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Review

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An update on cobalamin deficiency in adults

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Summary

Cobalamin (vitamin B_{12}) deficiency is particularly common in the elderly (>65 years of age), but is often unrecognized because of its subtle clinical manifestations; although they can be potentially serious, particularly from a neuropsychiatric and hematological perspective. In the general population, the main causes of cobalamin deficiency are pernicious anemia and food-cobalamin malabsorption. Food-cobalamin malabsorption syndrome, which has only recently been identified, is a disorder characterized by the inability to release cobalamin from food or its binding proteins. This syndrome is usually caused by atrophic gastritis, related or unrelated to *Helicobacter pylori* infection, and long-term ingestion of antacids and biguanides. Besides these syndromes, mutations in genes encoding endocytic receptors involved in the ileal absorption and cellular uptake of cobalamin have been recently uncovered and explain, at least in part, the hereditary component of megaloblastic anemia. Management of cobalamin deficiency with cobalamin injections is currently well codified, but new routes of cobalamin administration (oral and nasal) are being studied, especially oral cobalamin therapy for food-cobalamin malabsorption.

Introduction

Cobalamin or vitamin B_{12} deficiency is common in elderly patients,[1] but is often unrecognized or not investigated because the clinical manifestations of cobalamin deficiency are subtle. However, complications of cobalamin deficiency, particularly neuropsychiatric and hematological,[1–4] are potentially serious and therefore require investigation in all patients who present vitamin or nutritional deficiency. Classic disorders such as pernicious anemia are the cause of cobalamin deficiency in only a limited number of patients, especially elderly patients.[4] A more common problem is food-cobalamin malabsorption, a disorder characterized by the inability to release vitamin B_{12} from food or its binding proteins.[4] Since the description of this disorder, several authors have demonstrated that oral cobalamin therapy can be a pharmacotherapeutic option for the treatment of cobalamin deficiency.[4] This review summarizes the current knowledge on cobalamin deficiency, with a particular focus on oral cobalamin therapy.

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Review criteria

PubMed was systematically searched for articles published from January 1960 to January 2008, using the following key words or associations: 'cobalamin deficiency', 'vitamin B_{12} deficiency' and 'food-cobalamin malabsorption'. Articles were restricted to those containing human data that were published in English and French languages. Unpublished data from our working group, the 'Groupe d'étude des carences en vitamine B_{12} des Hôpitaux Universitaires de Strasbourg', has also been included.

Box 1. Definitions of cobalamin (vitamin B_{12}) deficiency[7,8]

- Serum cobalamin levels <150 pmol/l and clinical features and/or hematological anomalies related to cobalamin deficiency.
- Serum cobalamin levels <150 pmol/l (<200 pg/ml) on two separate occasions.
- Serum cobalamin levels <150 pmol/l and total serum homocysteine levels >13 µmol/l or methylmalonic acid levels >0.4 µmol/l (in the absence of renal failure and folate and vitamin B6 deficiencies).
- Low serum holotranscobalamin levels <35 pmol/l.

Definition of cobalamin deficiency

Literature of the last 10 years has provided several definitions of cobalamin deficiency, depending mainly on the population studied and on the particular assay kits used.[5–7] Varying test sensitivities and specificities result from the lack of a precise 'gold standard' for the diagnosis of cobalamin deficiency, especially in elderly patients. The definitions of cobalamin deficiency used in this review are shown in Box 1[7,8]. At present, cobalamin deficiency is often defined in terms of the value of serum cobalamin (<150 pmol/l or <200 pg/ml) and of homocysteine (>13 µmol/l) and methylmalonic acid (>0.4 µmol/l), two components of the cobalamin metabolic pathway (Figure 1B). It is important to note that only methylmalonic acid is specific for cobalamin deficiency. Increased homocysteine is also caused by folate and vitamin B_6 deficiency. In the future, new serum cobalamin assay kits such as holotranscobalamin may replace older assay kits and become the standard for testing for cobalamin deficiency.[9] However, to date, little and conflicting evidence is available about the effectiveness of these new tests in regular clinical practice.

Epidemiology of cobalamin deficiency

Epidemiological studies show that in the general population of industrialized countries, cobalamin deficiency has a prevalence of around 20%, ranging from 5% to 60% depending on the definition of cobalamin deficiency used.[4,9] The Framingham study demonstrated a prevalence of 12% among elderly people living in the community.[10] Other studies focusing on elderly people, particularly those who are in institutions or who are sick and malnourished, have suggested a higher prevalence of 30–40%.[11,12] Using stringent definition, we found that cobalamin deficiency had a prevalence of 5% in a group of patients followed or hospitalized in a tertiary reference hospital.[8]

Cobalamin metabolism and function

The different stages of cobalamin metabolism and corresponding causes of cobalamin deficiency are shown in Table 1.[4,13–15] Absorption depends mainly on intrinsic factor (IF), which is secreted by the gastric mucosa. IF binds cobalamin forming a complex that is absorbed by the terminal ileum (Figure 1B). This mechanism is responsible for the absorption of at least 60% of oral coblamin.[13–15] Cobalamin metabolism is complex and requires many processes, any one of which, if not present, may lead to cobalamin deficiency.[13–15] Once metabolized, cobalamin is a cofactor and coenzyme in many biochemical reactions, including DNA synthesis, methionine synthesis from homocysteine and conversion of propionyl into succinyl coenzyme A from methyl malonate.[4,8,9] A typical Western diet contributes 3–30 µg of cobalamin per day based on the recommended dietary allowance set by the Food and Nutrition Board of the Institute of Medicine (US) of 2.4 µg/day for adults and from 2.6 to 2.8 µg/day during pregnancy.[16] It has been estimated that there is a delay of 5 to 10 years between the onset of cobalamin deficiency and the appearance of clinical manifestations, due to important hepatic stores (>1.5 mg) and the enterohepatic cycle.[4,13] Of particular interest is the observation that about 1–5% of free cobalamin (or crystalline cobalamin) is absorbed along the entire intestine by passive diffusion. This absorption explains the mechanism underlying oral coblamin treatment of cobalamin deficiencies.[17,18]

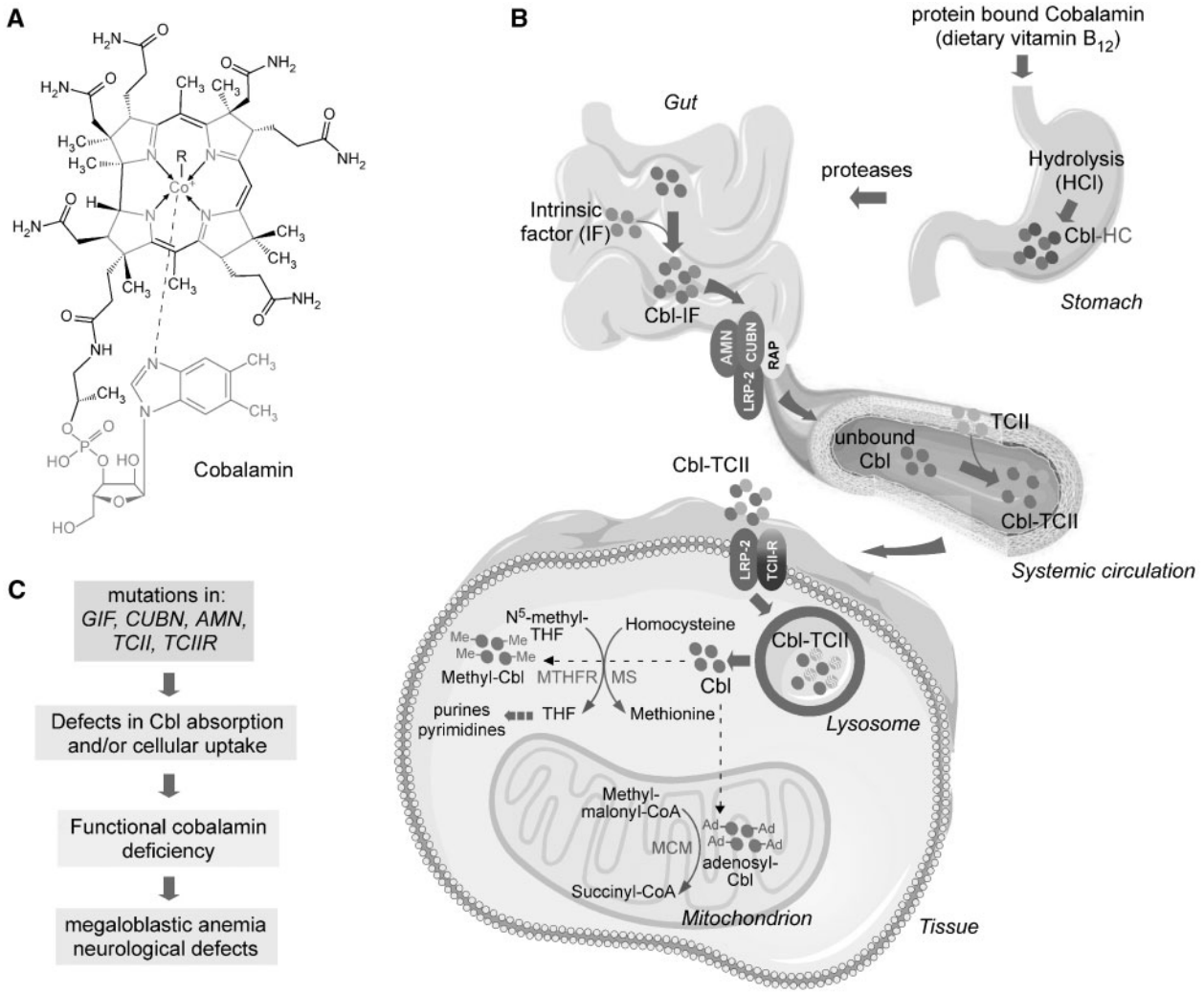

Figure 1. Cobalamin (cbl) absorption and metabolic pathway. (**A**) Structure of cobalamin (vitamin B_{12}) with a corrin ring bound to a central cobalt atom. (**B**) The metabolic journey of cbl from nutrient intake to its intestinal absorption. Endocytic receptors and proteins responsible for vitamin B_{12} intestinal absorption include cubilin (CUBN), amnionless (AMN), receptor-associated protein (RAP) and megalin (LRP-2). The membrane megalin/transcobalamin II (TCII) receptor complex allows the cellular uptake of cbl. Lysosomal-mediated degradation of TCII and subsequent release of free cbl is essential for vitamin B_{12} metabolic functions. MS: methonine synthase; THF: tetrahydrofolate; MTHFR: methyltetrahydrofolate reductase; MCM: methylmalonyl coA mutase. (**C**) Mutations in genes encoding the IF (GIF), CUBN, AMN, TCII or its receptor provoke defects in cbl absorption and/or cellular uptake which translates into functional cbl deficiency and its clinical manifestations.

Classical causes of cobalamin deficiency

In a recent study, we reported the principal causes of cobalamin deficiency in 172 elderly patients (median age: 70 years) hospitalized in the University Hospital of Strasbourg, France.[14] The main causes included food-cobalamin malabsorption (53%), pernicious anemia (33%), insufficient nutritional vitamin B12 intake (2%), postsurgical malabsorption (1%) and as much as 11% of the patients suffered from cobalamin deficiency of undetermined etiology. In elderly patients, cobalamin deficiency is classically caused by pernicious anemia.[1,11] The principal characteristics of pernicious anemia have been reported in detail in several reviews.[19–21] Cobalamin deficiency caused by dietary deficiency or malabsorption is rarer. Dietary causes of deficiency are limited to elderly people who are already malnourished, such as elderly patients living in institutions (they may consume inadequate amounts of vitamin B_{12}-containing foods) or in psychiatric hospitals (strict vegetarian).[4,13] Since the 1980s, the malabsorption of cobalamin has

Table 1 Stages of cobalamin metabolism and corresponding causes of cobalamin deficiency[13,15]

Stages and actors in cobalamin metabolism	Causes of cobalamin deficiency
Intake solely through food	Strict vegetarianism (patients who are sick in institutions or in psychiatric hospitals)
Digestion brings into play Haptocorrin Gastric secretions (hydrochloric acid and pepsin) Intrinsic factor Pancreatic and biliary secretions Enterohepatic cycle	Gastrectomies Pernicious anemia Food-cobalamin malabsorption
Absorption brings into play Intrinsic factor Cubilin, amnionless Calcium and energy	Ileal resections and malabsorption Pernicious anemia Food-cobalamin malabsorption
Transport by transcobalamins	Congenital deficiency in transcobalamin II
Intracellular metabolism based on various intracellular enzymes	Congenital deficiency in various intracellular enzymes

become rarer, owing mainly to the decreasing frequency of gastrectomy and terminal small intestine surgical resection.[4,8,14] Several disorders commonly seen in gastroenterology practice might, however, be associated with cobalamin malabsorption. These disorders include exocrine pancreas' function deficiency following chronic pancreatitis (usually alcoholic), lymphomas or tuberculosis (of the intestine), celiac disease, Crohn's disease, Whipple's disease and uncommon celiac disease.[11,15] Food-cobalamin malabsorption has been found to be the leading cause of cobalamin malabsorption, especially in elderly patients.[4,8,11,22] In our studies, we followed more than 300 patients with a documented cobalamin deficiency, and reported that food-cobalamin malabsorption accounts for about 60–70% of the cases of cobalamin deficiency in elderly patients, whereas pernicious anemia accounted for only 15–25%.[14,23]

Food-cobalamin malabsorption

First described by Carmel in 1995,[22] food-cobalamin malabsorption is a syndrome characterized by the inability of the body to release cobalamin from food or intestinal transport proteins, particularly in the presence of hypochlorhydria, where the absorption of 'unbound' cobalamin is normal ('maldigestion'). In our experience, this syndrome accounted for 60–70% of cases of cobalamin deficiency in elderly patients.[14,15] This syndrome is characterized by cobalamin deficiency in the presence of sufficient food-cobalamin intake and a normal Schilling test ruling out malabsorption or pernicious anemia (diagnosis of exclusion).[14,22,23] Thus in this syndrome, patients can absorb 'unbound' cobalamin through IF or passive diffusion mechanisms. Thus, the recognition of the syndrome permits new developments of oral cobalamin therapy.[4] The principal characteristics of this syndrome are listed in Table 2. Authors supporting the existence of this syndrome have employed a modified Schilling test, which uses animal protein-bound radioactive cobalamin (e.g. salmon, trout) and revealed malabsorption when the results of a standard Schilling test were normal.[4,14,23]

Some authors have speculated about the reality and significance of cobalamin deficiency related to food-cobalamin malabsorption,[4] because many patients displayed mild clinical or haematological features. However, we recently described several patients with serious features classically associated with pernicious anemia, including polyneuropathy, confusion, dementia, medullar-combined sclerosis, anemia and pancytopenia.[14] Nevertheless, the partial nature of this form of malabsorption may produce a more slowly progressive depletion of cobalamin than does the more complete malabsorption engendered by disruption of the IF-mediated absorption. The slower progression of cobalamin depletion probably explains why mild, preclinical deficiency is more frequently associated with food-cobalamin malabsorption than with pernicious anemia.[4,14]

Table 2 Food-cobalamin malabsorption syndrome[4,14,15]

Criteria for food-cobalamin malabsorption	Associated conditions or agents
Low serum cobalamin (vitamin B12) levels Normal results of Schilling test using free cyanocobalamin labeled with cobalt-58 or abnormal results of derived Schilling test[a] No anti-intrinsic factor antibodies No dietary cobalamin deficiency	Gastric disease: atrophic gastritis, type A atrophic gastritis, gastric disease associated with *Helicobacter pylori* infection, partial gastrectomy, gastric by-pass, vagotomy Pancreatic insufficiency: alcohol abuse Gastric or intestinal bacterial overgrowth: achlorhydria, tropical sprue, Ogylvie's syndrome, HIV Drugs: antacids (H2-receptor antagonists and proton pump inhibitors) or biguanides (metformin) Alcohol abuse Sjögren's syndrome, systemic sclerosis Haptocorrine deficiency Ageing or idiopathic

[a]Derived Schilling tests use food-bound cobalamin (e.g. egg yolk, chicken and fish proteins).

Food-cobalamin malabsorption is caused primarily by atrophic gastritis.[14] Over 40% of patients older than 80 years have gastric atrophy that might or not be related to *H. pylori* infection.[11,24] Other factors that contribute to food-cobalamin malabsorption in elderly people include chronic carriage of *H. pylori* and intestinal microbial proliferation, situations in which cobalamin deficiency can be corrected by antibiotic treatment,[24,25] long-term ingestion of antiacids such as H_2-receptor antagonists and proton-pump inhibitors,[26,27] particularly among patients with Zollinger–Ellison syndrome,[28,29] and biguanides (metformin).[30–32] In addition, other food-cobalamin malabsorption inducers include chronic alcoholism, surgery or gastric reconstruction (e.g. bypass surgery for obesity), partial exocrine pancreatic failure,[4,14] and Sjögren's syndrome or systemic sclerosis (Table 2).[33] In a serie of 92 elderly patients (mean age: 76 years) with food-cobalamin malabsorption, we have reported at least one of these associated conditions or agents in 60% of the patients.[14] These conditions mainly include atrophic gastritis (± *H. pylori* infection) in 30% of the patients and long-term metformin or antacid intake in 20% of elderly patients.

Clinical manifestations of cobalamin deficiency

The clinical manifestations are highly polymorphic and of varying severity, ranging from milder conditions such as fatigue, common sensory neuropathy, atrophic glossitis (Hunter's glossitis) and isolated macrocytosis or neutrophil hypersegmentation, to severe disorders, including combined sclerosis of the spinal cord, hemolytic anemia and even pancytopenia.[2,14,34–36] Frequently, neurologic signs and symptoms precede haematologic abnormalities or continue to be isolated. Several new studied or established manifestations of cobalamin deficiency are described in Table 3. In the aforementioned serie of 92 patients with food-cobalamin malabsorption,[14] we have found at least one clinical feature or haematological abnormalities in, respectively, 70% and 76% of the patients. Cobalamin deficiency appears to be more common among patients exhibiting a variety of chronic neurologic conditions such as dementia, Alzheimer's disease, stroke, Parkinson's disease and depression, although it remains unclear whether they are causally related.[4,37] In our own studies, we administered cobalamin to patients with dementia, but did not observe any improvement.[9,14] Other reports have yielded similar results.[18,38] At this time, a causal role of cobalamin deficiency in these conditions remains rather speculative.

Cobalamin deficiency: biochemical and molecular aspects

Vitamin B_{12}, from nutrients intake to the intestine

The molecular biology of cobalamin deficiency has been the subject of several studies investigating the genetics of cobalamin metabolism. Dietary vitamin B_{12} which is bound to proteins in food is released in the acidic environment of the stomach where it is rapidly complexed to the binding protein

Table 3 Main clinical features of cobalamin deficiency[2,4,14,15,34-36]

Hematological manifestations	Neuro-psychiatric manifestations	Digestive manifestations	Other manifestations
Frequent: macrocytosis, neutrophil hypersegmentation , aregenerative macrocytary anemia, medullar megaloblastosis (''blue spinal cord'') Rare: isolated thrombocytopenia and neutropenia, pancytopenia Very rare: hemolytic anemia, thrombotic microangiopathy (presence of schistocytes)	Frequent: polyneuritis (especially sensitive), ataxia, Babinski's phenomenon Classic: combined sclerosis of the spinal cord Rare: isolated thrombocytopenia and neutropenia, pancytopenia Under study: changes in the higher functions, dementia, stroke and atherosclerosis (hyperhomocysteinemia), parkinsonian syndromes, depression, multiple sclerosis	Classic: Hunter's glossitis, jaundice, LDH and bilirubin elevation (''intramedullary destruction'') Debatable: abdominal pain, dyspepsia, nausea, vomiting, diarrhea, disturbances in intestinal functioning Rare: resistant and recurring mucocutaneous ulcers	Frequent: Tiredness, loss of appetite Under study: atrophy of the vaginal mucosa and chronic vaginal and urinary infections (especially mycosis), hypofertility and repeated miscarriages, venous thromboembolic disease, angina (hyperhomocysteinemia)

and transporter haptocorrin (HC), also referred to as the R-binder or transcobalamin I (Figure 1B). About 80% of circulating cobalamin are bound to HC and serum cobalamin levels have been correlated with serum HC concentrations.[39,40] Although, some unexplained low serum cobalamin concentrations were reported to be caused by mild to severe HC deficiencies,[41,42] these abnormalities were not accompanied by pernicious anemia and are not thought to cause functional cobalamin deficiency. Cobalamin continues its route in the gastrointestinal tract and dissociates from HC under the action of pancreatic proteases, followed by it association in the intestine with the IF (also known as the S-binder) which is essential for ileal absorption of cobalamin (Figure 1B). Indeed, homozygous nonsense and missense mutations in the gene encoding the gastric IF (*GIF*) were reported to cause hereditary juvenile cobalamin deficiency.[43]

Endocytic receptors in cobalamin intestinal absorption

Essential nutrients such as cofactors and vitamins are transported to tissues following their binding to specific endocytic receptors (reviewed in[44]). Likewise, the absorption of IF-cobalamin does not occur passively and requires the presence of a complex of endocytic receptors at the ileal–blood barrier. This complex is located at the apical side of brush-border membranes (BBMs) of polarized epithelia, such as the intestinal apical BBM. It consists of the IF-vitamin B_{12} receptor cubilin, a 460 kDa peripheral membrane glycoprotein, encoded by the *CUBN* gene which was mapped to chromosomal region 10p12.33-p13,[45] and the 48 kDa amnionless protein encoded by the *AMN* gene, a gene essential for mouse gastrulation [46] and localized on human chromosome 14.[47] The human megalin/gp330/LRP-2 receptor, encoded by the *LRP-2* gene located on chromosome 2q24-q31,[48] is a giant endocytic receptor (600 kDa) of the low-density lipoprotein receptor (LDLR) family [49] that was strongly suggested to play an important role in the stability of the cubilin/AMN complex.[50] It is noteworthy that ligands for megalin include apoE, lipoprotein lipase, lactoferrin, receptor-associated protein (RAP) among other proteins and that this interaction is Ca^{2+}-dependent.[51–53] Importantly, the endoplasmic reticulum (ER)-localized 39 kDa protein RAP, which binds to all members of the LDLR family but also in a region contiguous to the cobalamin IF-binding region on the cubilin protein,[54,55] allows the processing of megalin where it binds to the newly synthetized megalin receptor in the ER and prevents the early binding of ligands and the aggregation of megalin receptors (reviewed in[44]). Intestinal-specific inactivation of megalin in *in-vivo* animal models would be of particular interest to establish a precise role of megalin in cobalamin-IF absorption at the intestinal BBM–blood barrier and its potential relationship with

hereditary megaloblastic anemia 1 (MGA1), a rare autosomal recessive disorder affecting human subjects with neurological symptoms and juvenile MGA.[56,57]

Mutations in cubilin and amnionless cause impaired cobalamin intestinal absorption

The endocytic receptor cubilin comprises a short N-terminal region followed by eight epidermal growth factor (EGF) repeats and a large cluster of 27 CUB domains. Deletion mutant and immunoprecipitation experiments identified the CUB1-8 region as the binding domain for the vitamin B_{12}-IF complex and the overlapping CUB13 and 14 domains as the binding region for the RAP protein.[55] Mutations in *CUBN* were reported to cause hereditary MGA1.[58] Two principal mutations were identified in finnish patients (FM), a 3916C → T missense mutation named FM1 changing a highly conserved proline to leucine (P1297L) in CUB domain 8, suggesting that this proline is functionally crucial in cubilin and one point mutation (FM2) in the intron interrupting CUB domain 6 responsible for in-frame insertions producing truncated cubilin. Interestingly normal size cubilin protein was identified in urine samples from homozygous FM1 patients, whereas a complete absence of the protein was reported in a patient homozygous for the FM2 mutation.[58] Other mutations were also uncovered but were subsequently identified as polymorphisms after their detection in normal individuals in the general population. The cubilin P1297L mutation associated with hereditary MGA1 was reported to cause impaired recognition of the cobalamin-IF complex by cubilin.[59] Moreover, mutation in *AMN* was reported in recessive hereditary MGA1,[60] and hence was demonstrated to be crucial for a functional cobalamin-IF receptor.[47] This study demonstrated that homozygous mutations affecting exons 1–4 of the human *AMN* gene translated into selective malabsorption of vitamin B_{12}, a phenotype associated with hereditary MGA1. Another study reported *AMN* deletion mutants in dogs with selective intestinal malabsorption of cobalamin associated with urinary loss of several low molecular weight proteins reminiscent of the human Imerslund–Gräsbeck syndrome (IGS a.k.a. MGA1). The authors showed that these mutations in the *AMN* gene abrogated AMN expression and blocked cubilin processing and targeting to the apical membrane. The essential AMN–cubilin interaction was recapitulated and validated in a heterologous cell-transfection model, hence explaining the molecular basis of intestinal cobalamin malabsorption syndrome.[61]

Tissular uptake of cobalamin requires intact megalin and transcobalamin II receptor

After cobalamin is absorbed at the BBM–blood barrier, it dissociates from the IF and reaches the systemic circulation where it associates with transcobalamin II (TCII). The kidney represents an essential organ were body vitamin B_{12} stores are maintained and studies demonstrated that kidney regulates plasma B_{12} levels by maintaining a pool of unbound cobalamin that can be released in case of B_{12} deficiency.[62–65] The tissular cobalamin-TCII complex uptake is achieved through megalin (LRP2)- and TCII receptor (TCII-R)-mediated endocytosis which plays a crucial role in cobalamin homeostasis (Figure 1B).[52,66] It is worth mentioning that TCII is responsible for the cellular uptake of B_{12} in most tissues and that TC deficiency is associated with severe MGA.[67,68]

Impaired megalin function has not been associated with cobalamin deficiency so far; however inappropriate megalin signaling has been shown to cause deleterious effects as a consequence of cobalamin uptake inhibition in tissues. This was particularly the case where mutations in the human *LRP2* gene encoding megalin were recently described to cause Donnai–Barrow and facio-oculo-acoustico-renal syndromes. Patients affected with these rare autosomal recessive disorders display severe malformations with proteinuria.[69] It is noteworthy that although essential, megalin and cubilin are not specific for cobalamin absorption and/or uptake, and are also receptors for haemoglobin,[70] albumin[71] and transferrin[72] among many other proteins (reviewed in[73]).

Intracellular metabolic functions of cobalamin

Following cobalamin-TCII cellular uptake, TCII undergoes lysosomal digestion allowing cobalamin separation from TCII and its cytoplasmic transfer. Part of the unbound cobalamin serves as a cofactor for methionin synthase-mediated homocysteine catabolism into methionine and methyltetrahydrofolate reductase (MTHFR)-mediated formation of the vitamin B_9 biologically active form, tetrahydrofolate, which is then involved in the synthesis of purines and pyrimidines (Figure 1B). The other part of free B_{12} is transferred to the mitochondria where it is transformed into adenosyl-B_{12}, an important cofactor in methylmalonyl-coenzyme A mutase-mediated

formation of succinyl-coA from methylmalonyl-coA, the product of odd-chain fatty acid and some amino acid catabolism. Hence, cobalamin deficiency will cause homocysteine accumulation, increased methylmalonyl-coA levels and decreased MTHFR activity. These changes translate into several abnormalities including folate deficiency and subsequent inhibition of purines and pyrimidines formation essential for RNA and DNA synthesis. The clinical manifestations of these metabolic abnormalities are MGA, neurological defects, malformations, increased cardiovascular thrombotic risk and renal disease and methylmalonic acidemia (reviewed in[65,74]). Functional cobalamin deficiency can also be caused by defects in the intracellular processing of cobalamin, such as abnormal lysosomal digestion of the TCII-cobalamin complex and subsequent defective lysosomal release of cobalamin, and abnormalities in intracytoplasmic cobalamin metabolism with all the consequences on biochemical reactions in which cobalamin acts as an important cofactor (reviewed in [75]).

Classical treatment of cobalamin deficiency

The classic treatment for cobalamin deficiency, particularly when the cause is not dietary deficiency, is parenteral administration—usually by intramuscular injection—of vitamin B_{12} in the form of cyanocobalamin and, more rarely, hydroxocobalamin.[1,17,18,34] In France, the recommended practice is to build up the tissue stores of cobalamin quickly and correct serum cobalamin hypovitaminosis, particularly in the case of pernicious anemia. The treatment involves the administration of 1000 µg of cyanocobalamin per day for 1 week, followed by 1000 µg per week for 1 month, then the dose is reduced to 1000 µg per month, normally for the rest of the patient's life.[8,11,1)] In the United States and the United Kingdom, dosages ranging from 100 to 1000 µg per month (or every 2–3 months when hydroxocobalamin is given) are used during the rest of the patient's life.[4,17]

Oral cobalamin therapy

In cases of cobalamin deficiency other than those caused by nutritional deficiency, alternative routes of cobalamin administration have been used: oral[17,18,76,77] and nasal.[78,79] These other routes of administration have been proposed as a way of avoiding the discomfort, inconvenience and cost of monthly injections. Our working group has developed an effective oral treatment for food-cobalamin malabsorption[80–83] and pernicious anemia[84] using crystalline cobalamin (cyanocobalamin). Our principal studies of oral cobalamin treatment (open, not randomized studies) are described in Table 4.[80–84] These data confirm the previously reported efficacy of oral crystalline cyanocobalamin, especially in food-cobalamin therapy.[18,36,76] All of our patients who were treated orally corrected their vitamin B_{12} levels and at least two-thirds corrected their haematological abnormalities.[80–84] Moreover, one-third of patients experienced a clinical improvement on oral treatment. In most cases of food-cobalamin malabsorption 'low' cobalamin doses (i.e. 125–1000 µg of oral crystalline cyanocobalamin per day) were used.

These data are in line with results from two prospective randomized controlled studies comparing oral cobalamin with intramuscular cobalamin therapy.[17,85] An evidence-based analysis by the *Vitamin B12 Cochrane Group* also supports the efficacy of oral cobalamin therapy, with a dose between 1000 and 2000 µg given initially daily and then weekly.[86] In this analysis, serum vitamin B_{12} levels increased significantly in patients receiving either oral vitamin B_{12} alone or patients receiving both oral and intramuscular treatment. In the two groups, patients exhibited neurological improvement of their symptoms.

In a randomized, parallel-group, double-blind and dose-finding trial, Eussen *et al*. showed that the lowest dose of oral cyanocobalamin required to normalize mild cobalamin deficiency is more than 200 times the recommended dietary allowance of ~3 µg daily (i.e. >500 µg per day).[87] The procedure for oral cobalamin therapy has, however, not been completely validated yet in clinical practice, most notably the long-term efficacy.[88] To date, as several authors suggest, oral cobalamin therapy remains one of 'medicine's best kept secrets'.[89] Nevertheless, the following can be proposed: ongoing supplementation until associated disorders are corrected (e.g. by halting the ingestion of the offending medication or exogenosis, or by treating *H. pylori* infection or pancreatic exocrine failure), lifelong administration or, when applicable, sequential administration.[4,14]

Concluding remarks

Until now, the definition of cobalamin deficiency needs to be established with precision since a consensus among members of the scientific community has not been reached yet. In this report, we

Table 4 Experience of oral cobalamin therapy for food-cobalamin malabsorption in the university hospital of Strasbourg, France

Study characteristics (number of patients)	Therapeutic modalities	Results	
Open prospective study of well-documented vitamin B_{12} deficiency related to food-cobalamin malabsorption ($n=10$)	Oral crystalline cyanocobalamin: 650 µg per day, during at least 3 months	Normalization of serum vitamin B_{12} levels in 80% of the patients Significant increase of hemoglobin (Hb) levels (mean of 1.9 g/dL) and decrease of mean erythrocyte cell volume (ECV) (mean of 7.8 fL) Improvement of clinical abnormalities in 20% of the patients No adverse effect	(81)
Open prospective study of low vitamin B_{12} levels not related to pernicious anemia ($n=20$)	Oral crystalline cyanocobalamin: between 1000 µg per day during at least 1 week	Normalization of serum vitamin B_{12} levels in 85% of the patients No adverse effect	(82)
Open prospective study of well-documented vitamin B_{12} deficiency related to food-cobalamin malabsorption ($n=30$)	Oral crystalline cyanocobalamin: between 1000 and 250 µg per day, during 1 month	Normalization of serum vitamin B_{12} levels in 87% of the patients Significant increase of Hb levels (mean of 0.6 g/dl) and decrease of ECV (mean of 3 fl); normalization of Hb levels and ECV in 54% and 100% of the patients, respectively Dose effect—effectiveness dose of vitamin B_{12} $\geqslant$500 µg per day No adverse effect	(80)
Open prospective study of low vitamin B_{12} levels not related to pernicious anemia ($n=30$)	Oral crystalline cyanocobalamin: between 1000 and 125 µg per day during at least 1 week	Normalization of serum vitamin B_{12} levels in all patients with at least a dose of vitamin $\geqslant$250 µg per day Dose effect—effectiveness dose of vitamin B_{12} $\geqslant$500 µg per day No adverse effect	(83)
Open prospective study of low vitamin B_{12} levels related to pernicious anemia ($n=10$)	Oral crystalline cyanocobalamin: 1000 µg per day, during at least 3 months	Significant increase of serum vitamin B_{12} levels in 90% of the patients (mean of 117.4 pg/ml Significant increase of Hb levels (mean of 2.45 g/dl) and decrease of ECV (mean of 10.4 fl) Improvement of clinical abnormalities in 30% of the patients	(84)

presented different aspects of cobalamin deficiency, including food cobalamin malabsorption syndrome and the classic treatment for cobalamin deficiency with a special focus on oral cobalamin therapy. Many causes of cobalamin deficiency have been uncovered to date, including mutations in genes encoding important proteins of the cobalamin metabolic pathway. However, many clinically diagnosed cobalamin deficiencies remain unexplained and molecular tools aimed at targeting genes involved in vitamin B_{12} absorption and cellular uptake signaling pathways will pave the way for new therapeutic approaches to efficiently treat functional cobalamin deficiency.

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