

Vitamin B₁₂ - “Active” B₁₂ and plasma MMA

Vitamin B₁₂ – function

As vitamins go, B₁₂ is a rather large molecule. One part of its structure is a “corrin” nucleus, which resembles the “haem” of haemoglobin. While the haem moiety in haemoglobin holds an atom of iron, in B₁₂ the corrin group holds an atom of cobalt. In order to be true B₁₂, cobalamin must have one of a number of attachments to the corrin group: depending on the attachment, cobalamin can be cyanocobalamin, hydroxycobalamin, aquacobalamin, nitritocobalamin, methylcobalamin, or adenosylcobalamin. Only two of these cobalamins are active as co-enzymes in the human body: methylcobalamin and adenosylcobalamin. Most supplemental B₁₂ is supplied as cyanocobalamin, in which form it is stable; cyanocobalamin must be converted to methyl- or adenosylcobalamin before it is biologically active.

Vitamin B₁₂ is synthesised by micro-organisms and enters the diet with food of animal origin. Plants do not require B₁₂ for any function, and therefore have no mechanisms to produce or store B₁₂. The biosynthesis of this nutrient never seems to have made the transition to the higher, eukaryotic forms of life. In humans, the vitamin is required in trace amounts (approximately 1 µg/day) to act as a co-enzyme to two enzymes, methionine synthase and (R)-methylmalonyl-CoA mutase [1,2]. B₁₂ is the only co-enzyme for methylmalonyl-CoA mutase, which catalyses the conversion of methylmalonyl-CoA to succinyl-CoA. When adequate B₁₂ is not available, methylmalonyl-CoA production increases. Because it is toxic, methylmalonyl-CoA is then rapidly converted to methylmalonic acid (MMA), which accumulates in the blood and urine. Since this reaction only requires B₁₂ as a co-enzyme, MMA levels are a good indicator of B₁₂ status. Certain rare genetic disorders can also cause high MMA levels in neonates.

Vitamin B₁₂ – digestion and absorption

When humans eat animal foods, the B₁₂ they consume is protein bound. When this protein-B₁₂ complex reaches the stomach, gastric acid and enzymes detach the B₁₂ from the protein. Then, in a process unique to B₁₂, another protein (R protein) picks up the B₁₂ and transports it through the stomach and into the small intestine. R protein is found in many human body fluids including saliva and stomach secretions. R protein can bind and transport up all corrinoid molecules in addition to true B₁₂.

The parietal cells of the stomach also produce a protein called intrinsic factor (IF). When B₁₂-R protein reaches the small intestine, B₁₂ is released from this complex by proteolytic enzymes secreted by the pancreas. B₁₂ then attaches to IF that has also found its way from the stomach into the small intestine. This B₁₂-IF complex protects B₁₂ against bacterial and digestive enzyme degradation and carries it to the ileum, the last section of the small intestine, where the enterocytes have surface receptors for B₁₂-IF complex. Uptake of the complex is by a calcium-dependent process; B₁₂ is then translocated through the enterocytes to the portal circulation, bound to another protein, transcobalamin II [1,2].

Vitamin B₁₂ deficiency

Deficiency states induced by poor dietary intake of B₁₂ (i.e. vegetarian or vegan diets) take up to 20 years to manifest. However, clinical deficiencies as a consequence of abnormalities in one of the multiple steps that regulate cobalamin absorption or its enterohepatic circulation present more rapidly (c. 2 years). Vitamin B₁₂ deficiency is relatively common in the U.K. population and occurs in patients with autoimmune disease (pernicious anaemia), severe primary hypothyroidism and ileal disease, as well as in subjects receiving chronic therapy with antacids, proton pump inhibitors (PPI) or H₂ antagonists. B₁₂ deficiency is also seen in chronic malnutrition states, including alcoholism, while other causes include vegetarian diets [3], pregnancy and lactation.

The timely detection, and correction, of vitamin B₁₂ deficiency prevents the development of macrocytic anaemia, as well as elevated homocysteine (which is a thrombotic risk factor), potentially irreversible peripheral neuropathy, memory loss and other cognitive deficits.

Vitamin B₁₂ status assessment

The measurement of serum 'total' levels of vitamin B₁₂ is commonly used as a marker of vitamin B₁₂ status. However, it is not widely appreciated that circulating vitamin B₁₂ is predominately bound to two different proteins (haptocorrin and holotranscobalamin) and that the commonly available laboratory assays are unable to discriminate between the '*Inactive*' (haptocorrin-bound) and '*Active*' (holotranscobalamin-bound) forms. Although some 80% of circulating vitamin B₁₂ is carried by haptocorrin (holohaptocorrin, abbreviated as HC), extra-hepatic cellular receptors for this form have not been described (hence the term '*Inactive*' B₁₂ for this form) [4]. Circulating levels of HC slowly decline in response to the onset of a deficiency state, typically taking 3-6 years to fall below the lower limit of the reference range. If the assessment is done using a B₁₂ assay that cannot discriminate between these two forms, then any decline in the less abundant '*Active*' form is masked by the more abundant '*Inactive*' variant. Tissue deficiency of vitamin B₁₂ is thus common among patients in whom the serum total ('*Inactive*' + '*Active*') vitamin B₁₂ level is within the reference range for total B₁₂ [5].

The '*Active*' Vitamin B₁₂ (holotranscobalamin or holoTC) assay

'*Active*' vitamin B₁₂ (holo TC) is the metabolically active portion of vitamin B₁₂. This assay measures holotranscobalamin in a form that can be taken up by the receptor-mediated process found in all DNA-producing cells [6]. The holoTC assay is the earliest and most sensitive laboratory marker of a negative vitamin B₁₂ balance and gives a better indication of vitamin B₁₂ deficiency than the previous commonly used markers.

Interpretation of '*Active*' Vitamin B₁₂ results using the Guy's and St.Thomas' Nutristasis Unit laboratory algorithm

Patients are defined as **deficient** if the '*Active*' vitamin B₁₂ concentration is **< 25 pmol/L**.

The status of patients with '*Active*' vitamin B₁₂ concentrations between **25 and 50 pmol/L** (the **intermediate** range) requires further investigation and this is best carried out using the serum methylmalonic acid (MMA) assay [7,8,9,10]. When the supply of vitamin B₁₂ is suboptimal production of succinyl CoA is blocked, leading to increased formation of MMA. Circulating levels of MMA are thus a functional indicator of vitamin B₁₂ status. Serum **MMA** concentrations of **<280 nmol/L** are considered to be within the reference range for healthy subjects under the age of 65 years. The upper limit of the serum MMA reference range for patients older than 65 years is **360 nmol/L**. However, one limitation is that serum MMA levels are not a reliable marker of vitamin B₁₂ status in patients with impaired renal function (which is more common in the elderly).

Patients with '*Active*' vitamin B₁₂ levels above **50 pmol/L** are replete and require no further investigation for at least 4 months. Please note that the diagnostic utility of this test can be limited immediately after vitamin B₁₂ supplementation [11]. If supplemental B₁₂ has been taken within one month prior to the test, this should be stated on the request form.

There is a transient increase in circulating levels of '*Active*' vitamin B₁₂ 6-8 hours after oral supplementation. Thus monitoring '*Active*' vitamin B₁₂ can identify subjects who are likely to benefit from oral treatment.

Specimen requirements

Serum separator tubes (plain gel tubes - available on request). If posted, samples must reach the laboratory within 24 hours.

Patient preparation

Patients should refrain from taking nutritional supplements for 24 hours before venipuncture. If supplements containing vitamin B₁₂ have been taken in the month prior to venipuncture, details should be given on the request form. Recent i.m. B₁₂ therapy may also complicate interpretation of the results.

Laboratory Methods

Serum 'Active' vitamin B₁₂ (holotranscobalamin) - Two-step chemiluminescent microparticle immunoassay.
Serum methylmalonic Acid - high pressure liquid chromatography (HPLC).

Turn around time: 10 working days.

References

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