ORIGINAL ARTICLE

Vitamin B12-fortified toothpaste improves vitamin status in elderly people: a randomized, double-blind, placebo-controlled study

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Abstract

Background Elderly people are at risk for vitamin B12 deficiency.
Aims We studied the ability of vitamin B12-enriched toothpaste vs. placebo to increase vitamin B12 status in elderly subjects.
Methods We conducted a randomized double-blind placebo-controlled intervention in 103 elderly subjects. Serum concentrations of vitamin B12, holotranscobalamin (holoTC), methylmalonic acid (MMA), and plasma total homocysteine (tHcy) were measured at baseline and after 3 months.
Results 92 subjects met the inclusion criteria, completed the 3 months study, and were included in the data analysis. After the intervention, concentrations of vitamin B12 were higher [mean (SD) = 368 (123) vs. 295 (123) pmol/L; $p = 0.005$] and holoTC tended to be higher [112 (48) vs. 91 (68) pmol/L; $p = 0.088$] in the vitamin B12 group compared with the placebo group. The changes of serum vitamin B12 [54 (74) vs. 3 (60) pmol/L, $p < 0.001$], holoTC [21 (34) vs. 2 (32) pmol/L, $p = 0.007$], and tHcy [−0.9 (2.3) vs. 0.3 (1.9) μmol/L, $p = 0.010$] were significantly different between the intervention groups. Mean percentage increase of serum vitamin B12 (+23% corresponds to +54 pmol/L) in the vitamin B12 toothpaste group suggests that the intervention had provided an additional daily intake of approximately +7 μg oral B12. Common diseases and drugs did not predict the change of blood markers in the vitamin group. No side effects were observed.
Conclusions The toothpaste enriched with 100 μg cyanocobalamin/g has increased vitamin B12 status and can thus be used for preventing vitamin B12 depletion in elderly people. The trial was registered at ClinicalTrials.gov: NCT02679833.

Keywords Vitamin B12 deficiency · Elderly · Holotranscobalamin · Supplementation

Abbreviations

holoTC Holotranscobalamin
MMA   Methylmalonic acid
tHcy   Total homocysteine

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Introduction

Vitamin B12 (cobalamin) status in elderly people has gained more attention in recent years following clinical studies showing associations with aging [1] or positive health effects after administrating the vitamin [2]. Elderly subjects constitute a classical risk group for subtle vitamin B12 deficiency [3]. A typical western diet provides adequate vitamin B12 intake to fulfill the adequate intake of 4 μg/day. However, food vitamin B12 malabsorption is a common cause of vitamin B12 deficiency in elderly people [4]. Examples of conditions that negatively impact vitamin B12 absorption or bioavailability in the elderly are: gastritis, achlorhydria, intrinsic factor antibodies, medications, or inability to release the vitamin from food proteins. Studies in elderly subjects with food-cobalamin malabsorption indicate that a daily vitamin B12 intake of approximately 6.0 μg/day is required to achieve normal serum concentrations of vitamin B12 biomarkers in the majority of the subjects [5, 6].

Deficient individuals who cannot absorb the vitamin may benefit from high oral doses of cyanocobalamin (0.5–1 mg/day) that can reach the circulation via passive diffusion. Consumption of vitamin B12-enriched cow milk (provided 1 mg B12/day) improved vitamin B12 status markers in blood of elderly people after 12 weeks [7]. Some supplement forms aimed to avoid the rate-limiting absorptive capacity in the intestine (believed to be approximately 1.5 µg/meal), the pH-sensitive absorption, and the intrinsic factor-dependent absorption path. Nasal spray has been used to deliver large amounts of the vitamin into the nasal mucosa and has been shown to be successful in raising blood vitamin levels [8, 9]. Moreover, vitamin B12-enriched toothpaste has been shown to increase vitamin B12 in vegan subjects [10]. In vegans, serum concentrations of vitamin B12 and holotranscobalamin (holoTC) increased within 3 months upon regular use of the enriched toothpaste compared with the placebo group [10]. The mechanisms of absorption in the mucosal membrane of the mouth are not known. Haptocorrin that is produced locally in the salivary [11] and the submandibular glands [12] could play a role in the absorption of vitamin B12 in the mouth, but this has not been proven yet. Improving vitamin B12 status or preventing its depletion via fortified toothpaste could avoid incompliance issues, especially in elderly people who commonly take several oral pills.

We hypothesized that vitamin B12-fortified toothpaste may deliver the vitamin into the blood and enhance vitamin B12 status in elderly people. The present study in elderly subjects investigated the effect of vitamin B12-fortified toothpaste (100 µg cyanocobalamin/g) versus placebo toothpaste on blood vitamin B12 status markers; total serum vitamin B12, holoTC, methylmalonic acid (MMA), and total homocysteine (tHcy).

Subjects and methods

Subjects and design

This is a randomized double-blind placebo-controlled intervention study that compared the effect of a cyanocobalamin-fortified toothpaste and a placebo toothpaste on vitamin B12 status markers in elderly people. The study was conducted at the Saarland University Hospital, Germany between March 2015 and January 2016. Candidates were invited to participate and were screened during their regular health care visits at their family physician (AZ). Potential participants received information about the study design and aims during the screening visit. Those who accepted to participate and met the inclusion criteria and none of the exclusion criteria were invited to attend the first study visit. The inclusion criteria were: men and women aged > 60 years with a stable general health condition (i.e. no foreseen surgical

interventions). The exclusion criteria were: current or past (during the last year) use of vitamin B12 or folate supplementation, anaemia or conditions that require therapeutic doses of vitamin B12, and all diagnosed conditions that can interfere with vitamin B12 markers such as pernicious anaemia, gastric or intestinal resection, Crohn disease, celiac disease, liver diseases or cancer. People who experienced a recent acute cardiovascular event (during the last 3 months before the study) or those who lost all of their natural teeth were not eligible for participation. The termination criteria were: acute event during the trial and medical indication for treatment with therapeutic doses of vitamin B12 or folate. A low baseline vitamin B12 status was not an inclusion criterion. The intervention was completed before measuring vitamin B12 status markers. After measuring all blood levels, participants who were judged to have received pharmacological doses of vitamin B12 or folate were excluded from the data analysis.

The study flow diagram is shown in Fig. 1. A total of 120 subjects were initially interested in joining the study. Later, five subjects refused to participate and eight were excluded because of meeting at least one of the exclusion criteria. 107 participants agreed to participate and were randomized on the day of first study visit (53 placebo and 54 vitamin). The allocation to one of the intervention arms was performed by the study nurse by providing sequentially numbered containers. The placebo and the vitamin B12 toothpaste had identical appearance, smell and taste, but carried two different codes. The recruiting staff, the participants and the analytical labs were blinded for the treatment. 103 subjects completed the second visit of the study after 3 months, while four participants dropped out (one died, one reported taking pharmacological doses of vitamin B12, and two had acute conditions and were hospitalized).

The participants were asked to report any new supplements or changes in their medications or lifestyle during the study. However, for subjects who consulted specialist physicians during the 3 months of intervention, no information was available about possible acute vitamin B12-injections. Of the 103 participants who completed the study, 11 subjects were excluded after completion of the 3 months intervention and measurement of the blood concentrations. One subject was excluded because of an extremely high serum folate concentration in the second visit (but not in the first visit), seven subjects were excluded because they had baseline serum vitamin B12 levels between 900 and 2000 pmol/L and they became strongly depleted during the study period. Those seven subjects were judged having been treated with pharmacological doses of vitamin B12 prior to the study. Finally, 3 out of the 11 subjects were excluded because they had a sharp increase in serum vitamin B12 and holoTC between visit 1 (baseline) and visit 2. This increase was judged to
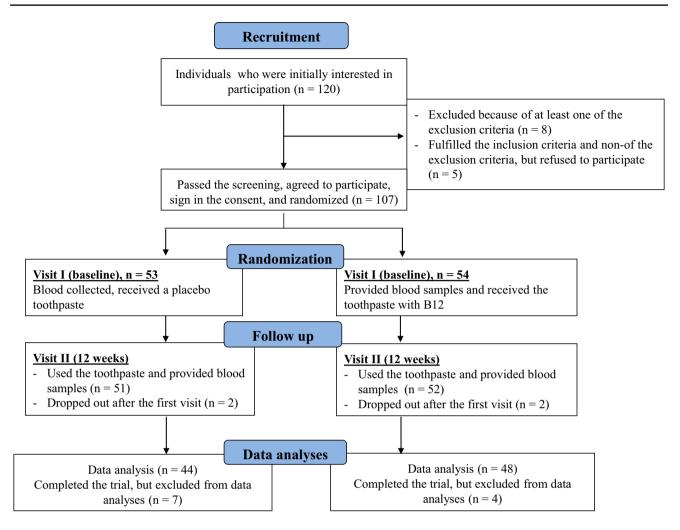
Fig. 1 Study flow diagram

be higher than what would be expected from the present intervention alone and was judged having been caused by treatment with pharmacological doses of vitamin B12 during the study. The final statistical analyses contained data from 92 subjects who completed the study visits and fulfilled all other criteria.

During the first study visit, blood samples were collected and information about health history, acute and chronic conditions and drugs was updated in the patient's documents.

Blood sampling and biochemical analyses

During the first (baseline) and second (after 3 months) study visits, fasting blood (≥ 8-h fasting period) was collected in tubes without anticoagulants (for serum) and those containing EDTA-K$^+$ (for plasma). Blood samples were centrifuged, and the serum and plasma samples were separated within a maximum of 40 and 30 min, respectively. All samples

were stored at $-70\ ^\circ$C until the end of the study and blood markers of vitamin B12 were measured at the end of the study. Samples were analysed after completion of the intervention, and samples from each participant were analysed in the same run to minimize analytical variations. In addition, blood count and plasma or serum markers (i.e. liver, kidney, inflammation marker) were measured from all participants at each of the study visits as part of the general care management.

Serum vitamin B12 concentrations were measured at the Labordiagnostik Mittelhessen (Germany) using the electrochemiluminescence immunoassay (Cobas; Roche Diagnostics GmbH, Mannheim, Germany) and that of holoTC [13] using chemiluminescence microparticle immunoassay (Architect, Abbott, Wiesbaden, Germany), respectively. The concentrations of tHcy and MMA were measured at the Central Laboratory of the Saarland University Hospital. Plasma tHcy was measured using chemiluminescence microparticle immunoassay (Architect, Abbott Laboratories, Wiesbaden,

Germany) and serum MMA was measured using an in-house method on gas chromatography–mass spectrometry device.

Vitamin B12-combined indicator (4cB12) was calculated for each participant from baseline and post-intervention data on: vitamin B12, holoTC, MMA and tHcy according to the equation published before [2, 14]. The 4cB12 was adjusted for age and serum folate concentrations that were measured in all participants.

Compliance and the estimated amount of vitamin B12 delivered to the oral cavity

Two packages of the toothpaste (each 75 mL) were handed out and participants were instructed to use the toothpaste twice per day for 2 min each time. Compliance was controlled by the completion of a written protocol that included the type of toothbrush used (i.e. manual or electric) and the time and duration of brushing over the study period. At the end of the intervention, all participants were considered to be highly compliant. The amount of vitamin B12 was analysed in the toothpaste and as in the previous study [10], the amount of the vitamin delivered to the oral cavity was estimated to be 130 or 290 mg/day for the two brushing sessions combined when using electrical or manual brushing tools, respectively.

Study outcomes and sample size

The primary outcomes of the present study were the differences between the intervention groups in the changes (delta) of vitamin B12 and holoTC over 3 months. The secondary outcomes were; between-groups differences in serum concentrations of vitamin B12 and holoTC over 3 months and the within-groups longitudinal changes of the markers within the same time. Serum concentrations of vitamin B12 and holoTC are known to show earlier response to vitamin B12 supplementation compared to MMA concentrations [15–18]. Changes of serum MMA depend largely on the vitamin dose, duration of the study, and baseline MMA levels. Therefore, MMA concentrations or changes of MMA were not considered as an outcome in the present study.

There are no available dose–response studies using the toothpaste as a vitamin B12 carrier. In practice, serum vitamin B12 concentrations are mostly stable upon repeated measurements [19]. In a repeated measurement study within 2 years, the within-person coefficient of variation has been reported to be 11.6% [19]. We assumed that variations in serum vitamin B12 that exceed the biological variations would be due to the intervention. From our studies in elderly people [20], we expected mean serum vitamin B12 at baseline to be approximately 285 pmol/L. Within-group variations of ± 33 pmol/L (i.e. 11.6%) over 3 months would be within the expected

biological variations [19]. A difference in serum vitamin B12 between the intervention groups of ≥ 50 pmol/L is expected to be exceeding the biological variations. Thus, if mean serum vitamin B12 would increase from 285 to 335 pmol/L in the vitamin B12 intervention arm within 3 months (SD 100 pmol/L), we would interpret this observation as due to the vitamin B12 in the toothpaste. Assuming a power of 0.80, $\alpha = 0.05$, and an allocation ratio of 1:1 we expected that we would need 53 participants in each arm (106 in total) to show a difference in serum vitamin B12 between the two study arms.

Statistical analyses

The results were analysed using SPSS (version 24; IBM, Chicago, USA). Continuous variables were tested for normality of distribution using the Kolmogorov–Smirnov test with Lilliefors significance correction. All vitamin B12 markers were not normally distributed and were log-transformed before applying statistical tests that require normal distribution of the data. All markers and their changes between the study visits (post-intervention − baseline) are expressed as mean ± SD. The percentage changes of the markers from baseline were calculated as: (post-intervention − baseline) × 100/baseline concentrations. The one-way analysis of variance test (ANOVA) was used to compare the log-transformed data between the study groups. For between-group comparisons that required adjustments, we used the general linear model univariate analysis of variance while including the possible confounders as covariates. Comparisons of the concentrations of vitamin B12 markers and their changes (post-intervention − baseline) between the study groups were adjusted for baseline concentrations of these markers, as recommended earlier [21]. Further adjustments were performed for: sex, gastrointestinal disorders (yes vs. no), using proton pump inhibitors (yes vs. no), diabetes status (no diabetes, diabetes receiving metformin, and diabetes not receiving metformin). We used the general linear model univariate analysis of variance test to study the interactions between treatment effect and baseline levels or treatment and other confounding variables stated before.

All tests were two-sided and p values < 0.05 were considered to be statistically significant.

Results

Population characteristics

The study included 92 subjects (48 subjects in the vitamin group and 44 in the placebo group). The mean (SD) of

age was 66.3 (4.8) years and the study included 49 women (53%). The main characteristics of the study population and the distribution of comorbidities and medications are shown in Table 1.

Table 1 Baseline characteristics, morbidities and medications of the study participants according to the randomization

	Vitamin B12, 48	Placebo, 44
Age, years [mean (SD)]	66.8 (4.6)	65.9 (5.0)
BMI, kg/m² [mean (SD)]	28.4 (5.2)	28.2 (4.1)
BMI ≥ 30 kg/m²	14 (29.2%)	15 (34.1%)
Serum creatinine, mg/dL	0.97 (0.53)	0.92 (0.19)
Hemoglobin, mg/dL	14.1 (1.0)	14.0 (1.3)
Hematocrit, %	43 (3)	42 (3)
Mean corpuscular volume, fl	92 (9)	91 (4)
Women, n (%)	27 (56.3%)	22 (50.0%)
Diabetes, n (%)	36 (75.0%)	25 (56.8%)
Hypertension, n (%)	38 (79.2%)	29 (65.9%)
Metabolic syndrome (IDF[a]), n (%)	16 (33.3%)	13 (29.5%)
Gastrointestinal diseases, n (%)		
Reflux or gastritis	12 (25.0%)	12 (27.3%)
Hypothyroidism, n (%)	17 (35.4%)	13 (29.5%)
Proton pump inhibitors, n (%)	15 (31.3%)	16 (36.4%)
Metformin, n (%)		
Metformin users	17 (35.4%)	13 (29.5%)
Non metformin users	19 (39.6%)	12 (27.3%)
No diabetes	12 (25.0%)	19 (43.2%)
Statins, n (%)	20 (41.7%)	14 (31.8%)

[a] The definition of metabolic syndrome according to the International Diabetes Federation (IDF) is the presence of diabetes, abdominal obesity, high cholesterol and high blood pressure

Baseline concentrations of vitamin B12 markers and their changes after 3 months

No side effects were observed during the intervention. Baseline concentrations of plasma tHcy and serum vitamin B12 and holoTC did not differ significantly between the groups (Table 2). At baseline, the concentrations of serum MMA were higher in the placebo group than in the vitamin B12 group ($p = 0.035$). Only 5 subjects out of the 92 had elevated baseline concentrations of MMA ≥ 300 nmol/L, while 4 had lowered holoTC < 36 pmol/L.

After the intervention, concentrations of tHcy remained not significantly different between the intervention groups. After 3 months, serum concentrations of MMA remained higher in the placebo group as compared with the vitamin B12 group; while serum concentrations of vitamin B12 and holoTC were higher in the vitamin B12 group as compared with the placebo group after the intervention (Table 2).

The changes of tHcy (post-intervention − baseline) over 3 months were stronger than in the vitamin B12 group than in the placebo group (mean = − 0.9 vs. 0.3 μmol/L, $p = 0.010$) (Table 3). The changes of MMA concentrations did not differ significantly between the groups. The changes of serum concentrations of vitamin B12 (mean = 54 vs. − 3 pmol/L, $p < 0.001$) and holoTC (mean = 21 vs. 2 pmol/L, $p = 0.007$) were stronger in the vitamin B12 group compared with the changes in the placebo group (Table 3). The treatment showed significant interactions with baseline concentrations of tHcy, MMA and vitamin B12 (but not with concentrations of holoTC). Moreover, there were no significant interactions between the treatment and possible confounders: sex, metformin, proton pump inhibitors and gastrointestinal diseases (Table 3).

The percentage change of serum vitamin B12 in the vitamin B12 group [mean (SD)] was + 23% (31%), whereas in the

Table 2 Serum concentrations of vitamin B12 markers and their changes according to randomization to the vitamin B12 or placebo toothpaste

	Baseline			After 3 months			Adjusted
	Vitamin B12, 48	Placebo, 44	p^a	Vitamin B12, 48	Placebo, 44	p^b	p^c
Hcy, μmol/L	12.8 (6.4)	11.9 (3.9)	0.492	11.9 (6.2)	12.2 (3.7)	0.445	0.014
MMA, nmol/L	175 (48)	208 (80)	0.035	178 (59)	223 (81)	0.002	0.062
Vitamin B12, pmol/L	313 (128)	298 (118)	0.557	368 (123)	295 (123)	0.002	< 0.001
HoloTC, pmol/L	91 (37)	89 (50)	0.411	112 (48)	91 (68)	0.006	0.001
Four markers cB12[4]	0.47 (0.44)	0.37 (0.54)	0.322	0.66 (0.40)	0.29 (0.57)	0.001	< 0.001

The results are shown as mean (SD)

cB12 combined B12 indicator, *holoTC* holotranscobalamin, *MMA* methylmalonic acid, *tHcy* total homocysteine

[a]p values were calculated using ANOVA test

[b]p values were calculated using general linear model analysis of variance test (unadjusted)

[c]p values were calculated using general linear model analysis of variance test including sex, metformin (yes, no, no diabetes), proton pump inhibitors (yes, no), gastrointestinal diseases (yes, no), and baseline concentrations of the corresponding marker as covariates

[d]cB12 was calculated from four markers (vitamin B12, holoTC, MMA and tHcy) and adjusted for age and total serum folate

Table 3 Changes of serum concentrations of vitamin B12 markers according to randomization to the vitamin B12 or placebo toothpaste

	Change from baseline[a]		p[b]	p[c]	p[d]	p[e]
	Vitamin B12	Placebo	Treatment	Adjusted	Interaction treatment × baseline	Interaction treatment × covariates
tHcy, μmol/L	− 0.9 (2.3)	0.3 (1.9)	0.010	0.024	0.002	0.783
MMA, nmol/L	3 (49)	15 (64)	0.300	0.185	0.040	0.092
Vitamin B12	54 (74)	− 3 (60)	< 0.001	< 0.001	< 0.001	0.481
HoloTC, pmol/L	21 (34)	2 (32)	0.007	0.032	0.703	0.381

The results are shown as mean (SD)

holoTC holotranscobalamin, *MMA* methylmalonic acid, *tHcy* total homocysteine

[a]Changes of the markers were calculated as (post − baseline concentrations)

[b]p values were calculated using general linear model analysis of variance test (unadjusted)

[c]p values for the differences in the changes between the groups were calculated using general linear model analysis of variance test including sex, metformin (yes, no, no diabetes), proton pump inhibitors (yes, no), gastrointestinal diseases (yes, no), and baseline concentrations of the corresponding marker as covariates

[d]p values for the interactions were calculated using general linear model analysis of variance test including levels of the baseline marker as covariate

[e]p values for the interactions were calculated using general linear model analysis of variance test including sex, metformin (yes, no, no diabetes), proton pump inhibitors (yes, no), and gastrointestinal diseases (yes, no) as covariates

placebo group it was − 1% (18%) ($p < 0.001$). The percentage change of serum holoTC was + 29% (49%) vs. − 1% (26%) ($p = 0.001$). The percentage change of tHcy was significantly different between the groups [−5% (16%) vs. +5% (20%) in the vitamin and placebo groups, respectively; $p = 0.008$]. There were no differences in the percentage change of serum MMA between the vitamin and placebo group [+4% (29%) and + 11% (25%), respectively; $p = 0.183$].

Supplemental Table 1 shows the results of vitamin B12 markers and their changes in the 103 participants who completed the study. The individual vitamin B12 markers and their changes either did not significantly differ between the groups or showed slight changes in the vitamin B12-toothpaste compared with the placebo toothpaste. However, mean 4cB12 increased in the B12-toothpaste group and declined in the placebo group. The change of 4cB12 (a combined indicator that includes vitamin B12, holoTC, tHcy and MMA) was significant between the study arms.

Discussion

The present study has shown that vitamin B12 administered to elderly people via a vitamin B12-fortified toothpaste (100 μg cyanocobalamin/g) had reached the blood and increased serum concentrations of vitamin B12 and holoTC and lowered plasma tHcy. The response of MMA concentrations to the vitamin B12 toothpaste was not significant.

The magnitudes of changes of vitamin B12 biomarkers in the present study are comparable with previous studies in elderly people using low oral doses of cobalamin (≤ 10 μg) [6, 22]. In a placebo-controlled trial, Hill et al. administered 10 μg/day, 100 μg/day, and 500 μg/day oral cyanocobalamin over 8 weeks in elderly subjects who had a low baseline plasma concentrations of vitamin B12 [23]. After supplementation, the group that received 10 μg/day had approximately 60 pmol/L higher plasma vitamin B12 levels as compared with the placebo group [23], while holoTC was approximately 25 pmol/L higher in the 10 μg/day group compared with the placebo [23]. In a cross sectional observational study in elderly Canadians [24], using supplements containing less than 10 μg/day vitamin B12 was associated with higher serum vitamin B12 concentrations as compared with non-vitamin users (geometric mean 348 vs. 285 pmol/L; difference 63 pmol/L), while using 10–25 μg/day or larger doses was associated with a mean vitamin B12 concentration of approximately 415 μg/day (the difference to the non-vitamin users 130 pmol/L) [24]. Additionally, a dose–response relationship has been reported between oral vitamin B12 (between 2.5 and 80 μg/day for 30 days) and serum vitamin B12 concentrations in subjects with low vitamin B12 and malabsorption [5]. The increase of serum vitamin B12 in response to vitamin B12 intake appears to level off at intakes above 20 μg/day, which is in line with the results of the Canadian study [24]. For a vitamin B12 dose ≤ 10 μg/day, serum vitamin B12 increased up to 50 pmol/L within 1 month [5]. Doses higher than 10 μg/day caused approximately 80 pmol/L increase in serum vitamin B12 compared to baseline levels. When vitamin B12 is provided orally at doses of ≤ 10 μg/day, it is likely to be absorbed via intrinsic factor receptor, while higher doses are likely to pass via passive diffusion. In the present study, the

magnitude of change of serum vitamin B12 (54 pmol/L) and holoTC (21 pmol/L) suggests that the oral dose provided by the toothpaste was below 10 μg/day.

The dose–response association between vitamin B12 intakes and serum/plasma markers (vitamin B12 and MMA) has been studied in a recent meta-analysis [25]. Dullemeijer et al. estimated that doubling the vitamin B12 intake would increase serum vitamin B12 concentration by 11.0% (95% CI 9.4%, 12.5%) [25]. This association was stronger in the meta-analysis of studies in elderly populations than in adult populations, which was assumed to be related to lower baseline serum concentrations of vitamin B12 in the elderly (i.e. higher margin for changes) [25].

The response of serum MMA to low doses of vitamin B12 is known to be less pronounced than that of serum holoTC or vitamin B12 [23;25]. Dullemeijer et al. have shown that the absolute changes of serum MMA (mean − 7%; 95% CI − 10 to − 4%) after doubling vitamin B12 intake were smaller than the changes of serum vitamin B12 [25]. Elevated baseline plasma MMA concentrations (> 260 nmol/L) declined by approximately 16% after supplementation with 2.5 μg/day or 100 μg/day cyanocobalamin for 16 weeks in elderly people [26]. Because the majority of our participants did not show elevated MMA at baseline, the lack of serum MMA reduction after vitamin B12 supplementation in our study is expected. In line with this, the response of serum MMA in the elderly was smaller than that in vegan subjects who had higher baseline MMA concentrations [10]. However, studies in elderly people who cannot absorb the vitamin (i.e. due to pernicious anaemia or gastritis) can provide more information on the absorption path of vitamin B12 applied in the mouth. In case of cobalamin malabsorption, it is not known whether the amount of the vitamin that reaches the circulation would be sufficient to maintain normal vitamin B12 status markers in blood.

Prediction of vitamin B12 intakes derived from vitamin B12 concentrations

The toothpaste contained 100 μg cyanocobalamin/g. We previously estimated the total amount of vitamin B12 delivered to the mouth cavity to be 130–290 μg cyanocobalamin/day [10]. Half of this amount is delivered during each of two brushing sessions and retained in the mouth for approximately 2 min. It would be interesting to estimate how much of this amount is absorbed. A direct comparison with studies using sublingual cyanocobalamin is not possible, because the retention time in the mouth is longer for the sublingual forms and vitamin B12 is more likely to be swallowed after sublingual application. Thus, we followed an indirect approach to estimate the intake from the observed changes of serum concentrations of vitamin B12 following the intervention with the vitamin B12 toothpaste. The regression-equation of the relationship between vitamin B12 intake and plasma vitamin B12 in non-supplemented elderly people has been published by Dullemeijer et al. [25] and was used in the present study to predict average vitamin B12 intakes (x) from average vitamin B12 concentrations (y):

$$\log_e(y) = 0.18 \times \log_e(x) + 5.47$$

Serum concentrations of vitamin B12 at baseline (y) were between 295 and 313 pmol/L in none-supplemented elderly subjects (Table 2, in both study groups). Thus, according to the equation above, the baseline vitamin B12 intake was estimated to be between 3.5 and 4.6 μg/day. This baseline intake is in line with intake estimates in elderly Germans not using additional supplements [27].

$$\rightarrow \log_e(295) = 0.18 \times \log_e(x) + 5.47$$
$$\rightarrow \log_e(313) = 0.18 \times \log_e(x) + 5.47$$

Using this equation, we also estimated the vitamin intake in the vitamin B12-fortified toothpaste group to be 11.4 μg/day: mean post-intervention serum vitamin B12 $(y) = 368$ pmol/L

$$\rightarrow \log_e(368) = 0.18 \times \log_e(x) + 5.47$$
$$\rightarrow \log_e(x) = \left[\log_e(368) - 5.47\right]/0.18 = 2.434$$
$$\rightarrow x = e^{2.434} = 11.4 \mu g/day \text{(total daily vitamin B12 intake from the diet plus the toothpaste).}$$

Accordingly, we predicted that the toothpaste that contains 100 μg cyanocobalamin/g has provided an additional average input of vitamin B12 equivalent to approximately

additional intake of $+7$ µg/day. Thus, we estimated that between 2.4 and 5.4% of the daily administered vitamin B12 amount (130–290 µg cyanocobalamin) were absorbed during two brushing sessions. When vitamin B12 is given orally, approximately 1% of the dose crosses the brush border barrier into the blood by simple diffusion [28] after saturation of intrinsic factor (max. 5 µg/meal is delivered via intrinsic factor) [29]. Our results may suggest that the absorbed amount of vitamin B12 from the toothpaste cannot be solely explained by simple diffusion.

The present study has some limitations. First, it was not possible to make knowledgeable guess of the sample size prior to the study, because we had no prior experimental evidence on the time-course and the amount of vitamin B12 that could enter the circulation via the toothpaste. Second, none of the participants had a mild or moderate vitamin B12 deficiency. Therefore, the results should be interpreted with caution because they may not be generalizable to elderly people with low vitamin B12 status or those with malabsorption disorders.

In summary, elderly subjects using toothpaste that contained 100 µg cyanocobalamin/g showed improved serum concentrations of vitamin B12 and holoTC within 3 months, compared with the placebo group. Thus, vitamin B12-fortified toothpaste could be used to improve vitamin B12 status in elderly people in general or to avoid vitamin depletion. Similar studies in elderly with vitamin B12 malabsorption are needed before the delivery pathway through the mouth mucosa can be generally recommended to prevent deficiency in elderly people.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

Ethical approval The study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki. The study protocol was reviewed and approved by the Ethics Committee of the Saarland Region (Approval Number: 244/14).

Statement of human and animal rights All procedures were approved by the review board of the Saarland Medical Committee.

Informed consent All participants provided written informed consent to the study.

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