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# SHORT COMMUNICATION

# Changes in markers of cobalamin status after cessation of oral B-vitamin supplements in elderly people with mild cobalamin deficiency

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Mildly cobalamin-deficient elderly were supplemented with 1000 $\mu$g cobalamin (group C, $n=34$), 1000 $\mu$g cobalamin with 400 $\mu$g folic acid (group CF, $n=31$) or a placebo ($n=30$) for 6 months. Participants provided one single blood sample 3, 5 or 7 months after cessation of supplementation to monitor early changes in plasma concentrations of cobalamin, holotranscobalamin (holoTC) and methylmalonic acid (MMA). At the end of supplementation (groups C + CF), one participant met our criteria for mild cobalamin deficiency, as did 13, 14 and 43% of the participants assessed at respectively 3, 5 and 7 months post-supplementation. Cobalamin and holoTC declined on average with 47 and 56% relative to concentrations at the end of supplementation for the group assessed at 7 months post-supplementation. Essentially similar declines were observed for those participants assessed at 3 and 5 months post-supplementation. Mean MMA concentrations increased by 15% ($P=0.07$) in those participants assessed at 3 and 5 months post-supplementation, and increased by 50% ($P=0.002$) in those participants assessed at 7 months post-supplementation. Considering MMA as a sensitive tissue marker for cobalamin status, oral supplementation may afford adequate cobalamin status for a period of up to 5 months after cessation in the majority of participants.
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Cobalamin deficiency is common in elderly people (van Asselt *et al*., 1998) and may cause anemia and a variety of irreversible neuropsychiatric symptoms (Lindenbaum *et al*., 1988; Stabler, 2000). Therefore, early diagnosis and treatment of cobalamin deficiency are important. There is no consensus about the definition of cobalamin deficiency (Schneede and Ueland, 2005), but in addition to low cobalamin concentrations, increased concentrations of methylmalonic acid (MMA) and total homocysteine (tHcy) (Stabler, 2000), and decreased concentrations of holotranscobalamin (holoTC) (Hvas and Nexo, 2005; Morkbak *et al*., 2005) are established as useful diagnostic markers. Several studies showed that oral cobalamin supplementation corrects these markers of cobalamin deficiency (Hathcock and Troendle, 1991; Kuzminski *et al*., 1998; Eussen *et al*., 2005). However, little is known about the duration of the effects of oral cobalamin treatment in elderly people with mild cobalamin deficiency. Therefore, we monitored early changes in markers for cobalamin status after cessation of oral cobalamin supplementation in a follow-up study, which was conducted after completion of a randomized placebo controlled intervention trial in elderly with mild cobalamin deficiency (Eussen *et al*., 2006). Cobalamin deficiency was

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defined as either cobalamin concentrations between 100 and 200 pmol/l (71% of participants), or as cobalamin concentrations between 200 and 300 pmol/l in combination with MMA concentrations $\geqslant 0.32\,\mu$mol/l (29% of participants) and creatinine concentrations $\leqslant 120\,\mu$mol/l to exclude severe renal impairment (Baik and Russell, 1999).

During the intervention study, participants were treated daily for 6 months with a capsule containing 1000 $\mu$g cobalamin (group C), 1000 $\mu$g cobalamin with 400 $\mu$g folic acid (group CF), or placebo (group P). Ninety-five participants were invited to provide one single blood sample either 3, 5 or 7 months post-supplementation (follow-up). Analytical coefficients of variation for the assays of cobalamin (Immulite, 2000), MMA (determined by a liquid chromatography electrospray ionization tandem mass spectrometry system), tHcy (Windelberg *et al*., 2005), holoTC (Morkbak *et al*., 2005) and red blood cell (RBC) folate were 6.3, <2.2, <2.2, 12 and 5.9%, respectively. The Medical Ethics Committee of Wageningen University approved the studies and participants provided written informed consent. Compliance of capsule intake during supplementation period was 99%; no adverse effects were reported. The mean (standard deviation) age of participants in the follow-up study was 82 (6) years, 22% were males and 52% were institutionalized.

To assess the duration of the effects of supplementation on each cobalamin marker we calculated the relative change for each participant between pretreatment and follow-up concentrations, and between post-treatment and follow-up concentrations. Statistical significance of these changes was calculated by a paired Student's *t*-test. After ascertaining that groups C and CF did not differ according to any of the cobalamin indices at either the start or end of the treatment period, we pooled group C and CF. The relative changes in the three follow-up groups were compared using analysis of variance with Tukey *post hoc* tests using SAS statistical software (version 9.1; SAS Institute Inc., Cary, NC, USA). The graph was performed by GraphPad Prism (version 4; GraphPad Software Inc., San Diego, CA, USA).

The mean concentrations of markers for cobalamin status in the placebo group remained stable throughout the supplementation period and during follow-up (Table 1). At the end of supplementation (groups C + CF), one participant met our criteria for mild cobalamin deficiency, as did 13, 14 and 43% of the participants assessed at 3, 5 and 7 months post-supplementation, respectively. In the pooled group assessed at 7 months post-supplementation, concentrations of cobalamin and holoTC were, respectively, 36 and 38% higher, and MMA 28% lower compared to their baseline

**Table 1** Cobalamin status in older people with mild cobalamin deficiency supplemented with B-vitamins[a]

| | *Participants according to time of post-supplementation assessment of cobalamin status* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *3 months* | | | *5 months* | | | *7 months* | | |
| | *Baseline* | *End* | *After* | *Baseline* | *End* | *After* | *Baseline* | *End* | *After* |
| *Groups C and CF combined* | | | | | | | | | |
| Total *n*, *n* (%) deficiency[b] | 16, 16 (100) | 16, 0 (0) | 16, 2 (13) | 28, 28 (100) | 28, 1 (4) | 28, 4 (14) | 21, 21 (100) | 21, 0 (0) | 21, 9 (43) |
| Cobalamin | | | | | | | | | |
| pmol/l | 206 ± 57 | 642 ± 271[c] | 339 ± 117[c,d] | 184 ± 41 | 563 ± 180[c] | 265 ± 48[c,d] | 190 ± 55 | 532 ± 194[c] | 257 ± 74[c,d] |
| % change | | 220 ± 128[c] | −43 ± 20[c,d] | | 212 ± 98[c] | 47 ± 34[c,d] | | 196 ± 113[c] | 36 ± 20[c,d] |
| HoloTC | | | | | | | | | |
| pmol/l | 65 ± 18 | 281 ± 198[c] | 101 ± 32[c,d] | 62 ± 26 | 261 ± 174[c] | 86 ± 33[c,d] | 59 ± 23 | 215 ± 105[c] | 79 ± 32[c,d] |
| % change | | 322 ± 214[c] | 63 ± 56[c,d] | | 338 ± 238[c] | 50 ± 50[c,d] | | 311 ± 205[c] | 38 ± 34[c,d] |
| MMA | | | | | | | | | |
| $\mu$mol/l | 0.37 ± 0.12 | 0.23 ± 0.07[c] | 0.24 ± 0.07[c] | 0.35 ± 0.17 | 0.22 ± 0.07[c] | 0.25 ± 0.09[c] | 0.50 ± 0.25 | 0.24 ± 0.06[c] | 0.35 ± 0.14[c,d] |
| % change | | −37 ± 12[c] | −34 ± 0[c] | | −32 ± 19[c] | −26 ± 18[c] | | −28 ± 19[c] | −28 ± 19[c,d] |
| *Placebo group (Group P)* | | | | | | | | | |
| Total *n*, *n* (%) deficiency[b] | 10, 10 (100) | 10, 10 (100) | 10, 10 (100) | 8, 10 (100) | 8, 8 (100) | 8, 8 (100) | 12, 12 (100) | 12, 12 (100) | 12, 12 (100) |
| Cobalamin | | | | | | | | | |
| pmol/l | 179 ± 57 | 156 ± 49 | 171 ± 45 | 180 ± 38 | 161 ± 40 | 162 ± 49 | 165 ± 48 | 162 ± 49 | 167 ± 55 |
| % change | | −12 ± 12 | −3 ± 12 | | −11 ± 10 | −10 ± 19 | | −5 ± 11 | 4 ± 13 |
| HoloTC | | | | | | | | | |
| pmol/l | 62 ± 30 | 54 ± 23 | 56 ± 24 | −10 ± 19 | 54 ± 23 | 58 ± 30 | 67 ± 31 | 59 ± 30 | 56 ± 23 |
| % change | | −10 ± 19 | −5 ± 21 | | −3 ± 42 | 3 ± 36 | | −5 ± 23 | −9 ± 13 |
| MMA | | | | | | | | | |
| $\mu$mol/l | 0.44 ± 0.20 | 0.45 ± 0.25 | 0.46 ± 0.24 | 0.33 ± 0.07 | 0.35 ± 0.07 | 0.32 ± 0.05 | 0.55 ± 0.33 | 0.60 ± 0.29 | 0.59 ± 0.45 |
| % change | | 0.6 ± 15 | (2 ± 26) | | 9 ± 17 | (3 ± 20) | | 12 ± 21 | 2 ± 36 |

Abbreviations: holoTC, holotranscobalamin; MMA, methylmalonic acid; s.d., standard deviation.
[a]Mean ± s.d., are given in absolute concentrations and as proportions (%) relative to the concentrations at baseline.
[b]Defined as either cobalamin concentrations between 100 and 200 pmol/l, or as cobalamin concentrations between 200 and 300 pmol/l in combination with MMA concentrations. $\geqslant 0.32\,\mu$mol/l and creatinine concentrations $\leqslant 120\,\mu$mol/l.
[c]Significantly different from start of supplement use, paired Student's *t*-test ($P<0.05$).
[d]Significantly different from end of supplement use, paired Student's *t*-test ($P<0.05$).

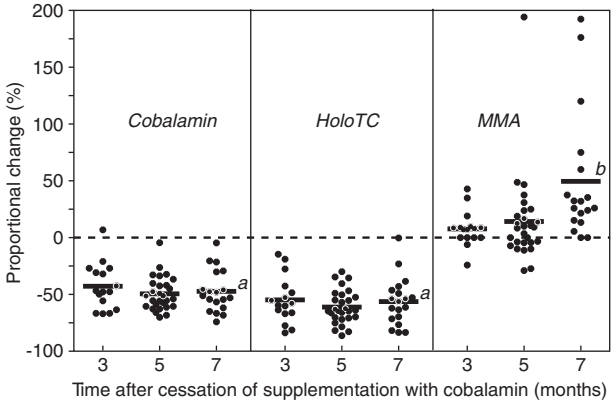


**Figure 1** Changes in markers of cobalamin status after cessation of oral vitamin B12 supplementation. Data are given as proportional changes relative to concentrations at the end of 6 months of supplement use. In each group, mean cobalamin and holoTC concentrations were significantly lower at follow-up than they were immediately post-treatment; however, mean MMA was significantly higher at follow-up only for the group assessed 7 months post-treatment. 'a' indicates no significant differences between 3, 5 and 7 months after cessation, Tukey's *post hoc* test ($P=0.48$ for cobalamin and $P=0.50$ for holoTC). 'b' indicated change at 7 months significantly different from changes at 3 and 5 months after cessation, Tukey's *post hoc* test ($P<0.01$). holoTC, holotranscobalamin; MMA, methylmalonic acid.

concentrations (Table 1). In group C, tHcy and RBC folate concentrations returned back to their corresponding baseline concentrations in all post-supplementation groups. Participants in group CF had significantly lower tHcy concentrations compared to baseline in all post-supplementation groups, and RBC folate concentrations returned to baseline concentrations only in those assessed at 7 months post-supplementation (data not shown).

The Figure 1 shows that cobalamin and holoTC decreased for virtually every subject compared to concentrations at the end of supplementation. The mean proportional declines in cobalamin and holoTC were 47 and 56%, respectively, for the group assessed at 7 months post-supplementation. This change was similar to that of participants assessed at 3 and 5 months post-supplementation. MMA showed no increase in 41% (18 out 44) of participants assessed at 3 and 5 months post-treatment, but was increased for almost every participant assessed at 7 months post-treatment. Overall, the mean proportional increase was greater for the group assessed at 7 months (50%, $P=0.002$) compared to the increase for the other two groups (15%, $P=0.07$). We observed a marked increase in MMA ($>50\%$) in one participant after 5 months and five participants after 7 months. Four of them had baseline MMA concentrations $>0.50\,\mu$mol/l, and changes during and after the intervention were more pronounced compared to other participants (data not shown).

The present study was designed *a posteriori*, and conducted after completion of an efficacy trial (Eussen *et al*., 2006). Unfortunately, we were not able to monitor longitudinal changes in individuals after cessation of supplements, and some subgroups were rather small for logistics reasons. Despite these weaknesses, we can evaluate the relative sensitivity of the different cobalamin markers by monitoring their early changes after cessation of supplementation when participants gradually attain a negative cobalamin balance. Moreover, this is the first study so far that allows assessment of the duration of treatment effects after oral cobalamin supplementation in elderly with mild cobalamin deficiency.

The most important findings from this follow-up study were the relatively rapid fall of cobalamin and holoTC concentrations (3 months post-supplementation). The parallel time courses for cobalamin and holoTC during follow-up suggest that these cobalamin fractions are equally sensitive to detect the recurrence of a negative cobalamin balance. MMA showed a marked increase in those assessed at 7 months post-supplementation, which may reflect that tissue deficiency was re-established within 7 months of cessation of supplementation.

Conceivably, the duration of treatment effects may depend on the severity of cobalamin deficiency before supplementation. One published investigation in elderly without cobalamin deficiency demonstrated that cobalamin returned to pretreatment concentrations and MMA and tHcy were still slightly reduced 9 months after injection with high doses of cobalamin, folic acid and vitamin B6 (Henning *et al*., 2001). Another study in patients with pernicious anaemia indicated that after treatment, serum cobalamin clearance was higher than clearance in vegans and people with an adequate cobalamin status. This can be explained by impaired reabsorption of biliary cobalamin due to absence of intrinsic factor in pernicious anaemia (Amin *et al*., 1980).

In summary, after cessation of daily oral supplementation with $1000\,\mu$g cobalamin with or without additional $400\,\mu$g folic acid for 6 months, there is a parallel decrease of serum cobalamin and holoTC concentrations. The decline precedes the recurrence of tissue cobalamin depletion, as measured by increased MMA concentrations. Oral supplementation may afford an adequate cobalamin status in most participants for a period of up to 5 months after cessation of supplementation. Thereafter, a negative cobalamin balance may reoccur.

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