Do we need to reconsider the desirable blood level of vitamin B12?

"The proscription that cobalamin deficiency should not be diagnosed unless megaloblastic changes are found is akin to requiring jaundice to diagnose liver disease." [1]. This statement by Carmel, one of the leaders in the vitamin B12 field, was made more than 10 years ago and yet many physicians and health authorities still insist on haematological changes before accepting a diagnosis of vitamin B12 (cobalamin) deficiency. It is not unusual that health authorities refuse prescriptions for vitamin B12 in patients with clinical signs of neuropathy because the patients have no haematological signs and their plasma vitamin B12 levels are reported as 'normal'. This situation is disconcerting in view of the long history of studies showing that neurological signs of deficiency may occur in patients who do not show anaemia [2]. Even in patients with clinical pernicious anaemia, up to 28% do not have anaemia and up to 33% have normal mean corpuscular volume [1]. How, then do we define which patients with clinical signs or symptoms should be treated and what is the desirable plasma vitamin B12 level below which treatment is required?

In mammals, vitamin B12 provides co-factors for two enzymes (methionine synthase and L-methylmalonylCoA mutase), and the substrates for these enzymes, homocysteine (tHcy) and methylmalonic acid (MMA) build-up when vitamin B12 status is low. Several large population studies have examined the relationship of tHcy and MMA to blood vitamin B12 concentrations [3-5]. It has been found that the concentrations of these metabolic markers start to increase at vitamin B12 levels considerably above the typical cut-off value used to define B12-deficiency, 148 pmol/L (200 pg/mL), as can be seen from the Figure. The key question is: do the metabolic markers indicate a clinically relevant functional deficit in vitamin B12, or are they simply reflecting that the enzymes are no longer saturated by their co-factors? If the latter, then elevated tHcy or MMA might just reflect a state that has been called 'subclinical cobalamin deficiency' (SCCD), defined by Carmel [5] as a state of 'mild metabolic abnormalities without clinical signs or symptoms'. SCCD may 'eventually progress sufficiently to produce clinical, symptomatic deficiency; or remit completely…; or accelerate and reach clinical deficiency more quickly; or fluctuate indefinitely between normal and mildly subclinical deficiency states.'[5] The unpredictable consequences of SCCD may be important for public health because it is relatively common, especially in the elderly [5], and it has been discussed whether attempts should be made to reduce its prevalence - for example by fortification of food with vitamin B12 [6-8]. However, if SCCD is a benign state, it is difficult to see any justification for treating it. In contrast, if vitamin B12 levels in this low-normal range are associated with clinical signs and

symptoms then we have to consider what should be done with patients who display these signs. In this Editorial, prompted by a report in the current issue of the Journal [9], we will draw attention to many observational studies and one clinical trial indicating that low-normal levels of vitamin B12 may well be associated with clinically significant outcomes.

A study in 161 community elderly from Sweden [10] reported that there was no increase in the prevalence of most of the typical vitamin B12 deficiency symptoms in those with raised tHcy or MMA levels, which is consistent with the concept of SCCD. The only correlation was between raised tHcy and an increase in tongue mucosa atrophy and mouth angle stomatitis. However, examination of the data shows several trends that did not reach statistical significance, and the authors pointed out that their study may have been underpowered to detect other associations, and that some of the tests used were not very sensitive. Using more sensitive cognitive tests, Hooshmand *et al.* [9] found that, in 274 community elderly, raised baseline tHcy is associated with cognitive decline over 7 y, and they also found that high baseline concentrations of holotranscobalamin (holoTC) are protective against cognitive decline. HoloTC i.e., vitamin B12 bound to transcobalamin, is the circulating form of vitamin B12 taken up by most cells [11]. It is noteworthy that Hooshmand *et al.* found that 'the protective effect of holoTC was present over the whole distribution of holoTC'. Thus, low vitamin B12 status, as reflected by low holoTC, is a risk factor for cognitive decline over its whole distribution. A similar result has been reported in two other prospective studies. In one, incident dementia was associated with the decrease in vitamin B12 status over a two-year period across the normal range of vitamin B12 [12], and in another study the lowest three quartiles of baseline holoTC were associated with cognitive decline over a 10 year period [13]. Thus, it is not just the lowest levels of vitamin B12 that are a risk factor for later cognitive decline, but levels above the traditional deficiency cut-off as well. Similar results have been reported from cross-sectional studies, such as the 'Banbury B12 study' on 1000 community elderly, where raised tHcy or MMA and low holoTC across the normal range were associated with cognitive deficit and with impaired reflexes [14]. We have reviewed such studies [15, 16] and some are listed in the Table.

It is mainly outcomes that affect the functioning of the nervous system that have so far been associated with vitamin B12 status in the low-normal range (Table). Nevertheless, these studies were usually observational, and so causality cannot be assumed. Thus, it will be necessary to perform clinical trials to see whether administering vitamin B12 will prevent such outcomes in those with low-normal vitamin B12 status.

A striking relationship between vitamin B12 or holoTC levels across the entire range (vitamin B12: 160 – 700 pmol/L) was found for progressive brain atrophy over 5 y in a community population of 107 elderly: the lower the B12 status, the faster the brain atrophied [17]. To see whether the rate of atrophy could be modified, elderly subjects with mild cognitive impairment were recruited into the VITACOG trial [18] and the rate of brain atrophy was assessed over 2 y: half the participants were treated with a combination of vitamin B12, B6 and folic acid in order to lower their tHcy levels. This treatment, which lowered tHcy by 30% and increased plasma vitamin B12 from a mean of 330 to 672 pmol/L, was associated with an average slowing of brain atrophy of 30% compared with the placebo group. The treatment effect was dependent on the baseline level of tHcy, with a 53% slowing of the rate of atrophy in those in the top quartile of tHcy [18]. Those in the B vitamin treated group with high baseline tHcy also showed a slowing of cognitive decline and an improvement in clinical status compared with the placebo group [19]. Using three vitamins in combination, it is not, of course, possible to conclude that it was the increase in vitamin B12 level that caused the beneficial effects on brain atrophy and cognition. However, the trial does demonstrate that subjects who are not classically deficient in vitamin B12 but who have raised tHcy and show progressive brain atrophy and cognitive decline can benefit from B vitamin treatment.

Subject to further clinical trials on the other outcomes in the Table, we suggest that the concept of SCCD may need to be revised: people with elevated MMA or tHcy and low-normal B12 status may sometimes have subtle clinical signs that could reflect underlying disease processes due to low vitamin B12 status. In such cases, what level of vitamin B12 is desirable in order potentially to slow down or prevent such disease processes? One approach is to examine the association of vitamin B12 with tHcy and MMA levels, as shown in the Figure, and identify the level of vitamin B12 at which tHcy or MMA levels start to rise steeply. Several large community studies have presented such figures and the inflection points usually fall between about 200 and 500 pmol/L [3, 4, 20, 21]. In the Hordaland study (Figure), Vogiatzoglou *et al.* used segmented regression analysis to obtain breakpoints of 334 pmol/L and 393 pmol/L for vitamin B12, based on MMA and tHcy, respectively [21] and defined a vitamin B12 replete group as individuals with vitamin B12 ≥400 pmol/L. A similar approach was used by Selhub *et al.* [4], who suggested that the use of disease-based epidemiological data might ultimately replace or augment the use of the metabolic markers.

The emphasis on disease to determine desirable levels is important. While it is relatively uncontroversial to give vitamin B12 to those with disease, or with symptoms or signs consistent

with "deficiency", it is not so simple to use the metabolic markers to define new desirable levels of vitamin B12 for the entire population. The findings of studies like that of Hooshmand *et al.* [9] are challenging because they raise the question whether something needs to be done for the elderly who have a low-normal vitamin B12 status without clinical signs, but who have an increased risk of future cognitive decline. Mandatory fortification of foods with vitamin B12 is one approach [6-8], but this should not be introduced without large-scale trials to look for efficacy as well as potential adverse effects. For subjects showing signs or symptoms on the other hand, there is a good case for improving their vitamin B12 status and the question of what value we should aim for is a matter for international discussion between experts. We believe that the traditional cut-off value of 148 pmol/L is too low. We suggest that physicians should consider treating patients who show symptoms but have vitamin B12 levels above this value, particularly those in the low-normal range up to ~300 pmol/L, in order to see whether their symptoms are relieved. It has now been shown that oral vitamin B12 is effective [22] and so such interventions are simple and likely to be cost-effective if successful.

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Conflict of interest

The authors have no conflicts to declare.

Figure. Relationship between plasma vitamin B12 and plasma total homocysteine (tHcy) or methylmalonic acid (MMA) in 3,262 community-dwelling people aged 71-74 y in Norway. Disease associations are discussed in the text and references are in the Table. Based on Figure 1 in Vogiatzoglou [21]. SCD, sub-acute combined degeneration of the spinal cord.

Table. Associations between low-normal vitamin B12 status* and different outcomes

Outcome	Type of study	References
Neural tube defect	Prospective (in mothers)	[23]
Cognitive deficit in elderly	Cross-sectional	[14]
Cognitive decline in elderly	Prospective	[9, 13]
Alzheimer's disease	Cross-sectional	[24, 25]
White matter damage	Cross-sectional	[26]
Whole brain atrophy	Prospective, Cross-sectional	[17, 27]
Depression	Prospective	[28]
Response to treatment of depression	Prospective	[29]
Stroke	Prospective	[30]
Low bone mineral density in women	Cross-sectional	[31]
(Autonomic dysfunction – B12 deficient)	Treatment intervention	[32]
DNA damage in lymphocytes	Treatment intervention	[33]
Uracil mis-incorporation into DNA	Cross-sectional	[34]

*Low-normal vitamin B12 status defined as >148 pmol/L and was assessed by measuring serum/plasma vitamin B12, holoTC, or MMA. See individual studies for details. This is not intended to be a comprehensive list.

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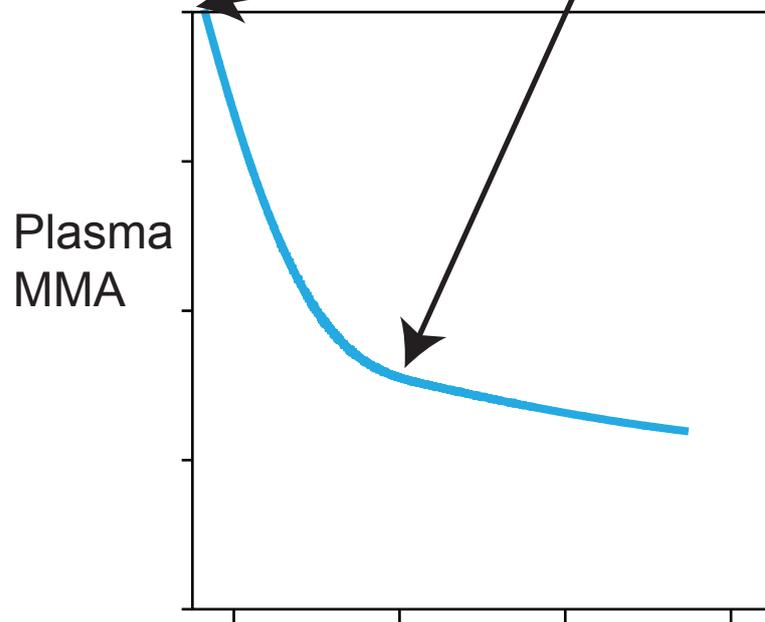Mansfield Rd.
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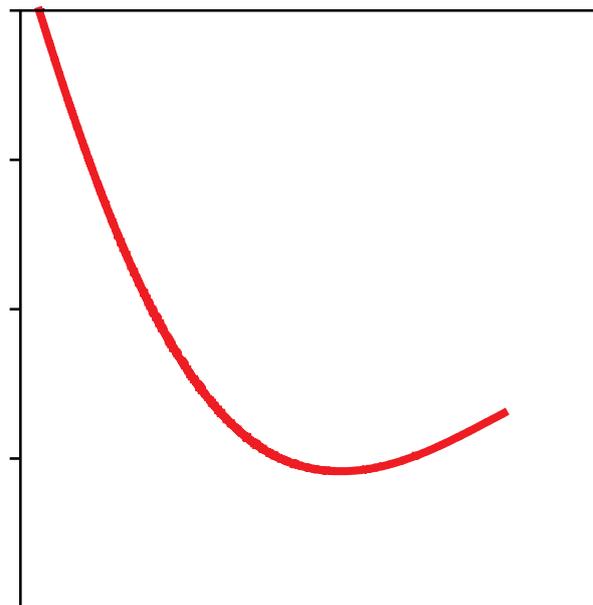Tel +44-1865-271617

Metabolic insufficiency
Neural tube defects
Cognitive deficit/decline
Alzheimer's disease
Brain atrophy
White matter damage
Stroke
Depression
Neuropathy
Low bone mineral density
DNA damage

Classical deficiency
Haematological signs
SCD

Plasma MMA

Plasma tHcy

200   400   600   800
Plasma vitamin B12 (pmol/L)