Original Communication

Efficacy of a 28-Day Oral Cyanocobalamin Supplementation on Vitamin B Status in Spanish Institutionalized Elderly

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Abstract: *Background:* Cobalamin deficiency is a common problem in the elderly. There is no consensus about adequate doses for supplementation. *Subjects/Methods:* We performed an intervention study in order to establish the efficacy of a supplement providing 500 μg cyanocobalamin for four weeks in sixty-four institutionalized elderly residents, over 60 years of age, in Madrid (Spain). Before and after treatment, concentrations of serum cobalamin, serum holotranscobalamin, serum total homocysteine, and serum and red blood cell folate were analyzed. Clusters were built according to the initial cobalamin status and differences in the effect of supplementation were checked using a general linear model for repeated measures. *Results:* Cobalamin and holotranscobalamin increased highly significantly from 308 to 558 pmol/L and from 54 to 96 pmol/L ($p < 0.001$) in the whole study group as well as in each subgroup (clustered by initial cobalamin levels, all $p < 0.01$), with the highest relative change in the subgroup with the lowest initial cobalamin values. Total homocysteine decreased from 15 to 13 μmol/l, $p < 0.001$). Only the change of cobalamin ($F = 4.61$, $p < 0.01$), but not of holotranscobalamin nor total homocysteine, depended on the initial serum cobalamin status. *Conclusions:* A supplementation with an oral supplement solution of 500 μg cyanocobalamin daily for only four weeks, a shorter period than that found in former studies, may be considered suitable in institutionalized elderly.

Key words: vitamin B12, cyanocobalamin, elderly, supplementation, subclinical deficiency

Introduction

The elderly represent a high-risk group for vitamin B12 deficiency, with prevalence ranging from 10–25 % depending on the criteria used for classification [1–4]. In contrary to other high-risk groups such as vegetarians where food cobalamin intake usually is reduced, low cobalamin concentrations in the elderly are usually not due to low dietary intake but mainly due to gastric and intestinal malabsorption [5, 6], which accounts for more than 60 % of the cobalamin deficiency in that population [7]. After folate deficiency, vitamin B12 represents the second most important nutritional determinant for homocysteine status. Elevated homocysteine status has been recognized as an independent risk factor for cardiovascular, peripheral vascular, and cerebrovascular disease [8]. The Institute of Medicine of the National Academies in the United States is also aware of the public health importance of improving the vitamin B12 status of the elderly and therefore recommends B12-fortified foods or B12-containing supplements to individuals over 50 [9], even though it is still controversial with respect to which of these measures is the best from the public health point of view [10]. Regarding supplementation, there is also some controversy about the best pharmaceutical formulation that should be used to improve this status: intramuscular injections or oral supplements. Traditionally, cobalamin deficiency is treated with intramuscular injections of 1000 μg cyanocobalamin, consisting of initial loading doses followed by monthly injections for maintenance [11]. However, this approach has several disadvantages; i. e. administration needs to be done by health professionals, it may be painful in very thin people [12], it seems to be more allergenic than pills [13], and it shows a lower compliance compared to oral treatment [12]. Among the alternative options, oral treatment is the one most commonly used. Several studies have proven that oral doses of 1 and 2 mg are clinically effective and can be a valid substitute for intramuscular injections for patients with megaloblastic anemia [14], with severe cobalamin deficiency [15], and even with mild cobalamin deficiency [16, 17]. But even smaller doses may be effective [18]. The minimal effective dose and how long it should be used is still a hot topic in the literature [1, 2, 4, 13].

Therefore, the aim of this study was to assess the influence of an oral daily supplementation (500 μg cyanocobalamin) on serum cobalamin (sCbl), holotranscobalamin (holoTC), and total homocysteine (tHcy) status in institutionalized elderly for a time period of four weeks.

Experimental methods

The study was performed in the frame of a broader study dealing with early diagnosis and treatment of vitamin B12 deficiency in Spanish elderly [4]. The study was performed in accordance with the Helsinki Declaration of 1964 and later amendments, the last one revised in 2000 in Edinburgh, with the Convention on Human Rights and Biomedicine of Oviedo in 1997, and approved by the Human Research Review Committee of the Universidad de Granada. A written informed consent was obtained from all study participants, family representatives, or guardian if participants were unable to provide consent.

Subjects and design

Sixty-four residents were recruited from two old people's homes in the Region of Madrid (Spain). Elderly homes were contacted through the *Asociación Familiares Alzheimer* (AFAL, Spain) and the intervention trial performed with the participation of the responsible physician. An oral solution supplement of 500 μg cyanocobalamin1 was administered daily for 28 days with breakfast. Inclusion criteria were institutionalized participants of both genders aged ≥60. Exclusion criteria were vitamin B12 and folate supplement intake, neuropathy, and Hunter's glossitis. The medication intake (for chronic as well as acute diseases) was collected from the data management systems of the old people's home [19]. The concomitant intake of medication related to affect Cbl absorption (such as metformin, H2-antagonists, and proton pump inhibitors) was not considered as an exclusion criterion if the medication had been started at least three months before the study and was taken continuously by the study participant for the whole duration of the intervention period. Furthermore, splitting the whole study population into two groups (taking or not taking medication with a possible influence on vitamin B12 status) did not result in statistical differences neither of the initial status nor of the increase produced by the supplement between those two groups (data not shown). The intake of the oral supplement was monitored by caregivers of the two old people's homes.

[1] Active ingredients: cyanocobalamin 500 μg, glutamine 60 mg, D,L-phosphonoserine 40 mg; other ingredients: sorbitol 2.5 g, methyl-4hydroxybenzoate, sodium hydroxide, D-mannitol, raspberry flavor, purified water. Whitehall Much GmbH; Münster (Germany).

Analytical methods

Blood was collected from the participants after an overnight fast the day before starting the trial, and the day after having received a daily oral supplementation for 28 days. Blood specimens were collected into Vacutainer Tubes containing EDTA or gel for serum. The EDTA tubes were used for whole blood count and red blood cell folate (RBC folate) and the gel tubes were immediately placed on ice. After blood clotting, they were centrifuged at 3000 × g for 15 minutes, aliquoted for serum samples and these samples were frozen at –86ºC until analysis in the biochemical laboratory of the Faculty for Sport Sciences, UPM, Madrid (Registered Lab number 242, *Red de Laboratorios de la Comunidad de Madrid*). Total sCbl concentrations were determined by a Microparticle Enzyme Immunoassay (MEIA; Abbott AxSYM, Abbott Park, USA, total CV ≤11 %) [20]. Serum holoTC was analyzed by a two-step sandwich MEIA (Abbott AxSYM, total CV ≤10 %) [21]. Serum folate (sFolate) and RBC folate were measured by ion-capture immunoassay (ICIA; Abbott AxSYM, total CV ≤19 %) [22]. RBC folate was determined in whole blood from EDTA-coated tubes. Serum tHcy was determined by a fluorescence polarization immunoassay (FPIA; Abbott AxSYM, total CV ≤6 %) [23]. The samples for sCbl, holoTC, sFolate, RBC folate, and tHcy of each subject were measured within one run to avoid between-run variation.

Hemoglobin (Hb, CV ≤1.5 %), hematocrit (HCT), mean corpuscular volume (MCV, CV ≤2.0 %), and erythrocyte (CV ≤2.0 %) values were obtained by an automated hematology analyzer (Coulter HMX, IZASA, Alcobendas; Madrid, Spain). Serum creatinine concentrations were analyzed with the Hitachi 912 (Roche Diagnostics; Mannheim, Germany.) and were conducted by a local clinical laboratory (CLIMESA, Gabinete Medico Conde-Duque; Madrid, Spain).

Body weight was measured to the nearest 0.1 kg on a Seca scale (709, Seca; Germany). The knee height was measured to the nearest 1 mm using a GPM spreading anthropometer (GPM; Switzerland) at the left side in the sitting position, lower legs relaxed, with the knee flexed at a 90° angle. Height was calculated by the formulas of Chumlea [24].

Statistical analyses

Continuous variables were checked for Gaussian normal distribution with the Kolmogorov-Smirnov test and natural log(ln) transformation was applied for holoTC, sFolate, RBC folate, and Hb. Geometric and arithmetic means with standard deviations of pre- and post-supplementation concentrations were computed for tables. In addition, the trimmed mean was also calculated to check the degree of variation. Differences between means were analyzed with a *t*-test for paired samples and the Wilcoxon matched-pairs signed-rank test, in the case of sCbl. Since there is still no consensus on cut-off values to define Cbl deficiency, neither for serum cobalamin nor for holoTC values, and given that the distribution of cobalamin concentration values was not normal, participants were grouped in clusters (k-means clustering) according to the initial cobalamin status in order to achieve maximum homogeneity within the groups. Generalized linear model (GLM) repeated measures analysis with "sCbl clusters" as a fixed factor and post-hoc Bonferroni analysis was used in order to assess the interaction of the initial sCbl status on the effect of the supplementation. Results were reported as arithmetic means, all tests were two-tailed, and a p value ≤0.05 was considered statistically significant. SPSS for Windows software (version 15.0, SPSS Inc., Chicago, USA) was used for data analysis.

Results

The characteristics of the whole study population are summarized in Table I. The study group comprised 64 participants, 40 (63 %) women and 24 (37 %) men, with a mean age of 82 (range 63 – 93 years). At baseline, 13 (20 %) participants of the study population showed sCbl values lower than 200 pmol/L and 23 (36 %) showed serum holoTC levels lower than 40 pmol/L. Anemia, defined as hemoglobin concentrations < 13 g/

Table I: Characteristics of the study population (n = 64).

Characteristics	N	Mean ± SD
Age (y)	64	82 ± 7
Gender (Male/Female; n)	64	24/40
Weight (kg)	64	64 ± 14
Knee Height (cm)	64	47 ± 3
Height (cm)	64	153 ± 8
BMI	64	27 ± 6
Creatinine (mg/dL)	64	0.9 ± 0.3
Hemoglobin (g/dL)	64	13.3 ± 1.7
Hematocrit (%)	64	41.4 ± 5.1
MCV (fL)	64	89.8 ± 5.6
Erythrocytes (10^{12}/L)	64	4.6 ± 0.5

Table II: Wilcoxon test (z) for pre- and post-mean values for sCbl and its absolute and relative changes.

Variables	N	Pre [a]	Post [a]	Wilcoxon (z)	Change	(%)
sCbl (pmol/L)	64	308.4 (132.9)	558.3 (262.2)	–6.95***	249.9 (172.8)	88 (59)

*** $p < 0.001$
sCbl, serum cobalamin, to convert to pg/mL, divide by 0.738.
a. Values are presented as mean (SD).

Table III: t-test for pre- and post-mean values (geometric and arithmetic) for holoTC, serum and RBC folate, tHcy, MCV, HCT, Hb, and erythrocytes and its absolute and relative changes.

Variables	N	Pre GM	Pre [a]	Post GM	Post [a]	t	Change	(%)
HoloTC (pmol/L) [b,c,d]	64	45.77	53.8 (32.5)	84.49	95.7 (47.2)	–14.11***	41.8 (28.0)	97.3 (85.5)
sFolate (nmol/L) [b,c,d]	64	19.4	21.0 (8.6)	19.2	21.02 (9.1)	0.3	0.04 (4.8)	1.5 (21.3)
RBC folate (nmol/L) [b,c,d]	56	779.7	859.2 (417.7)	863.8	945.3 (434)*	–2.6*	86.1 (334.8)	15.8 (37.4)
tHcy (µmol/L) [b,c]	64	–	15.1 (4.5)	–	13.1 (3.5)	8.6***	v2.0 (1.9)	–11.9 (10.9)
MCV (fL) [b]	64	–	89.7 (5.6)	–	89.4 (5.6)	1.7	–0.34 (1.6)	–0.4 (1.7)
HCT (%) [b]	64	–	41.4 (5.2)	–	41.0 (5.0)	1.4	–0.4 (2.1)	–0.7 (5.1)
Hb (g/dL) [b,d]	64	13.2	13.3 (1.7)	13.5	13.6 (1.6)	–2.7**	0.2 (0.7)	1.9 (5.4)
Erythrocytes (10^{12}/L) [b]	64	–	4.6 (0.5)	–	4.6 (0.5)	0.7	–0.02 (0.2)	–0.36 (5.2)

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$
GM, geometric mean; HoloTC , holo transcobalamin; sFolate, serum folate; RBC folate, red blood cell folate; tHcy, total homocysteine; MCV, mean corpuscular volume; HCT hematocrit; Hb, hemoglobin.
a. Values are presented as mean (SD).
b. Pre- and post-treatment means compared by *t*-test
c. SI conversion factors: To convert holoTC to ng/L, multiply with 1.3554; folate to ng/mL divide by 2.266; tHcy to mg/L, divide by 7.397.
d. Data ln-transformed for analysis.

dL in men and <12 g/dL in women, was present in 28 % of the study group. Only one participant had MCV levels above 100 fL. Folate deficiency, defined as serum folate ≤6 ng/mL (13.6 nmol/L) was present in 12 participants (19 %). For each analyzed parameter the trimmed mean at 5 % was calculated and no significant differences were found between their means and trimmed means. Creatinine values were within reference range.

Mean sCbl levels improved from 308.4 to 558.3 pmol/L ($p < 0.001$, Table II) except in one participant whose serum cobalamin levels decreased slightly (from 484 to 459 pmol/L), whereas the same participant's holoTC values increased (from 35 to 79 pmol/L). After supplementation, from the above-mentioned 13 subjects with inadequate sCbl levels, 11 (85 %) reached normal levels (defined as >200 pmol/L) and from the 23 with inadequate holoTC levels, 18 (78 %) reached normal values (defined as >40 pmol/L).

The quick cluster analysis delineated four clusters according to initial sCbl values. The "low" sCbl cluster consisted of eight subjects who had sCbl values of 126.8 (25.3) pmol/L, the "medium" cluster consisted of 17 with mean sCbl levels of 220.3 (26.6) pmol/L, the "high" cluster comprised 30, and the "very high" nine participants with mean sCbl concentrations of 332.6 (38.2) and 555.5 (107.3) pmol/L, respectively. The "low" sCbl cluster had serum holoTC values of 21.4 (15.2) pmol/L, the "medium" cluster of 46.0 (16.7), and the "high" and "very high" of 54.4 (21.6) and 95.8 (52.3) pmol/L, respectively. The "low" sCbl cluster had serum tHcy values of 18.5 (6.6) µmol/L, the "medium" cluster of 15.1 (3.7), and the "high" and "very high" of 13.8 (3.8) and 16.2 (4.9) µmol/L, respectively.

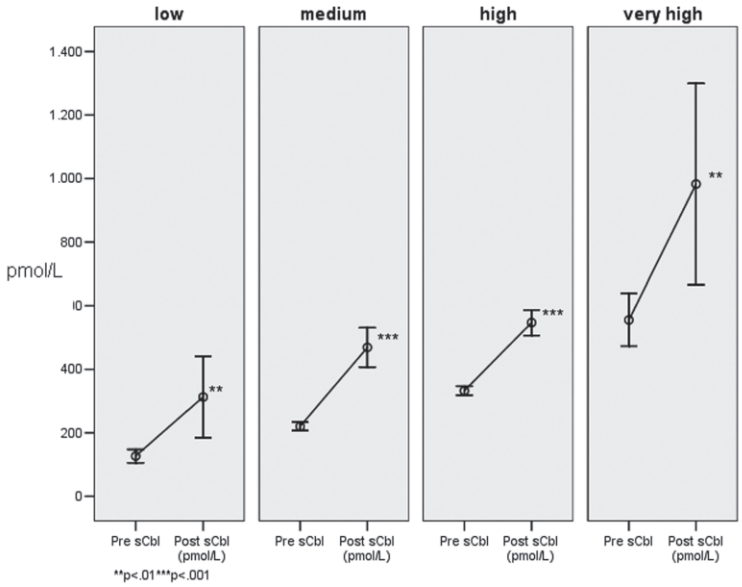

Figure 1: Change from pre- to post-treatment mean serum cobalamin (sCbl) concentrations by clusters of initial sCbl concentrations.

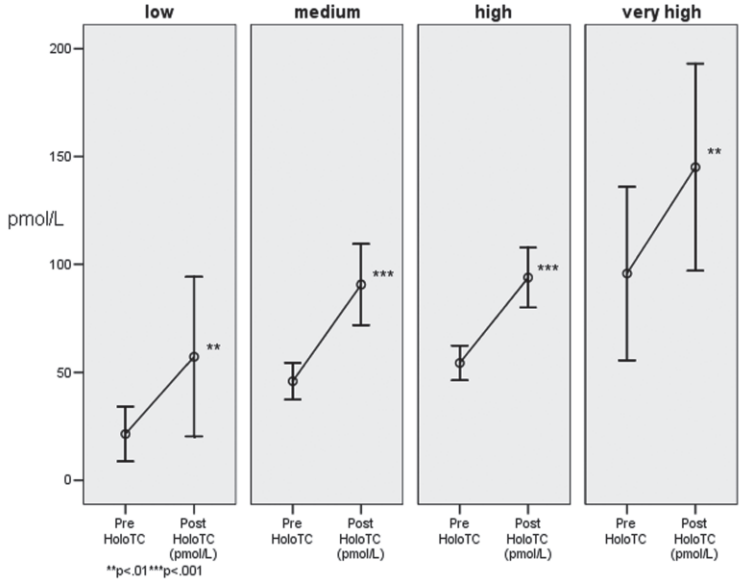

Figure 2: Change from pre- to post-treatment mean holotranscobalamin (holoTC) concentrations by clusters of initial serum cobalamin (sCbl) concentrations.

There was a significant increase from pre- to post-treatment sCbl values (308.4 to 558.3 pmol/L, $z = -6.95$, $df = 63$, $p < 0.001$, Table II). Similarly, holoTC concentrations rose significantly (53.8 to 95.7 pmol/L, $t = -14.11$, $df = 63$, $p < 0.001$, Table III) as a result of the treatment. Cobalamin supplementation lowered significantly mean tHcy concentrations (15.1 to 13.1 µmol/L, $t = 8.55$, $df = 63$, $p < 0.001$, Table III). RBC folate increased significantly from pre- to post-supplementation (859.2 to 945.3 nmol/L, $t = -2.6$, $df = 55$, $p < 0.05$, Table III). The hemoglobin values increased significantly (13.3 to 13.6 g/dL, $t = -2.7$, $df = 63$, $p < 0.01$). No significant changes were found for sFolate, MCV, HCT, and erythrocytes values (Table III).

The comparison of the pre- and post-sCbl, holoTC, and tHcy values within each cluster showed significant changes (all $p < 0.01$); sCbl and holoTC concentrations increased whereas tHcy values decreased in each cluster (Figures 1–3). Regarding tHcy, the "low" and "very high" sCbl clusters showed pre- as well as post-treatment tHcy levels with a high variation, and the "very high" sCbl cluster had higher initial tHcy concentrations than the "high" and "medium" group but without statistical significance. When applying the

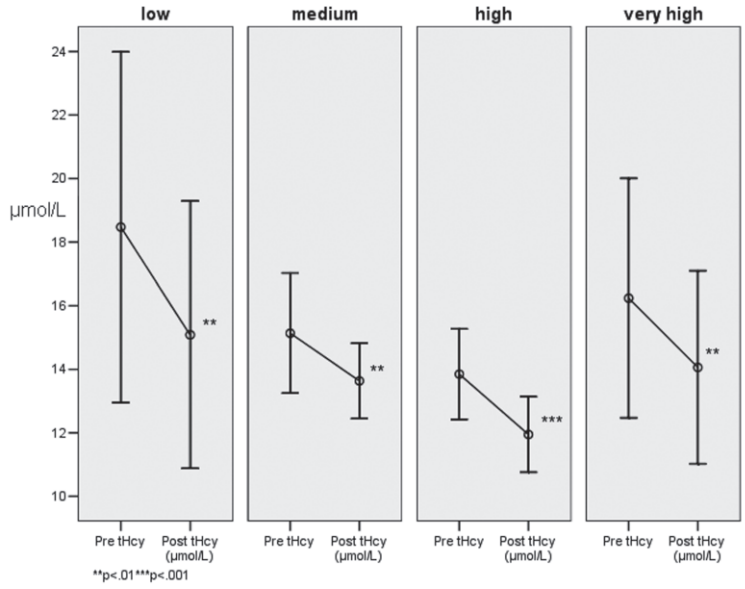

Figure 3: Change from pre- to post-treatment mean total homocysteine (tHcy) concentrations by clusters of initial serum cobalamin (sCbl) concentrations.

Table IV: Summary table One-Way ANOVA with repeated measures and one fixed factor (serum cobalamin cluster) for mean serum cobalamin (sCbl), holotranscobalamin (holoTC), and total homocysteine (tHcy) concentrations.

Source	Sum of Square	df	Mean Square	F
sCbl	1763,621.618	1	1763,621.618	138.5***
sCbl * Cluster sCbl	176,145.652	3	58,715.217	4.61**
Error (sCbl)	764,275.388	60	12,737.923	
HoloTC	43,688.135	1	43,688.135	108.83***
HoloTC * Cluster sCbl	549.343	3	183.114	0.46
Error (HoloTC)	24,084.607	60	401.410	
tHcy	122.437	1	122.437	71.81***
tHcy * Cluster sCbl	10.047	3	3.349	1.96
Error (tHcy)	102.300	60	1.705	

** p<0.01 *** p<0.001
sCbl, serum cobalamin; HoloTC , holotranscobalamin; tHcy, total homocysteine.

GLM model for repeated measurements with the initial sCbl as a fixed factor in order to compare the effect of the supplement according to the pre-treatment sCbl status, only the increase of sCbl depended on the initial status ($F = 4.61$, $p < 0.01$). The effect of the supplement on holoTC and tHcy was not significantly affected by the initial sCbl status ($F = 0.46$ and $F = 1.96$, respectively, both $p > 0.05$, Table IV).

Discussion

The main results of the study were that tHcy values decreased significantly whereas sCbl and holoTC values increased significantly after only four weeks of daily 500-µg cyanocobalamin supplementation. The observed effect improves upon the results of a prior investigation of a dose-finding trial conducted by Eussen *et al.* [18], who concluded that when comparing a daily oral dosage of 500 µg vs. 1000 µg cyanocobalamin administered for eight weeks that 500 µg was the lowest oral dose associated with a maximum increase of holoTC levels in mild vitamin

B12-deficient subjects, with deficiency defined as serum vitamin B12 levels of 100–300 pmol/L. It must be noted that in the study of Eussen *et al.* [18], they did not analyze a period of four weeks, as was done in our study. The increase in the subjects included in the "low" cluster in our study was slightly higher than the one presented by the group of the dose-finding trial of Eussen *et al.* [18], which received the same amount of oral cyanocobalamin (186 vs. 182 pmol/L) but for twice the duration of the present study. In addition, the increase of the "medium" cluster even reached the same effect as the group receiving 1000 μg/day of Eussen's *et al.* dose-finding trial [18] for eight weeks and the milk trial of the study performed by Dhonukshe-Rutten [16], who administered a milk drink enriched with 1000 μg crystalline Cbl or the same amount containing Cbl capsules for 12 weeks (249 vs. 248 vs. 250 pmol/L) to mildly cobalamin-deficient elderly, defined as serum vitamin B12 levels of 100–300 pmol/L.

Highly significant increases were also observed for holoTC values, both for the whole study group and for each cluster. The increase of the "low" and "medium" clusters were smaller (36 vs. 45 vs. 49 pmol/L) than the one presented by the 500-μg oral Cbl-receiving group of the dose-finding trial [18].

Regarding tHcy, mean values of the "medium" cluster decreased by a lesser degree than in the 500-μg oral Cbl-administered subgroup of the dose-finding trial [18] (–1.5 vs. –1.9 μmol/L) and the capsule trial (–1.8 μmol/L) of the above-named study performed by Dhonukshe-Rutten [16]. In contrast, the "low" cluster showed a higher response of –3.4 μmol/L. But it should be mentioned that both pre- and post-treatment values presented a great variation. The "very high" sCbl cluster had higher pretreatment tHcy concentrations than the ones for the "high" and even "medium" group. It has to be mentioned that in our study, high tHcy values were mostly independent of Cbl status. In relationship to folate, folate deficiency according to serum folate levels was present in 12 subjects (18.5 %) of the whole study population. Only two subjects had tHcy values < 13 μmol/L. This seems to indicate that other factors beyond Cbl and folate status, such as lifestyle factors [25], are affecting tHcy concentrations in this population group. Furthermore, that cluster consisted of few people and showed a big variance and that pre-treatment value did not reach statistical significance compared to the pre-treatment values of the other clusters.

The oral vitamin B12 supplementation did not significantly change mean sFolate. RBC folate levels increased ($p < 0.05$) in the whole study population. These findings are supported by a double-blind, placebo-controlled trial [17] where oral vitamin B12 was supplemented alone or together with folic acid. In the latter study, mildly vitamin B12-deficient subjects ($n = 195$) were randomly assigned to receive 1000 μg vitamin B12, 1000 μg vitamin B12 in combination with 400 μg folic acid, or a placebo. After 12 weeks of supplementation, mean RBC folate values increased significantly in the vitamin B12 group and in the vitamin B12-plus-folic acid group. In the above-mentioned study performed by Dhonukshe-Rutten *et al.* [16], after 12 weeks both intervention sets also demonstrated an increase of RBC folate, but only in the milk trial did the increase reached statistical significance. After having performed the quick cluster analysis, in our study the significant increase of RBC folate disappeared (data not shown).

To the best of our knowledge, only two studies included hematological parameters in the outcome. The results of the present study are consistent with previous trials that reported no significant changes in hematological parameters within one month of Cbl administration, such as the study conducted by Seal *et al.* [26] who compared the effectiveness of 10 and 50 μg oral cyanocobalamin, or placebo, administered daily for four weeks in 31 older patients with deficient or borderline serum vitamin B12 levels between 100 and 150 pmol/L. Hvas *et al.* [27] found no significant changes in blood Hb and MCV between the treatment group ($n = 70$) and the placebo group ($n = 70$) in a Danish clinical trial with participants older than 18. It should be noted that these studies administered much lower doses than was the case in our study.

Our results support the fact that a daily dose of 500 μg of cyanocobalamin during 28 days improves vitamin B12 status in institutionalized elderly. No control group was included because foods provided at the nursing homes followed a regular pattern, thus dietary factors were unlikely to be important. Additionally, as it has been mentioned above, dietary vitamin B12 intake as a determinant of blood concentrations loses importance during the ageing process because of increasing malabsorption problems [5–7].

Conclusions

Concerning the choice of supplementation of a representative elderly care facility population group, an oral supplement of 500 μg for only four weeks was effective in increasing the sCbl and holoTC levels and in decreasing tHcy levels significantly. Being a shorter

treatment period, the oral treatment was very easy to apply to a high-risk group such as institutionalized elderly people. The fact that the treatment of four weeks was adequate for producing an effect similar to that of longer treatments with the same amount of cyanocobalamin supplements makes this treatment less expensive. Given the prevalence of clinical and especially of subclinical deficiency among this population group, from a public health point of view, oral B12 supplementation may be considered as an alternative to injections, and from a practical point of view it could reduce personal costs. For evaluating its long-term effects, specifically the effect on liver stores, further investigations among the elderly should be conducted.

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Conflict of interest

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List of non-standard abbreviations

Cbl	cobalamin
MMA	methylmalonic acid
holoTC	holotranscobalamin
sCbl	serum cobalamin
tHcy	total homocysteine
MEIA	microparticle enzyme immunoassay
sFolate	serum folate
RBC	red blood cell folate
ICIA	ion-capture immunoassay
FPIA	fluorescence polarization immunoassay
Hb	hemoglobin
HCT	hematocrit
MCV	mean corpuscular volume

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