

# Quantitative target metabolomics using LC-MS/MS improves the diagnosis of vitamin B<sub>12</sub> deficiency & saves cost and time

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## Introduction

Vitamin B<sub>12</sub> is a water-soluble vitamin with a key role in the vital functioning of the brain and nervous system, as well as in the formation of blood. Furthermore, it is also involved in cell metabolism, energy production, fatty acid synthesis, DNA synthesis and regulation. Vitamin B<sub>12</sub> deficiency is common in the general population, particularly in elderly people and vegetarians [1]. It causes hematologic and/or neuropsychiatric diseases and increases the risk of age-associated disorders like dementia, depression and bone fractures. The vitamin B<sub>12</sub> deficiency-related neurological sequela are mostly non-specific, but can cause irreversible damage if not treated rapidly. Diagnosis of vitamin B<sub>12</sub> deficiency at an early stage is crucial for preventing the development of irreversible clinical complications. Total serum B<sub>12</sub> fails to detect the deficiency in the majority of cases with intracellular B<sub>12</sub> deficiency [2]. Low dietary intake of vitamin B<sub>12</sub> explain the deficiency only in vegetarians, but not in elderly people where only 5 % of them are known to show low serum vitamin B<sub>12</sub> [3], while up to 20 % show biochemical evidence of deficiency [4,5]. The metabolic marker methylmalonic acid (MMA), which increases in plasma and urine in the case of vitamin B<sub>12</sub> deficiency, fulfils both the sensitivity and specificity criteria [6]. By analysing plasma MMA levels, it is possible to distinguish persons according to their dietary habits (vegetarians, omnivores etc.) to diagnose vitamin B<sub>12</sub> deficiency in Metformin users, a diabetic drug, and to optimise the success of vitamin B<sub>12</sub> supplementations. However, until now, MMA levels have mainly been measured with GC-MS methods, which can be expensive and are often not available in modern laboratories.

The aim of the study was to compare the performance of combining MMA, with active B<sub>12</sub> (holotranscobalamin, holoTC) or the traditional assay of total serum B<sub>12</sub>. MMA was measured by GC-MS in a 2-step-diagnostic algorithm after total or active B<sub>12</sub> in 1034 samples referred to our laboratory for B<sub>12</sub> status testing. In addition, a reagent kit for the specific determination of MMA in plasma/serum and urine using LC-MS/MS (Chromsystems GmbH) was compared with the GC-MS method.

## Methods

1034 samples that were referred to our laboratory for determination of total vitamin B<sub>12</sub>, were additionally tested for MMA and holoTC. The LC-MS/MS method for MMA was compared to the GC-MS method using plasma (n = 131) samples. The GC-MS reference method was performed as described elsewhere [7]. Briefly, sample mixture consisting of plasma/serum, water and internal standard (MMA-D4) were transferred to an anion exchange chromatography column and after several washing steps with water and acidic acid in methanol, MMA was eluted using a solution of acidic acid and HCl from the resins. Finally, MMA derivatisation was performed with MTBDSFA in acetonitrile and measured by GC-MS. Sample preparation using LC-MS/MS reagents (Chromsystems GmbH) involves the use of clean-up tubes and clear eluates for direct injection into an LC-MS/MS system.

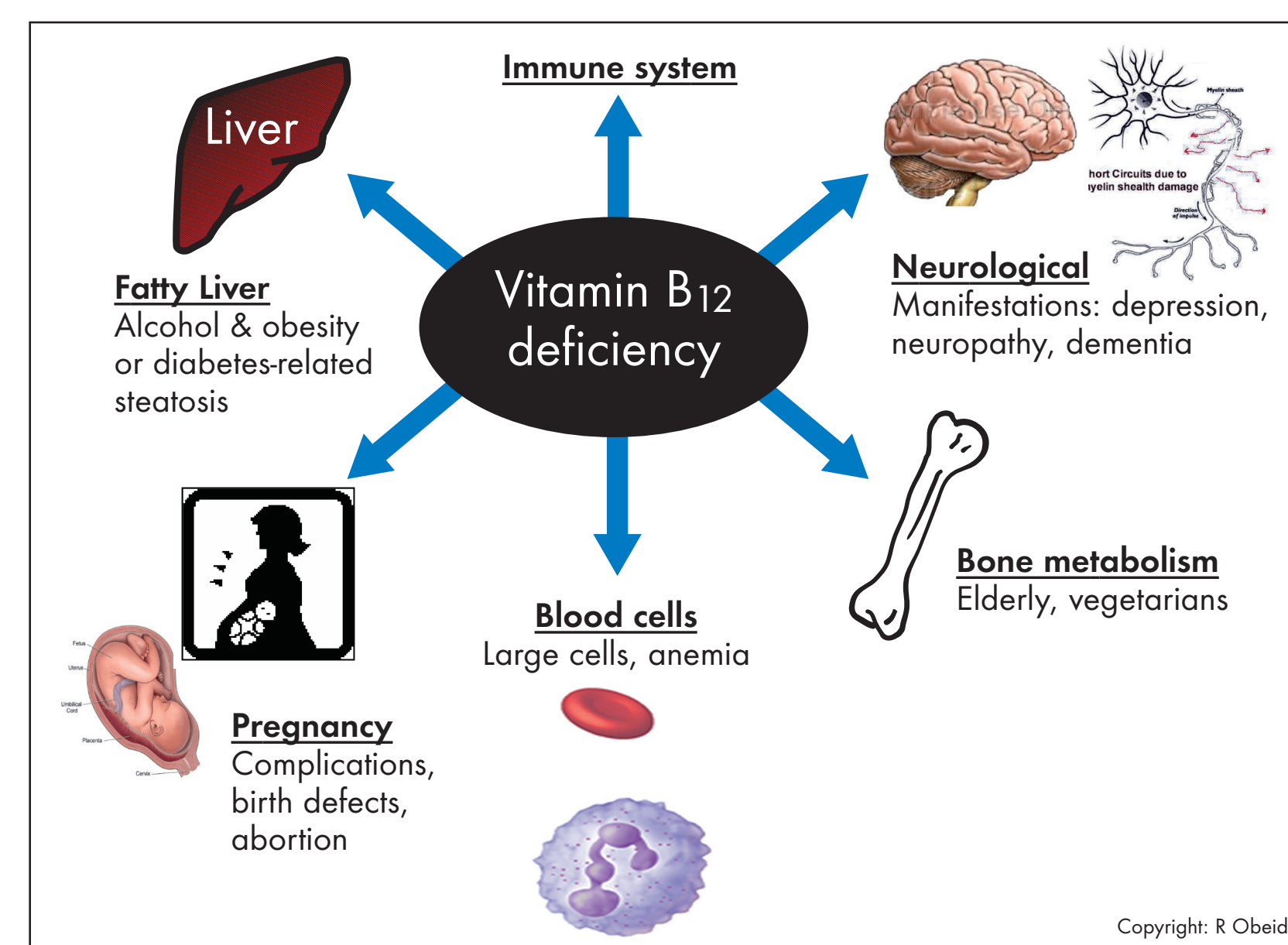


Figure 1: B<sub>12</sub> deficiency clinical outcome.

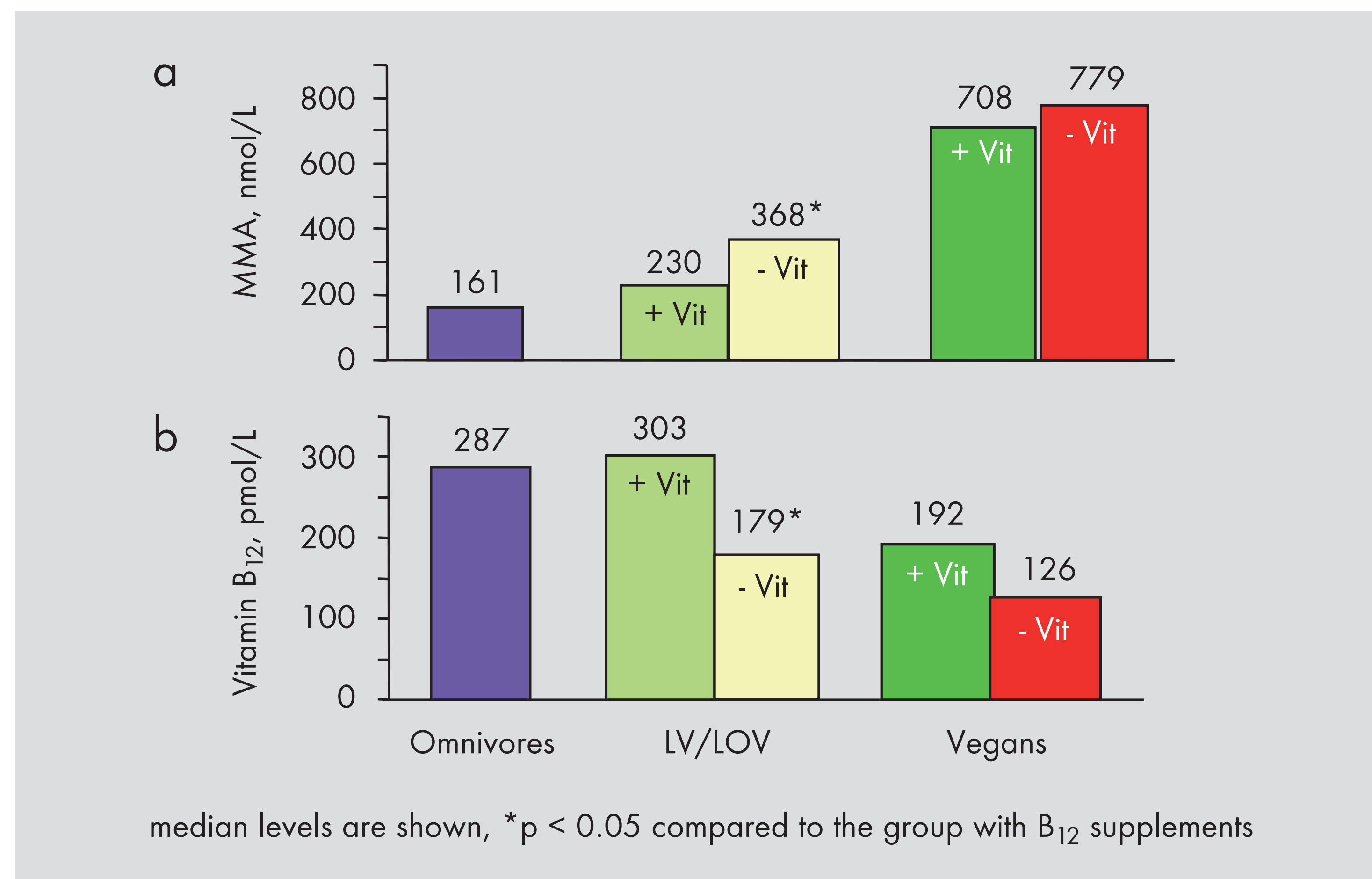


Figure 2a/2b: MMA levels are the best marker to show vitamin B<sub>12</sub> deficiency that is related to dietary causes. LV/LOV = lactovegetarians/lacto-ovo-vegetarians; normal MMA < 271 nmol/L; B<sub>12</sub> > 156 nmol/L.

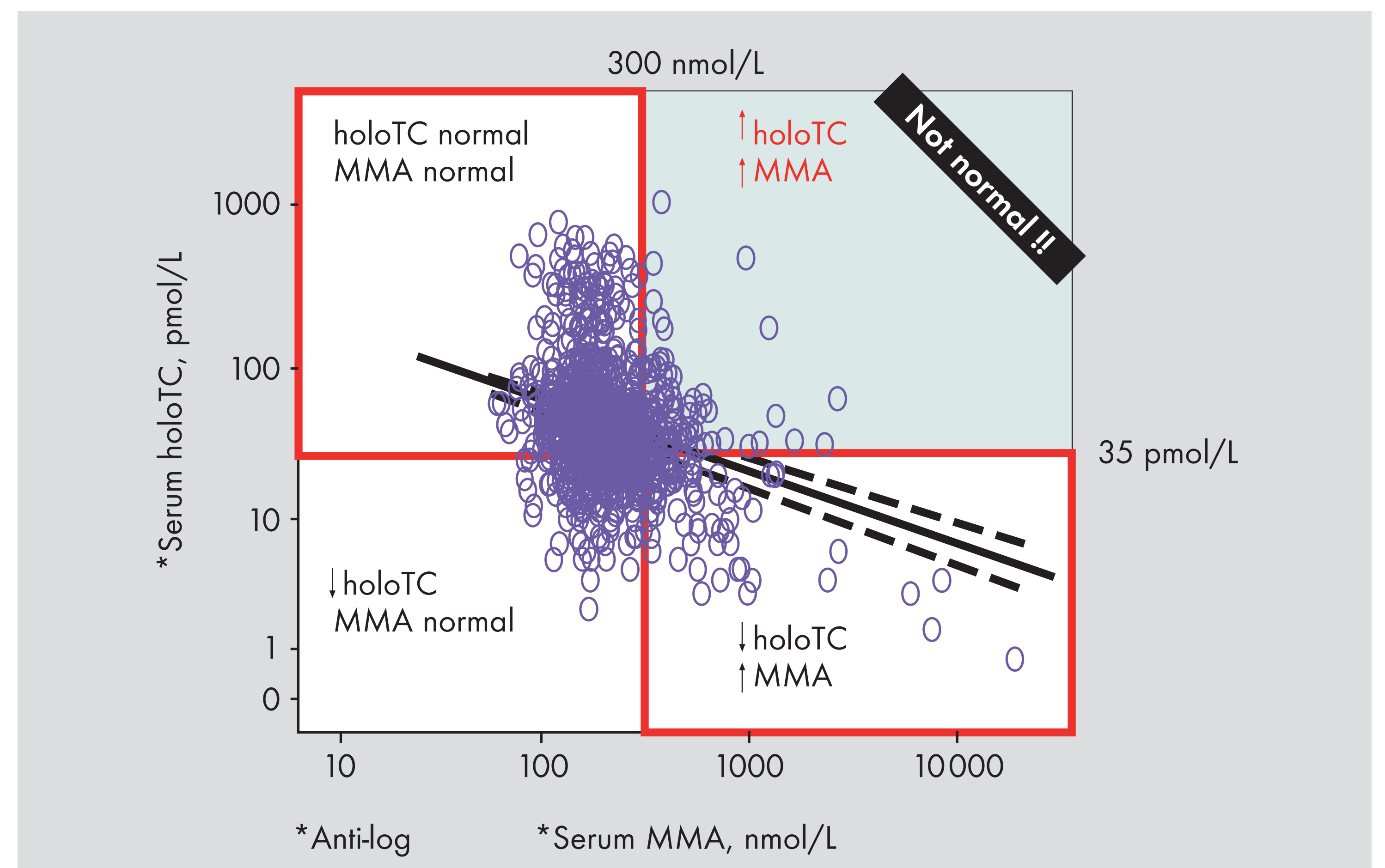


Figure 3: MMA and holoTC offer a better diagnostic tool for vitamin B<sub>12</sub> deficiency [2].

## Results

Serum levels of MMA were higher in vegetarians, and in particular in those who avoid all kinds of animal products (vegans), especially, when they did not receive any kind of B<sub>12</sub> supplements (Fig. 2a). MMA levels were > 271 nmol/L (the upper limit of the reference range) in approximately 65 % of vegetarians and 85 % of vegans who were not taking additional supplements. In contrast median vitamin B<sub>12</sub> showed less pathological findings compared to MMA, suggesting that total vitamin B<sub>12</sub> in plasma does not reflect intracellular B<sub>12</sub> status (Fig. 2b).

We obtained a better results when combining MMA and holoTC levels (Fig. 3). In this case, samples can be classified to either deficient (MMA elevated, holoTC decreased), depleted (only holoTC decreased) or normal (both MMA and holoTC are normal). In a subgroup of the population (~15 %) holoTC was normal and MMA was elevated, suggesting contradicted finding. Lowering MMA after B<sub>12</sub> treatment in this group can provide the final evidence for B<sub>12</sub> deficiency.

Plasma MMA concentrations measured using the commercial kit (LCMS/MS) and the GC-MS method revealed strong correlation (linear regression curves  $y = 0.941x + 41.681$  ( $r^2 = 0.994$ )), indicating good agreement between both methods. The LCMS/MS method has 1.5 fold lower costs and requires 20-fold less time from sample preparation to reporting results.

## Conclusion

Vitamin B<sub>12</sub> deficiency in screening studies can be detected using modern metabolites adapted to LC-MS/MS instrumentation. Using this approach by routine laboratories improves early diagnosis of vitamin B<sub>12</sub> and therapy monitoring, hence reducing irreversible complications. Early diagnoses and treatment are cost effective compared to treating diseases caused by the deficiency. The LCMS/MS method is characterised by fast sample preparation, minimal matrix effects, high precision, robustness and cost-effectiveness.

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