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Invited Commentary

Invited commentary in response to: 'Identification of vitamin B_{12} deficiency in vegetarian Indians'

The proportion of humans adopting a plant-based lifestyle is on the rise worldwide. Benefits on economics, climate and health have been documented and projected to continue over the next decades[1–3]. Apart from its sustainability[4], well-planned plant-based diets are nutritionally complete for human beings of all ages[5], with vitamin B_{12} being the only micronutrient not found in plants[6–8]. Vitamin B_{12} is only synthesised by a few bacteria and archaea[9–11]; hence, humans adhering to a plant-based lifestyle can obtain the micronutrient from foods fermented with bacteria that naturally produce vitamin B_{12}, foods fortified with vitamin B_{12} and over-the-counter supplements. However, the high prevalence of vitamin B_{12} insufficiency in vegetarians across nations[12] suggests that food literacy programmes[13] are not yet meeting the needs of the current dietary shift. Vitamin B_{12} deficiency, whether acquired or inherited, leads to the inactivation of the two vitamin B_{12}-dependent enzymes, cytosolic methionine synthase and mitochondrial methylmalonyl-CoA mutase[14,15]. This manifests with elevated homocysteine (Hcy) and methymalonic acid (MMA), and in severe cases with reduction of total serum vitamin B_{12} and its bioactive fraction holo-transcobalamin (holo-TC).

The study by Naik *et al.*[16] examined vitamin B_{12} status in a cohort of 119 young, healthy unsupplemented vegetarian Indian graduates. Using standard cut-off values for the assessment of vitamin B_{12} status Naik *et al.* determined that 50 % were vitamin B_{12} deficient, 50–70 % exhibited low plasma holo-TC and 70–90 % presented elevated plasma total Hcy (tHcy). All participants in this study were asymptomatic of clinical vitamin B_{12} deficiency[16]. These findings are in good agreement with a previous study by Refsum *et al.* in a different Indian cohort[17]. Interestingly, nineteen participants with plasma vitamin B_{12} concentrations between 113 and 122 pmol/l had normal values of holo-TC (34–52 pmol/l), while exhibiting elevated tHcy according to standard cut-off reference values (discussed in Hannibal *et al.*[18] and references therein). Naik *et al.*[16] propose a new set of cut-off values to improve the diagnosis of vitamin B_{12} deficiency in young vegetarian Indians. The authors propose the use of a combination of biomarkers and cut-off values of 100 and 19·6 pmol/l for plasma vitamin B_{12} and holo-TC, respectively, and values of tHcy of 17·6 and 27 μmol/l for females and males, respectively[16]. This proposal appears in line with previously documented data in a study performed on a cohort of American vegetarians by the late Dr Victor Herbert[19]. Herbert divided vitamin B_{12} status in vegetarians in four distinct stages, namely I, II, III and IV[19]. Herbert and independent colleagues established that vegetarians can withstand long-term insufficient intake of vitamin B_{12} due to up-regulation of enterohepatic circulation and intestinal reabsorption of traces of vitamin B_{12}[19–22]. In healthy vegetarians, this represents a mechanism to optimise the recycling of the scarce micronutrient.

One strength of the study by Naik *et al.* is that the level of accuracy for identifying vitamin B_{12} deficiency using Hcy as a metabolic biomarker did not depend on the concentration of vitamin B_{12} chosen (see fig. 2, ROC, tHcy in Naik *et al.*[16]). Using the current standard cut-off of 150 pmol/l for plasma vitamin B_{12}, the sensitivity was 91·8 % and the specificity 79·31 %. Applying the new proposed cut-off value of 100 pmol/l for plasma vitamin B_{12}, the sensitivity was 82·72 % and the specificity 89·47 %[16].

An important consideration that emerges from this analysis concerns the origin of reference intervals used worldwide to diagnose vitamin B_{12} deficiency. Reference ranges have been established by examining plasma vitamin B_{12}, Hcy, methylmalonic acid and holo-TC, in healthy individuals residing in Western industrialised nations of whom the vast majority (>95 %) pursue an omnivorous lifestyle. An omnivorous diet provides sufficient vitamin B_{12} to keep tHcy and MMA at a minimum, and holo-TC above the established cut-off for deficiency (<35 pg/ml). Are these reference ranges established in omnivores appropriate cut-offs to assess vitamin B_{12} status in populations that adhere to vegetarian diets? More generally, what are the optimal intracellular concentrations of Cbl, Hcy and MMA required to support function? And how well do serum levels of Cbl, Hcy and MMA reflect cellular cobalamin status?

What follows is the question of whether slightly elevated tHcy and MMA as seen in asymptomatic vegetarians represent a prelude to clinical deficiency of vitamin B_{12} or if instead, these are metabolically satisfactory levels of metabolites that will cause no harm in the long term, that is subclinical cobalamin deficiency. According to Carmel, subclinical cobalamin deficiency is a condition where mild biochemical changes are documented (elevated tHcy and MMA, low vitamin B_{12} and holo-TC) but the patient is asymptomatic[23]. According to this definition, asymptomatic individuals presenting with elevated tHcy and low vitamin B_{12} and holo-TC in the study by Naik *et al.*[16] would classify as having subclinical cobalamin deficiency[23]. Carmel described that subclinical deficiency of

cobalamin rarely progresses into clinical deficiency[23], which brings us to the next issue: should vegetarian individuals with subclinical cobalamin deficiency receive treatment with cobalamin? If so, what should be the dose and form of administration? A possibility exists that what classifies as subclinical cobalamin deficiency in omnivores may not represent a status of abnormal vitamin B_{12} homoeostasis in plant-based individuals. Conceivably, applying reference values and cut-offs established from studying omnivorous populations may lead to the over-diagnosis of vitamin B_{12} deficiency in vegetarians and vegans. More broadly, the meaning of elevated tHcy and the impact of hyperhomocystinaemia on health remain a debate[24]. The finding that patients with classical homocystinuria (deficiency of the enzyme cystathionine β-synthase) sustain good health by staying at a target level of tHcy under 120 μM, that is well beyond the accepted normal range[25], questions the suitability of the tHcy reference values in plasma whereby 15 μM is defined as the upper limit. Vegetarian populations who naturally consume lower amounts of vitamin B_{12} may exhibit tHcy concentrations higher than 15 μM, without it representing a health threat.

Should a new reference interval for assessing vitamin B_{12} status be considered when examining human populations that pursue a predominantly plant-based lifestyle as proposed by Naik *et al.*? In light of current knowledge this proposal is a reasonable one, yet the definite answer to this question demands further study in larger cohorts of humans who have adopted vegetarianism both short and long term. Further, a mathematical expression that combines two, three or four biomarkers of vitamin B_{12} status, namely the cB_{12} index, has been shown to be a more reliable indicator of vitamin B_{12} status[26–28]. It would be valuable to examine the performance of the cB_{12} index with and without the introduction of new cut-off points for the accurate and timely diagnosis of vitamin B_{12} deficiency in vegetarians.

Another consideration that emerges from this analysis is what supplemental dose of cobalamin should be recommended to support good health in vegetarians? The most recent recommendation for strict vegetarians is a small oral dose of 2–6 μg daily[29]. Higher doses were only recommended if absorption problems are confirmed in individual cases. Further, patients who recovered from a clinical cobalamin deficiency and have no absorption problems can be maintained safely with low-dose daily supplements in the range of 5–10 μg of cobalamin[29]. Therapeutic doses of cobalamin (1 mg daily doses, 4 weeks[29]) are recommended to individuals with subclinical cobalamin deficiency only when the patient exhibits 'sufficiently suspicious clinical findings'[29].

Special populations, whether omnivore or vegetarian, where vitamin B_{12} demands may not be met satisfactorily should be considered with caution. This includes the elderly and women in reproductive age attempting to conceive or who are pregnant. Elders with inefficient absorption of vitamin B_{12}, a natural condition that together with lower dietary intake may increase the risk of acquiring a deficiency in vegetarians more so than in omnivores. In the case of women attempting to conceive or already pregnant, it must be remembered that depletion of serum vitamin B_{12} occurs much later than cellular depletion and elevation of metabolites in serum, making serum vitamin B_{12} a poor stand-alone marker of cobalamin status[19,30]. Therefore, a conceiving woman with depleted cellular vitamin B_{12} may seriously compromise embryogenesis and early development, as the fetus relies entirely on the maternal supply of vitamin B_{12} via the placenta. It is highly recommended that vegetarian and vegan women in reproductive age take a vitamin B_{12} supplement before conception as well as during pregnancy and breast-feeding to ensure sufficient supplies of the micronutrient to the baby.

With the worldwide increase of humans invoking a vegetarian lifestyle, a demand exists to address questions concerning the diagnosis, interpretation and management of vitamin B_{12} insufficiency in these populations. The study by Naik *et al.*[16] sets the stage for further large-population studies both at the epidemiological and basic research fronts to reassess the criteria for diagnosing vitamin B_{12} deficiency in vegetarian populations. Careful examination of these criteria will allow clinicians to define the appropriate chemical form, mode of administration and dose of cobalamin supplementation required to prevent the onset of acute vitamin B_{12} deficiency, as well as the timely implementation of therapy.

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