Vitamin B₁₂ Treatment Normalizes Metabolic Markers But Has Limited Clinical Effect: A Randomized Placebo-controlled Study

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Background: The clinical significance of increased plasma methylmalonic acid (P-MMA) is unclear. We assessed the efficacy of vitamin B₁₂ treatment in reducing P-MMA and plasma total homocysteine compared with the clinical benefits of treatment.

Methods: We studied 140 individuals with mildly to modestly increased P-MMA (0.40–2.00 μmol/L), not previously treated with vitamin B₁₂, in a randomized, placebo-controlled study. A detailed medical history was obtained, and laboratory tests as well as an objective neurologic disability score were performed at baseline and 3 months after the start of intervention.

Results: P-MMA (P <0.001) or plasma total homocysteine (P <0.001) decreased in the treatment group vs the placebo group, but no significant difference was found in the change of blood hemoglobin (P = 0.18) and mean cell volume (P = 0.71). Changes in symptom scores did not differ between the groups for symptoms of anemia (P = 0.63), neurologic symptoms (P = 0.21), gastroenterologic symptoms (P = 0.32), or the Neurological Disability Score (P = 0.85).

Conclusions: Treatment with vitamin B₁₂ reduces P-MMA and plasma total homocysteine, but individuals with a mild to modest increase in P-MMA may have only limited clinical benefit from vitamin B₁₂ treatment, at least in the short term.

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No consensus exists about diagnosing vitamin B₁₂ deficiency. Some authors have suggested that measurement of plasma cobalamins has limited value in diagnosing patients with suspected vitamin B₁₂ deficiency (1–6); therefore, the metabolic markers plasma methylmalonic acid (P-MMA) and plasma total homocysteine (P-tHcy) are increasingly used as additional diagnostic tests (7–11).

Vitamin B₁₂ (as 5'-deoxyadenosylcobalamin) is an essential cofactor in the enzymatic conversion of methylmalonyl-CoA to succinyl-CoA (12). When vitamin B₁₂ is lacking, methylmalonyl-CoA accumulates and is hydrolyzed to MMA, producing increased P-MMA (9). Vitamin B₁₂ (as methylcobalamin) is needed to convert Hcy to methionine; thus, P-tHcy also increases in vitamin B₁₂ deficiency (9, 13, 14). Unlike P-MMA, however, P-tHcy is not a specific marker of vitamin B₁₂ deficiency because it is also increased in folate and vitamin B₆ deficiency (13, 15). Finally, both markers are influenced by renal function; reduced renal function is correlated with increased P-MMA and P-tHcy (16–19).

Among elderly people, estimates of the prevalence of vitamin B₁₂ deficiency, defined as increased P-MMA, vary from 15% to 44%, depending on the population groups studied and the reference interval used for P-MMA (7, 20–23). The majority of these elderly people have mild to modestly increased P-MMA, but it is unclear whether this increase reflects a clinically significant condition that would benefit from treatment.

We recently found a high variation in P-MMA over time among individuals with increased P-MMA not followed by treatment. Almost one-half the participants had a spontaneous decrease in P-MMA to values within the reference interval, and the P-MMA concentration was not associated with clinical manifestations of disease (24). These findings indicate severe limitations in the use of P-MMA as the sole biochemical marker for the presence of clinically significant vitamin B₁₂ deficiency.

Several studies, summarized by Björkergren and Svärdssudd (25), have shown that treatment with B vitamins in different combinations can normalize serum vitamins, P-MMA, and P-tHcy, but randomized placebo-controlled studies are needed to clarify whether vitamin B₁₂ influences the clinical outcome of the patients as well as decreasing P-MMA and possibly P-tHcy. The present
randomized trial was carried out as a first step to clarify the clinical value of P-MMA and P-tHcy measurements. Our aim was to assess the efficacy of parenteral vitamin B₁₂ in reducing P-MMA and P-tHcy, as compared with the clinical benefits of treatment in individuals with moderately increased P-MMA.

**Materials and Methods**

**STUDY POPULATION**

From the laboratory information system, we obtained information on individuals with increased P-MMA, based on requests by the physicians in charge of the individuals. We examined 860 participants with an increased P-MMA that was not followed by treatment (visit 1). Participants who at follow-up (visit 1) qualified for the randomized trial (n = 140; P-MMA, 0.40 –2.00 μmol/L) were examined again at the date of randomization (visit 2) and finally 3 months later (visit 3; Fig. 1).

The 140 participants included in the randomized trial were 18 years or older and lived in the Aarhus municipality. They were enrolled during a period of 18 months, from November 1998 until March 2000. To recruit participants, we used two different procedures. The first group consisted of 769 individuals who despite an increased P-MMA (>0.28 μmol/L) were not treated. These participants were included 1–3.9 years after the initial finding. This procedure has been described in detail in a previous publication (24). The second group consisted of 91 individuals recruited during 1999 on the basis of daily information on the P-MMA results from the laboratory information system. For individuals with P-MMA ≥0.40 μmol/L, we asked the general practitioner to inform the individuals about our study, and individuals who agreed to participate were referred to our study.

The following exclusion criteria were established before the randomized study began: P-MMA >2.00 μmol (n = 5); blood hemoglobin ≤6.0 mmol/L and erythrocyte mean cell volume ≥110 fL (n = 0); blood hemoglobin ≤6.0 mmol/L and plasma ferritin ≤10 μg/L (males) or ≤8 μg/L (females; n = 0); plasma thyroid-stimulating hormone ≥4.1 mIU/L (n = 6); plasma creatinine >120 μmol/L (females) or >133 μmol/L (males; n = 41); incapacity to give informed consent (n = 6); life-threatening disease (n = 1); hypersensitivity to cyanocobalamin (n = 0); treatment with anticoagulants (n = 4); Leber hereditary optic neuropathy (n = 0); tobacco-alcohol amnobyopia (n = 0); tropical atoxic neuropathy (n = 0); deafness or severe visual impairment (n = 8); non-Danish speaking (n = 11); severe aphasia (n = 2); and current participation in another clinical trial (n = 1). Three individuals fulfilled two exclusion criteria.

Written informed consent was obtained from all participants. The study was approved by the Research Ethics Committee of Aarhus County (1998/4207) and the Danish Medicines Agency (2612-472). We followed the guidelines of Good Clinical Practice (26), and the trial was monitored by the Unit for Good Clinical Practice at Aarhus University Hospital.

**THERAPEUTIC REGIMENS**

The participants were randomized to receive intramuscular injections of either 1 mg of cyanocobalamin (Betolvex®) or a placebo preparation containing 1 mL of isotonic sodium chloride. The two preparations were supplied by Dumex-Alpharma in packets of four vials each. The only visible difference between the packets was a code number, and the packets were delivered in two batches, each having an equal number of Betolvex and placebo packets, in random sequence. The code was kept by Dumex-Alpharma during the entire trial.

Randomization was based on the P-MMA value at visit 1 and took place at visit 2. We used two strata to ensure a balanced distribution of participants: (a) a mild increase in P-MMA (0.40–0.59 μmol/L; n = 95) and (b) a modest

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**Fig. 1. Study population.**

The study included individuals with increased P-MMA.
increase in P-MMA (0.60–2.00 μmol/L; n = 45). When a participant fulfilled the inclusion criteria, the next available packet was drawn in the batch corresponding to the P-MMA value. Because we followed the Danish standard treatment regimen, the participants were given one injection of Betolvex or placebo weekly for 4 weeks, the first injection at the hospital and the subsequent injections in the participant’s home. A research nurse administered all injections, and the investigator (A.M.H.) and the participants were blinded to the treatment regimens.

**LABORATORY INVESTIGATIONS**

Baseline blood tests for the randomized trial were performed at visit 2, just before the first injection, and repeated at visit 3, three months after the start of intervention. Blood was drawn by standard antecubital venipuncture with subjects in the sitting position. The participants were not fasting. Blood was collected into tubes without anticoagulants for measurement of P-MMA and plasma creatinine, into heparin fluoride-containing tubes for measurement of P-tHcy, and into EDTA-containing tubes for measurement of plasma cobalamins, blood hemoglobin, erythrocyte mean cell volume, erythrocyte folate, platelet count, and white cell count.

P-MMA concentrations were measured by stable-isotope-dilution capillary gas chromatography–mass spectrometry. The analytical imprecision (CV) for the method was <8% (27), and the reference interval was 0.08–0.28 μmol/L (28).

P-tHcy was measured by an immunologic method using an IMx (Abbott) instrument; the analytical imprecision (CV) for the method was <5%. Plasma was separated from the blood cells <2 h after venipuncture. The reference interval was 5.80–11.90 μmol/L. Plasma cobalamins and erythrocyte folate were determined on the ACS/Centaur™ Automated Chemiluminescence System (Bayer A/S) by a competitive protein-binding assay (CV <10%). The reference intervals were 200–600 pmol/L for plasma cobalamins and >350 nmol/L for erythrocyte folate.

Standard methods were used for determination of hematologic values. The reference intervals for blood hemoglobin were 7.4–9.6 mmol/L for females and 8.4–10.8 mmol/L for males; for erythrocyte mean cell volume, the reference interval was 85–100 fl. Plasma creatinine was measured by the Jaffe method on a Roche Cobas Integra 700 automated analyzer [analytical imprecision (CV) <3%]. The plasma creatinine reference intervals were 44–115 μmol/L for females and 62–133 μmol/L for males.

**INTERVIEW AND CLINICAL EXAMINATIONS**

Information on symptoms was obtained in a structured interview. We recorded symptoms of anemia (daily fatigue, palpitations, shortness of breath, and angina on effort), gastrointestinal symptoms (reduced sense of taste, sore mouth or tongue, daily reduced appetite, daily nausea, and daily diarrhea), and neurologic symptoms, using a slightly modified version of the Neurological Symptom Score (29), which includes standardized questions regarding (a) symptoms of muscle weakness [bulbar (extraocular, facial, tongue, throat) and limbs (shoulder girdle and upper arm, hand, glutei and thigh, legs)]; (b) sensory disturbances [negative (difficulty identifying objects in mouth, difficulty identifying objects in hands, unsteadiness in walking) and positive symptoms (“numbness”, “asleep feeling”, “like Novocain”, “prickling”, and pain)]; and (c) autonomic symptoms (postural fainting, male impotence, loss of urinary control, night diarrhea). The symptoms were scored as present or absent. Symptoms of anemia, gastrointestinal symptoms, and neurologic symptoms were summed to a total symptom score.

The clinical examination included a finger-nose test, a heel-knee-shin test, a test for dysdiadochokinesis, the Romberg test, and assessment of gait, general health, and nutritional state, as described in detail previously (24). In addition, we used a slightly modified version of the Neurological Disability Score (a summed score of muscle strength, reflexes, and sensory loss) to quantify the degree of peripheral neuropathy (29). The Neurological Disability Score was the sum of 28 item scores, each ranging from 0 (normal) to 4 (high degree of impairment).

The vibratory sensation was measured by a vibrometer (Vibrameter type IV; oscillations of 100 Hz; Somedic). The equipment ensured a constant application pressure, and the vibration amplitude (μm) was read from a digital display. The vibration perception threshold was determined as described by Roos (30) with two measurements at the pulp of the index finger and the big toe, at the processus styloideus radii, and at the medial malleolus, on both sides. The vibration disappearance threshold was not measured because many participants had difficulty reporting this. All participants were interviewed and examined by the same investigator (A.M.H.).

As primary outcome measure, we chose the relative reduction in vibration perception threshold, considering a threshold reduction of 25% to be clinically relevant. We assumed a standard deviation of the log-transformed threshold of 0.5 (actual SD, 0.51). With a significance criterion of 0.05 and a power of 0.90, the sample size needed was 60 participants in each group.

**STATISTICAL ANALYSIS**

Pearson correlation was used to analyze the associations between the biochemical markers at the time of randomization and to analyze the associations between the different sites of measurements of the vibratory sensation. We used the Student t-test and linear regression analysis to analyze the changes in results of blood tests comparing the treatment and placebo groups. The Levene test was used to test for equal variances. We used the $\chi^2$ test, linear...
data were log-transformed when appropriate. To fulfill the criteria for gaussian distribution, the analyses were done according to the intention-to-treat principle. To analyzed using SPSS 10.0 (SPSS Inc.).

**Results**

**STUDY GROUPS**

As shown in Fig. 1, a total of 860 individuals volunteered to participate in the follow-up (visit 1). At the time of randomization (visit 2), the two differently recruited groups of participants did not differ regarding laboratory test results.

On the basis of the physical examination, interview, and the blood tests, the 140 participants were included in the randomized trial according to the criteria described in Materials and Methods. The participants were randomized into treatment or placebo groups (visit 2). The P-MMA value measured at visit 2 was comparable to the value obtained at visit 1 (mean decrease in P-MMA from visit 1 to visit 2 was 0.01 μmol/L in the treatment group and 0.05 μmol/L in the placebo group). The treatment and placebo groups were very similar at the time of randomization (visit 2) as shown in Table 1; in particular, no significant difference was found in the P-MMA distribution. In the treatment group, 50 (71%) were females, and 48 (69%) in the placebo group were females.

At the final visit 3 months later (visit 3), 137 participants showed up for laboratory retesting (treatment group, n = 65; placebo group, n = 70), and 134 had the clinical examinations repeated (treatment group, n = 65; placebo group, n = 69).

**Laboratory Tests**

Associations between and distribution of the laboratory tests at the time of randomization (baseline values; visit 2). P-MMA was significantly but not strongly associated with both P-tHcy (r = 0.24; P = 0.006, data log-transformed) and plasma cobalamins (r = −0.18; P = 0.037; data log-transformed). Sixty-five (46%) participants had P-tHcy within the reference interval, and 122 (87%) had plasma cobalamins >200 pmol/L. Significant associations were found between P-tHcy and plasma cobalamins (r = −0.20; P = 0.018, data log-transformed) as well as between P-tHcy and erythrocyte folate (r = −0.39; P < 0.001, data log-transformed). Twelve (9%) participants had erythrocyte folate <350 nmol/L.

As shown in Table 1, the treatment and placebo groups had very similar distributions of blood hemoglobin, mean cell volume, white cell count, and platelet count. Sixteen (23%) participants in the treatment group and 11 (16%) in the placebo group had anemia. The mean cell volume was >97 fL in 13 (19%) and 8 (11%) participants in the treatment and placebo groups, respectively. P-MMA, P-tHcy, plasma cobalamins, and erythrocyte folate were not associated with blood hemoglobin or mean cell volume.

**Effect of vitamin B12 treatment on metabolite and vitamin concentrations.** Both P-MMA and P-tHcy were significantly decreased in the treatment group compared with the placebo group (Table 2).

In the treatment group, we found a significant association between the change in P-MMA and the change in P-tHcy (P = 0.04, data log-transformed; Fig. 2). P-MMA decreased to within the reference interval (<0.29 μmol/L) in 46 (69%) participants of the treatment group after treatment, but remained above 0.28 μmol/L in 21 (31%) participants (Fig. 3).

| Table 1. Medians and ranges of age and laboratory tests of the study population at the time of randomization (visit 2: n = 140). |
|-------------------------------------------------|-----------------|-----------------|-----------------|
| **Treatment (n = 70)**                           | **Placebo (n = 70)** | **Comparison of means** |
| Age, years                                      | Median, Range    | Median, Range    |                |
|                                                 | 75, 19–92        | 74, 33–88       | 0.89<sup>a</sup> |
| P-MMA, μmol/L                                  | 0.54, 0.27–2.00<sup>b</sup> | 0.48, 0.26–1.30<sup>c</sup> | 0.12<sup>d</sup> |
| P-tHcy, μmol/L                                 | 13.52, 5.26–46.63 | 12.80, 5.80–31.28 | 0.50<sup>d</sup> |
| Plasma cobalamins, pmol/L                      | 278, 143–1348    | 254, 137–724    | 0.36<sup>d</sup> |
| Erythrocyte folate, nmol/L                     | 543, 255–1685    | 633, 199–1550   | 0.56<sup>d</sup> |
| Blood hemoglobin, mmol/L                       | 8.30, 6.20–10.50 | 8.45, 6.80–10.10 | 0.18            |
| Mean cell volume, fL                           | 91, 69–111<sup>e</sup> | 91, 80–112     | 0.71            |
| Platelet count, 10<sup>9</sup>/L               | 284, 129–421     | 274, 161–503    | 0.43            |
| White cell count, 10<sup>9</sup>/L             | 7.05, 2.80–16.60 | 6.55, 3.90–14.10| 0.10<sup>d</sup> |
| Plasma creatinine, μmol/L                      | 84, 55–123       | 89, 60–148      | 0.28<sup>d</sup> |

<sup>a</sup> Means compared by t-test.
<sup>b</sup> Transformation: age squared.
<sup>c</sup> The randomization was performed on the basis of P-MMA at visit 1 and took place at visit 2 (baseline). All of the participants had P-MMA of 0.40–2.00 μmol/L at visit 1.
<sup>d</sup> Data log-transformed.
<sup>e</sup> The participant with a mean cell volume of 69 fL had concomitant iron deficiency and started treatment with iron simultaneously with vitamin B12 treatment.
Fifty-two (78%) participants of the treatment group had P-tHcy $< 13 \mu$mol/L after treatment. Among the 15 participants with a persistently increased P-tHcy, only 1 had erythrocyte folate $< 350 \text{nmol/L}$. As shown in Table 2, the erythrocyte folate concentration increased in both groups, but significantly more in the treatment group. The decrease in P-tHcy in the treatment group remained significant after controlling for the increase in erythrocyte folate ($P < 0.001$, data log-transformed).

No association was found between baseline plasma cobalamins and change in P-MMA in the treatment group ($P = 0.32$). In the placebo group, we found no significant association between baseline plasma cobalamins and change in P-MMA during the following 3 months ($P = 0.23$).

Effect of vitamin $B_{12}$ treatment on the hematologic values. The change in blood hemoglobin and mean cell volume did not differ between the treatment and placebo groups (Table 2). This was also the case for participants who were anemic ($P = 0.67$) or had an increased mean cell volume at the time of randomization. Table 3 shows the characteristics of the three participants who had macrocytic anemia before initiation of vitamin $B_{12}$ treatment.

The platelet count in the treatment group was slightly increased after 3 months, whereas the white cell count was almost unchanged (Table 2).

Clinical manifestations
Associations between clinical manifestations and laboratory tests at the time of randomization (baseline values; visit 2). Symptoms possibly related to vitamin $B_{12}$ deficiency were prevalent among the participants: 82 (59%) had at least one symptom compatible with anemia, 112 (80%) had more than one neurologic symptom, and 37 (26%) had at least one gastrointestinal symptom. Using linear regression controlled for age and sex, we found no association between the P-MMA concentration and symptoms of anemia ($P = 0.47$), gastrointestinal symptoms ($P = 0.62$), neurologic symptoms ($P = 0.29$), or the total symptom score ($P = 0.66$) at visit 2.

Regarding the Neurological Disability Score, 23 (16%) participants had a normal score of 0, and 67 (48%) had a score of $> 10$ points, 112 points being the highest possible score, indicating the highest degree of disability. We found no association between P-MMA and the Neurological Disability Score ($P = 0.89$, linear regression) or other signs related to vitamin $B_{12}$ deficiency: finger-nose test ($P = 0.07$, inverse), knee-shin-heel test ($P = 0.97$), dysdiadochokinesis ($P = 0.25$), Romberg sign ($P = 0.63$),

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**Table 2. Comparison of biochemical changes among participants randomized to vitamin $B_{12}$ treatment or placebo, retested after 3 months ($n = 137$).**

<table>
<thead>
<tr>
<th>Blood value</th>
<th>Treatment ($n = 67$)</th>
<th>Placebo ($n = 70$)</th>
<th>$P$ (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-MMA,$^b$ $\mu$mol/L</td>
<td>-61</td>
<td>4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P-tHcy,$^b$ $\mu$mol/L</td>
<td>-23</td>
<td>2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma cobalamins,$^b$ pmol/L</td>
<td>236</td>
<td>2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Erythrocyte folate,$^a$ nmol/L</td>
<td>23</td>
<td>5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood hemoglobin, mmol/L</td>
<td>1</td>
<td>1</td>
<td>0.95</td>
</tr>
<tr>
<td>Mean cell volume, fl</td>
<td>0</td>
<td>0</td>
<td>0.70</td>
</tr>
<tr>
<td>Platelet count, $10^9$/L</td>
<td>3</td>
<td>-3</td>
<td>0.054</td>
</tr>
<tr>
<td>White cell count,$^b$ $10^9$/L</td>
<td>2</td>
<td>0</td>
<td>0.58</td>
</tr>
</tbody>
</table>

$^a$ Percentage of change and $P$ values are cited.

$^b$ Data log-transformed.
abnormal gait \( (P = 0.38, \text{inverse}) \), or nutritional state \( (P = 0.39) \), using logistic regression controlled for age and sex.

The two vibrometer measurements at each site were highly correlated \((r \sim 0.90 \text{ for all four sites})\) as well as the right- and left-sided measurements at each side \((r = 0.76–0.87)\). The measurements at both upper extremity sites \((r = 0.85)\) and at both lower extremity sites \((r = 0.83)\) were also highly correlated. We therefore found it justified to use an average measurement for the upper and lower extremities in the analysis. We found a weak and insignificant association between P-MMA and the degree of reduced vibratory sensation \((\text{upper extremities}, P = 0.36; \text{lower extremities}, P = 0.31)\).

**Effect of vitamin B\textsubscript{12} supplement on symptoms and signs.**

When we compared the changes in symptom scores, no difference was found between the treatment and placebo groups (Table 4). We also found no association between P-MMA, P-tHcy, or plasma cobalamin concentrations at the time of randomization and change in symptom scores (Table 4; data not shown for P-tHcy and plasma cobalamins). When we compared the treated anemic participants with the anemic participants in the placebo group, we found no improvement in symptoms of anemia \((P = 0.72, \chi^2 \text{ test})\). Paresthesias were included in the Neurological Symptom Score, but no improvement was reported among the treated participants compared with the placebo group \((P = 1.0)\).

When we subdivided the treatment and placebo groups into two groups based on baseline biochemical markers, we found a significant association between improvement in neurologic symptoms in the treatment group compared with the placebo group for participants with P-MMA \( \geq 0.60 \mu\text{mol/L} \) \((n_{\text{treatment}} = 24; n_{\text{placebo}} = 21)\) and participants with P-tHcy \( \geq 15 \mu\text{mol/L} \) \((n_{\text{treatment}} = 25; n_{\text{placebo}} = 26; \text{Table 5})\). We found a slight improvement in neurologic symptoms among several participants, but it was not attributable to one particular symptom. The improvement in neurologic symptoms in the treatment group was not associated with the plasma cobalamin concentration at randomization. We used a cutoff point of 250 pmol/L for plasma cobalamin to obtain an almost equal distribution of participants within the groups. Use of a lower cutoff point did not strengthen the association between low plasma cobalamins and change in symptoms. The combination of two abnormal baseline biochemical markers \( (\text{P-MMA} \leq 0.60 \mu\text{mol/L}; \text{P-MMA} \leq 0.60 \mu\text{mol/L} \text{ and P-tHcy} \leq 15 \mu\text{mol/L} \text{ and P-tHcy} \leq 15 \mu\text{mol/L}) \) did not predict an improvement in symptoms after treatment \((\text{data not shown})\). The 21 participants who had increased P-MMA after treatment did not differ from the rest regarding symptom scores.

No significant differences were found between the treatment and placebo groups with respect to a change in the Neurological Disability Score \((P = 0.85)\). In the treatment group, we found no association between changes in the Neurological Disability Score and the P-MMA \((P = 0.60), \text{P-tHcy} (P = 0.59), \text{or plasma cobalamin} (P = 0.71)\) concentration at the time of randomization. When we divided the treatment and placebo groups into two subgroups based on the baseline values for biochemical markers, we found no significant improvement in the treatment group compared with the placebo group regarding the Neurological Disability Score \((\text{Table 5})\). Finally, using the McNemar test, we found no significant improvement in other signs in the treatment group after 3 months \((P = 0.11–1.0)\).

Regarding examination of the vibratory sensation, we found small improvements in both the treatment \( (\text{upper extremities}, P = 0.04; \text{lower extremities}, P = 0.89)\) and the placebo group \( (\text{upper extremities}, P = 0.01; \text{lower extremities}, P = 0.08)\). We found no improvement in the treated group compared to the placebo group \( (\text{upper extremities}, P = 0.44; \text{lower extremities}, P = 0.41)\). No improvement was found in the treated group with P-MMA \( \geq 0.60 \).
μmol/L compared to the placebo group (upper extremities, \( P = 0.32 \); lower extremities, \( P = 0.59 \)).

**Discussion**

To examine the clinical impact of increased P-MMA, we conducted a randomized placebo-controlled trial that included 140 participants with \( 0.40 \)–\( 2.00 \) μmol/L P-MMA, well above the reference interval. We report that vitamin B\(_12\) alone was efficient in decreasing P-MMA and P-tHcy, even when P-tHcy was within the reference interval. We found little if any improvement in clinical manifestations related to vitamin B\(_12\) deficiency after treatment.

The application of sensitive metabolic tests such as measurement of P-MMA and P-tHcy has expanded our view of vitamin B\(_12\) deficiency beyond the question of megaloblastic anemia. Mild vitamin B\(_12\) deficiency has been identified as the state in which metabolic evidence of insufficiency exists in a person who is without symptoms or anemia (31). However, the clinical importance of mild vitamin B\(_12\) deficiency and how to respond to it are still uncertain. Although everyone with clinically overt vitamin B\(_12\) deficiency must at one point have had mild deficiency, it is uncertain whether a biochemical deficiency will always progress rather than fluctuate or reverse (24, 32).

In the present study, P-MMA was normalized in 69% of the participants after treatment, leaving 31% with increased P-MMA. This 31% did not differ clinically from the rest. The persistently increased P-MMA could not be explained by renal insufficiency: all participants had plasma creatinine within the reference interval. We also do not attribute it to the Danish standard treatment regimen, in which the body’s stores are initially replenished with 1 mg of vitamin B\(_12\) weekly for 4 weeks, followed by injections at 3-month intervals, which is considered adequate maintenance therapy using a depot preparation (33). At present we do not know the clinical implications of a P-MMA not normalized by vitamin B\(_12\) treatment.

Our results support earlier studies (1, 3, 7, 34, 35) showing that vitamin B\(_12\) decreases P-tHcy. In the present study, the median P-tHcy was \(<15 \) μmol/L before treatment, and even in those cases where P-tHcy was not increased, vitamin B\(_12\) had a lowering effect on P-tHcy. Erythrocyte folate might be reduced in vitamin B\(_12\) deficiency and corrected after treatment with vitamin B\(_12\) (36), but the significant effect on P-tHcy remained after adjustment for the increasing erythrocyte folate concentration. Our finding might be of importance in the discussion of treating and preventing hyperhomocysteinemia. Many epidemiologic studies have shown that Hcy is an independent predictor of vascular occlusive disease (37, 38). What remains unknown is whether decreasing the Hcy concentration reduces the vascular occlusive risk that accompanies hyperhomocysteinemia.

We first recruited participants, via the laboratory information system, up to 3.9 years after the prestudy P-MMA measurement (24) and had no information on the duration of symptoms and signs related to vitamin B\(_12\) deficiency. The participants represented individuals with mild to moderately increased P-MMA, determined at least twice, and who had been suspected of having vitamin B\(_12\) deficiency. Because the clinical impact of mild vitamin B\(_12\) deficiency is unknown, we found it reasonable to examine the association between treatment with parenteral vitamin B\(_12\) and changes in clinical manifestations related to vitamin B\(_12\) deficiency in this population.

Apart from a significant effect of treatment on neurologic symptoms among participants with P-MMA >0.60 μmol/L or P-tHcy >15 μmol/L, we found no effects of vitamin B\(_12\) treatment. To assess the neurologic deficits and to test the efficacy of vitamin B\(_12\) treatment, we used the Neurological Disability Score, which consists of selected items from the conventional neurologic examination. The sensitivity and reliability of this test are not as high as could be desired, but that such scored evaluations can provide meaningful values had been demonstrated previously (39). We did not find any improvement in the Neurological Disability Score in the treatment group. To
achieve a sensitized measurement of the vibratory sensation, we used a standardized method for vibration threshold determination \( (40, 41) \). We found no improvement in the treated group compared with the placebo group, not even for participants with P-MMA >0.60 \( \mu \)mol/L. Nor did we find any associations between biochemical markers and other clinical manifestations after treatment. The symptoms and signs related to vitamin B\(_{12}\) deficiency are often vague and quite prevalent among the elderly. However, we did expect an improvement in symptom scores and signs among the treated participants compared with the placebo group.

We found an improvement in neurologic symptoms in a subgroup but no improvement in the objective neurologic examinations, including measurement of the vibratory sensation. We therefore hesitate to put too much emphasis on this. The improvement in neurologic symptoms suggests that individuals with a P-MMA >0.60 \( \mu \)mol/L and neurologic symptoms may benefit from vitamin B\(_{12}\) treatment.

In conclusion, our study confirms and expands earlier observations showing that treatment with vitamin B\(_{12}\) is efficient in reducing the metabolic markers P-MMA and P-tHcy. To decrease P-tHcy may be a goal in itself to reduce the risk of cardiovascular diseases. Because we observed only small improvements in clinical manifestations after treatment with vitamin B\(_{12}\), our results cast into question whether decreasing mild to modestly increased P-MMA is clinically significant. Obviously, based on our results, we cannot judge whether prolonged treatment of patients with increased P-MMA will prevent the development of symptoms and signs of vitamin B\(_{12}\) deficiency. However, in light of those results, we agree with others \( (32, 42) \) expressing concern about widespread testing for mild vitamin B\(_{12}\) deficiency and about the use of a slight to moderate increase in P-MMA as the only indicator for treating people with vitamin B\(_{12}\).

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