Q&A
Metformin and vitamin B12 deficiency

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What role does vitamin B12 play in the body?

Vitamin B12, also known as cobalamin, is a water-soluble vitamin that plays a crucial role in DNA synthesis and haematopoiesis. It functions as an enzymatic co-factor in two pathways to exert these effects: methylation of homocysteine to produce methionine, which is required for protein synthesis, and converting methylmalonyl-coenzyme A to succinyl-coenzyme A, which is involved in the tricarboxylic acid cycle (Shenkin and Roberts, 2012). The main dietary sources of vitamin B12 are meat and dairy products, shellfish, fish and fortified cereal products.

What are the typical signs and symptoms of vitamin B12 deficiency?

Vitamin B12 deficiency can have a variety of clinical presentations affecting the haematological and neurological systems. There is classically megaloblastic anaemia with raised mean corpuscular volume (MCV) on the full blood count, which can lead to symptoms such as general fatigue and shortness of breath as the anaemia becomes more severe. At its most severe, symptoms of congestive heart failure can present.

Neurological symptoms can be the presenting complaint and may predate haematological signs (Briani et al, 2013). Various neurological syndromes are recognised, from neuropsychiatric disturbance such as depression, psychosis, mania and cognitive impairment, to myelopathy, neuropathy, optic nerve atrophy and a characteristic spinal cord syndrome known as subacute combined degeneration of the cord (SCD). SCD affects the long tracts of the spinal cord, resulting in sensory disturbance, impaired position sense and spastic paraparesis (Hemmer et al, 1998).

What is the normal reference range for vitamin B12?

Reference ranges are somewhat controversial for vitamin B12 as there are no clear data to denote concentrations associated with definite deficiency and definite sufficiency. Some laboratories have tried to highlight this issue by providing a non-dichotomous reference range. For example, at University Hospital Southampton, concentrations greater than 160 ng/L are said to exclude deficiency, and concentrations of less than 130 ng/L are said to be consistent with deficiency. That leaves concentrations of 130–160 ng/L in a "grey zone". Here, clinical judgement is required. For instance, high pre-test probability, such as megaloblastic anaemia with neurological symptoms and positive anti-intrinsic factor antibodies, then such a result is likely to indicate deficiency and should be acted upon. Similar algorithms for dealing with the spectrum of vitamin B12 results have been published (Harrington, 2017).

It is important to note that automated vitamin B12 assays are known to have issues in patients with pernicious anaemia, and in such cases may generate false-normal results in 22–35% of individuals (Carmel and Agrawal, 2012). This occurs due to anti-intrinsic factor antibodies in the patient's sample interfering with competitive binding vitamin B12 assays (Wainwright and Cook, 2015). Due to this phenomenon it is crucial that clinicians treat normal vitamin B12 results in high-probability

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Glossary

- **Mean corpuscular volume**: Average volume of a red cell.
- **Megaloblastic anaemia**: An anaemia that results from inhibition of DNA synthesis during red cell production. Cells continue to grow without division, resulting in larger than normal cells.
- **Pernicious anaemia**: Anaemia due to vitamin B12 deficiency caused by lack of intrinsic factor impairing B12 absorption.
- **Zollinger-Ellison syndrome**: A rare condition in which one or more tumours form in the pancreas or duodenum. These tumours, called gastrinomas, secrete large amounts of the hormone gastrin, which causes the stomach to produce too much acid.

patients with suspicion. In some instances, it may be necessary to use a functional marker of vitamin B12 status such as plasma homocysteine or methylmalonic acid, and the local biochemistry laboratory will be able to advise where this might be appropriate.

Q What are the most common causes of vitamin B12 deficiency?

There are various causes of vitamin B12 deficiency, the commonest cause being impaired gastric absorption resulting from autoimmune pernicious anaemia (Hunt et al, 2014). Surgical gastrectomy and Zollinger-Ellison syndrome can also lead to this. Deficiency can result from impaired intestinal absorption such as that associated with Crohn's disease or small bowel bacterial overgrowth. There is a clear association between chronic alcoholism and vitamin B12 deficiency. It can also result from pancreatic insufficiency or decreased dietary intake associated with malnutrition or adhering to a strict vegan diet. There are various inherited disorders of cobalamin handling; however, these are rare. Finally, deficiency is associated with the use of several drugs such as proton pump inhibitors, H2 receptor blockers and metformin.

Q What is the role of intrinsic factor in vitamin B12 deficiency and what can anti-intrinsic factor antibody testing tell us?

Anti-intrinsic factor antibodies are a useful test for the diagnosis of pernicious anaemia, which is the commonest cause of vitamin B12 deficiency. It should be considered, however, that anti-intrinsic factor antibodies have a sensitivity of 50% in this setting (Andres and Serraj, 2012), indicating that only one in two affected individuals with pernicious anaemia will have positive antibodies. However, they are highly specific with a specificity of 95–100% for a diagnosis of pernicious anaemia (Andres and Serraj, 2012). Some advocate use of anti-gastric parietal cell antibodies either alone or in combination with anti-intrinsic factor antibodies. Combining the two has been reported to provide 73% sensitivity and 100% specificity for diagnosing pernicious anaemia (Lahner and Annibale, 2009).

Q What is the link between the use of metformin and vitamin B12 deficiency?

It has long been known that there is an association between the use of metformin and vitamin B12 deficiency. This was first reported when Tomkin et al (1971) observed that 21 out of 71 people with diabetes taking metformin demonstrated malabsorption of vitamin B12 as measured by an isotope-labelled functional technique. There have been multiple studies since then that have confirmed this association, although data vary somewhat in terms of estimated incidence of vitamin B12 deficiency in association with metformin use. A recent systematic review has synthesised the findings of six randomised controlled trials that have addressed this issue (Liu et al, 2014). The authors found that serum vitamin B12 concentrations were significantly lower in people treated with metformin than those who received either placebo or rosiglitazone, with a mean difference of 73 ng/L. They also noted that this relationship was dose dependent, with those receiving a higher dose of metformin experiencing a significantly greater mean reduction in vitamin B12 than those receiving a lower dose. Subgroup analysis showed that this effect was seen with short-term metformin use (treatment duration <3 years) and long-term use (treatment duration >3 years).

Vitamin B12 deficiency may be of particular importance in people with diabetes as both conditions can result in a peripheral neuropathy and the concern is that use of metformin may result in more cases of peripheral neuropathy in people with diabetes. Two recent small studies have investigated whether or not use of metformin is associated with an increase in the incidence of peripheral neuropathy or a worsening of existing neuropathy, and both concluded that no differences were seen when compared with people who have diabetes and were not treated with metformin (Chen et al, 2012; Ahmed et al, 2016). However, it is clear that larger studies are required to conclusively address this question.

The mechanism underlying this phenomenon is unclear. It has been suggested that it may be due to changes in bacterial flora affecting intestinal absorption; however, there is little

research to support this. It is possible that it is at least partially due the effect metformin has on calcium-dependent ion flux across membranes, as vitamin B12 bound to intrinsic factor is taken up across the ileal cell membrane in a calcium-dependent manner. This was investigated some 17 years ago by a group who showed that calcium supplementation reversed the impaired absorption of vitamin B12 in those taking metformin (Bauman et al, 2000). However, this was a very small study of just 21 people and sufficient evidence does not exist to justify additional calcium supplementation in all individuals taking metformin as a preventative measure against metformin-induced vitamin B12 deficiency. This is an area where further work is clearly required to fully understand the underlying pathophysiology.

Q Should people on metformin be routinely checked for vitamin B12 deficiency?

There is no evidence currently to suggest that routine screening for vitamin B12 deficiency in asymptomatic people with diabetes who take metformin would be cost-effective or clinically useful. Clearly, vitamin B12 testing in those with a high likelihood of deficiency, (such as those with macrocytosis, megaloblastic anaemia, unexplained neuropathy or a clinical malabsorption syndrome) is sensible. It is important that clinicians are aware of the increased risk of vitamin B12 deficiency for those taking metformin, and it may be useful to have a lower threshold for testing in such patients. For example, it would be sensible for all people with diabetes who develop a peripheral neuropathy to have their vitamin B12 levels checked to ensure that deficiency is not missed.

Q What is the preferred treatment for B12 deficiency?

For vitamin B12 deficiency, it is generally accepted that symptomatic individuals require parenteral treatment with intramuscular injections. This includes all of those diagnosed with pernicious anaemia. A standard replacement schedule in this context would be 1 mg hydroxycobalamin three times per week for 2 weeks, followed by 1 mg every 3 months thereafter.

For individuals who have a vitamin B12 concentration consistent with deficiency but have no suggestive signs or symptoms, then the treatment is less straightforward. In general terms, any individual found to have a vitamin B12 concentration suggestive of deficiency should be treated with replacement, even if they are not displaying any signs or symptoms. The rationale for this is that the consequences for untreated vitamin B12 deficiency are potentially severe, with the possibility of irreversible neurological damage, and treatment is simple, cheap and generally well tolerated. There is no clear evidence to guide management; however, treatment in such cases could be in the form of oral replacement and, indeed, there is evidence that oral replacement has equal efficacy with parenteral replacement and has the added advantage of being considerably less expensive and more acceptable to the patient (Masucci and Goeree, 2013).

Principles of treatment for those with vitamin B12 deficiency related to metformin treatment should not differ from that arising from any other indication, and there is certainly no indication to stop metformin if such deficiency develops. There is no clear guidance regarding whether treatment for people on metformin should be in the form of oral tablets or intramuscular injections, and this should be a decision on a case-by-case basis, taking into account patient preference. If a patient has clear symptoms of vitamin B12 deficiency, then intramuscular treatment is likely to be indicated, whereas oral treatment with biochemical monitoring is likely to be sufficient in asymptomatic cases. Additionally, given the increased pre-test probability for deficiency in those taking metformin, treatment is likely to be required for all individuals whose vitamin B12 concentrations fall within the "grey zone".

Q For those on replacement, when should vitamin B12 concentration be rechecked?

Once an individual is established on parenteral vitamin B12 replacement, there is no need for

"It is important that clinicians are aware of the increased risk of vitamin B12 deficiency for those taking metformin."

Take-home messages on metformin and vitamin B12 deficiency for primary care healthcare professionals.

- Vitamin B12, also known as cobalamin, is a water-soluble vitamin that plays a crucial role in DNA synthesis and haematopoiesis.
- Symptoms of vitamin B12 deficiency can be haematological (i.e. anaemia leading to general fatigue and shortness of breath, and at its most severe, symptoms of congestive heart failure) and neurological (i.e. psychological and peripheral neuropathy).
- The reference range for vitamin B12 deficiency is controversial; however, at University Hospital Southampton
  - **<130 ng/L**: deficient
  - **>160 ng/L**: not deficient
  - **130–160 ng/L**: grey zone (requires clinical judgement).
- The commonest cause of vitamin B12 deficiency is impaired gastric absorption resulting from autoimmune pernicious anaemia. Other causes include surgical gastrectomy, Crohn's disease, a strict vegan diet, or drug therapies such as PPIs, H2 blockers or metformin.
- B12 deficiency should be treated with intramuscular injections for symptomatic deficiency and all those with confirmed pernicious anaemia, oral treatment may be considered in asymptomatic individuals.
- It is important that clinicians are aware of the increased risk of vitamin B12 deficiency for those taking metformin.
- If someone is taking metformin and presents with the symptoms or signs of possible vitamin B12 deficiency, such as megaloblastic anaemia or peripheral neuropathy, it is sensible to check their B12 levels.
- At present there is no evidence to suggest routine vitamin B12 testing for people with diabetes taking metformin would be clinically useful.

routine monitoring of B12 concentrations. For those having oral vitamin B12 replacement, there are no set guidelines regarding treatment monitoring. A reasonable approach would be to monitor the initial response to treatment by checking vitamin B12 concentration 2 months after treatment starts. An ongoing response to treatment could then be monitored in the form of vitamin B12 concentrations every 6–12 months thereafter. ■

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