87 ± 4	8.4 ± 2.1 to 13.8 ± 0.7	4/6 <sup>c</sup>	3/3 <sup>h</sup>
164	1 mg/d <sup>b</sup>	20	1.5	No	<300	>271 (x2)	>13.9	ND	20/20	ND	ND	ND	ND
230	1.5 mg/d <sup>f</sup>	8	1.5	Yes (N = ?)	<259	ND <sup>e</sup>	ND	“Megalo” (Hgb ≤ 10.1 MCV ≥ 106)	ND <sup>e</sup>	7/8	7/7	?? <sup>d</sup>	?? <sup>d</sup>
All	—	104	—	>20	—	—	—	—	37/37 100%	26/30 87%	22/34 65%	9/13 69%	4/4 100%

Refs. [226,227] were randomized comparisons of parenteral and oral therapy. Inclusion criteria for Ref. [226] were a serum Cbl <160 pg/ml on 2 occasions and an elevated MMA or HCys value. Inclusion criteria for Refs. [228,229] were low serum Cbl on 2 occasions or a low serum Cbl on one occasion and an elevated HCys value. Units are: Cbl, pg/ml; MMA, nmol/l; HCys, μmol/l.

Abbreviations: Ref, reference number; N, number of subjects studied; Mo, months; Cbl, cobalamin; MMA, methylmalonic acid; Hgb, hemoglobin; MCV, mean corpuscular volume; Neuro, neuropathy; M.S., mental status; ND, not done; wk, weeks.

<sup>a</sup> Method of testing not stated.

<sup>b</sup> Treatment with 1 mg/day followed 6 weeks of treatment with 25 mcg/day and 6 weeks of treatment with 100 mcg/day.

<sup>c</sup> Objective testing reported in 3 of 4 responders.

<sup>d</sup> All 8 patients had mild neurologic or mental status changes which improved but details not provided.

<sup>e</sup> 24 hour urine MMA excretion was elevated in 4 patients tested and returned to normal in the one patient with a post-treatment value.

<sup>f</sup> Includes 3 patients on cyanocobalamin, 4 patients on hydroxocobalamin and 1 patient on methylcobalamin.

<sup>g</sup> Used cyanocobalamin liquid for injection mixed with 20 ml fruit juice.

<sup>h</sup> Determined by MMSE.

1–3 months to replete body stores followed by a maintenance regimen. Specific recommendations depend on the form of Cbl used and the route of administration selected.

### Form of cobalamin and frequency of administration

Intramuscular cyanocobalamin and hydroxocobalamin have distinct pharmacologic properties. Hydroxocobalamin has greater systemic retention and availability to cells than cyanocobalamin and cyanocobalamin therapy may be ineffective in some patients with inborn errors of metabolism.<sup>215–218</sup> Thus, while 1 mg maintenance doses of hydroxocobalamin may be given every 1.5–3 months, 1 mg doses of cyanocobalamin may be required as often as every 2 weeks.<sup>219</sup> Importantly, individual patients require more frequent injections of either Cbl derivative to maintain normal serum Cbl levels and prevent clinical relapse.<sup>219–221</sup>

### Oral vs parenteral administration

Since Cbl is absorbed by intrinsic factor-independent passive diffusion in the small intestine, daily

high-dose oral cyanocobalamin can induce and maintain remissions in patients with megaloblastic anemia due to pernicious anemia.<sup>222–224</sup> More recently, a Cochrane Review based on 2 randomized trials concluded that daily oral therapy “may be as effective as intramuscular administration in obtaining short term haematological and neurological responses in vitamin B12 deficient patients”.<sup>225–227</sup> However, of 104 patients in reported series, only 9 patients had pernicious anemia and 10 patients had atrophic gastritis (which causes both food Cbl malabsorption and intrinsic factor deficiency) while at least 66 patients had food Cbl malabsorption or decreased dietary Cbl intake (Table 2).<sup>164,226–230</sup> More than 20 patients had antecedent Schilling tests involving a parenteral dose of Cbl. Moreover, most of the patients in these studies were selected only for the presence of low Cbl and high metabolite levels, some were selected for the presence of megaloblastic anemia and none were selected for the presence of neurologic abnormalities.<sup>164,226–230</sup> While neurologic responses were observed, these were often not objectively measured. Thus, since delay in effective therapy can lead to irreversible neurologic dysfunction, parenteral therapy should be strongly considered. Finally, while studies with

**Table 3** Classification of cobalamin-responsive disorders.

CATEGORY	MECHANISM	TESTS	
		Cbl	MMA/HCys
DEFICIENCY	1) Decreased intake 2) Decreased absorption 3) Increased destruction (e.g. Nitrous Oxide)	Low	High
SYSTEMIC	1) Impaired utilization a) Hereditary - Inborn errors of Cbl metabolism b) Acquired i) Age $\geq$ 70 yrs ii) Renal insufficiency iii) Diabetes mellitus 2) Increased Requirements - Systemic a) Pregnancy ?? b) Abnormal plasma binders (TC; HC) c) Cbl analogues	Normal	High
FOCAL RESISTANCE	3) Increased requirements - local i) Multiple Sclerosis ii) Alzheimer’s disease iii) AIDS-myelopathy	Normal	Normal
PHARMACOLOGIC	4) Unknown: (Nucleic Acid Binding; Binding of Metabolic Intermediates; Enzyme Induction; Enzyme Inhibition; Cytokine/ Growth factor regulation?)	Normal	Normal

Abbreviations: Cbl, cobalamin; MMA, methylmalonic acid; HCys, homocysteine; TC, transcobalamin; HC, haptocorrin.

oral Cbl involved immediate-release tablets or liquid formulations, many over-the-counter high dose Cbl tablets are “timed release” preparations which have not been adequately studied.<sup>231</sup>

## Summary and conclusions

No single test has emerged as the gold standard for diagnosis of Cbl deficiency.<sup>232</sup> Moreover, Cbl requirements in normal subjects may be higher than previously appreciated; Cbl resistance may increase Cbl requirements in the setting of diabetes, renal insufficiency and advanced age; and Cbl may have physiologic and pharmacologic effects which are as yet poorly understood. Thus, Cbl-responsive disorders may result from true Cbl deficiency, systemic or local Cbl resistance; or nonspecific pharmacologic effects of high-dose Cbl therapy (Table 3). As a result, blood Cbl, MMA and HCys values often fail to predict whether or not a patient will respond to Cbl therapy. However, most clinical studies of high-dose Cbl therapy and neurologic function lacked appropriate control groups. Pending the completion of randomized, long-term, placebo-controlled trials of high-dose hydroxocobalamin, cyanocobalamin and methylcobalamin in neurologic disorders, *prolonged* (6–12 months)<sup>102</sup> therapeutic trials with *pharmacologic doses of parenteral Cbl* (at least 1,000 µg intramuscularly 1–3 days/week) are warranted when clinical findings consistent with Cbl deficiency are present. Finally, the role of oral Cbl therapy in patients with neurologic abnormalities has not yet been established.

### Practice points

- The current RDI may underestimate cobalamin requirements in normal subjects.
- Higher cobalamin levels may be needed in the elderly and in patients with diabetes or renal failure to prevent functional cobalamin deficiency.
- Common transcobalamin polymorphisms may also cause functional cobalamin deficiency at normal serum cobalamin levels.
- Since cobalamin, methylmalonic acid and homocysteine levels fluctuate and neither predict nor preclude responses to cobalamin, cobalamin therapy is suggested for symptomatic patients regardless of the results of these diagnostic tests.

- High-dose cobalamin therapy may improve neurologic function even in the absence of cobalamin deficiency.
- Low serum cobalamin levels associated with pregnancy, oral contraceptive use, multiple myeloma or HIV infection warrant further evaluation.
- High serum cobalamin levels in liver disease or myeloproliferative disorders may mask functional cobalamin deficiency.
- While the significance of subtle cobalamin deficiency is uncertain, therapy is suggested because of the presence of neurophysiologic abnormalities in some patients.
- Individual patients with cobalamin deficiency may require more frequent parenteral dosing to prevent relapse than is usually recommended.
- While oral cobalamin is effective in many patients, its benefit for neurocognitive dysfunction is uncertain and timed-release preparations should be avoided.

### Research agenda

- Further define the role of cobalamin in nucleic acid metabolism and genomic stability in normal subjects.
- Determine biochemical effects of pharmacologic doses of cobalamin in vivo on metabolic processes in normal subjects and in subjects with neurologic disorders.
- Determine the role of cobalamin in cytokine and growth factor regulation and on immune function.
- Define the mechanism of neurotoxicity in patients with cobalamin deficiency.
- Determine mechanisms of cobalamin resistance in diabetes, renal disease and the elderly.
- Evaluate the role of the kidney in the regulation of cobalamin metabolism.
- Explore pharmacologic role of cobalamin in neurologic disorders (including neuropathy associated with diabetes and HIV infection; multiple sclerosis; and Alzheimers disease) with particular attention to the form of Cbl (cyanocobalamin; hydroxocobalamin; or methylcobalamin), the dose of Cbl and the duration of therapy (i.e. 6–12 months).

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