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ORIGINAL CONTRIBUTION

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Effect of multivitamin supplementation on the homocysteine and methylmalonic acid blood concentrations in women over the age of 60 years

Summary *Background* Deficiency of folic acid, vitamin B_6 and/or vitamin B_{12} can result in elevated total plasma homocysteine concentrations (tHcy), which are considered to be a risk factor for vascular disease. Studies have shown that supplementation of the three vitamins can lower tHcy even in subjects with tHcy in the normal range. *Aim of the study* The aim of this study was to evaluate the effect of a 6 month supplementation with vitamin B_6, B_{12} and folate on the concentrations of total plasma homocysteine and serum methylmalonic acid (MMA) of elderly women. *Methods* The study was designed as a randomized placebo controlled double-blind trial, and 220 healthy women (aged 60–91 years) were involved. The vitamin and mineral capsule contained pyridoxine (3.4 mg), folic acid (400 µg) and cobalamin (9 µg) in addition to other micronutrients. Blood concentrations of folate, cobalamin, tHcy, MMA and the activity coefficient of erythrocyte alpha-aspartic aminotransferase (alpha-EAST) were measured at baseline and after 6 months of supplementation. Dietary intake was evaluated at the beginning and the end of the intervention by two 3-day diet records. *Results* Median concentrations of serum cobalamin, serum folate and erythrocyte folate increased significantly and tHcy and alpha-EAST activity (indicative of improved status of vitamin B_6) coefficient decreased significantly in the supplemented group. Median MMA concentration of the supplemented group was significantly lower than that of the placebo group after the intervention. The vitamin supplementation had a greater decreasing effect on the tHcy concentration of volunteers with lower vitamin and higher tHcy initial concentrations. In a linear regression model, baseline tHcy, serum folate, age and alpha-EAST activity coefficient were significantly correlated with the change in tHcy. The change in MMA in the supplement group was significantly associated to the baseline MMA values. *Conclusions* Our results show that a 6 month supplementation including physiological dosages of B vitamins improves the status of these nutrients and reduces tHcy in presumed healthy elderly women.

Key words elderly – women – vitamins – folate – vitamin B_6 – vitamin B_{12} – pyridoxine – cobalamin – homocysteine – methylmalonic acid

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Introduction

Numerous studies have shown that elevated blood concentration of the non-proteinogenic amino acid homocysteine is a risk factor for coronary heart disease (CHD) [1, 2]. A meta-analysis of prospective studies indicates that a 25% lower total plasma homocysteine (tHcy) is associated with an 11% lower ischemic heart disease risk and a 19% lower stroke risk [3]. Although the influence of the tHcy concentration on the vascular risk is lower than previously believed [4, 5], it is thought

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that lowering tHcy with vitamin supplementation can significantly reduce the health risk even in a well-nourished population [3].

High tHcy concentrations can be caused by genetic defects [6], but also by a deficiency of the vitamins that are involved in the tHcy metabolism: vitamins B_6, B_{12}, and folate. Hence, the adequate intake of these vitamins (at least RDA) is believed to optimize the tHcy concentration and thereby possibly reduce the risk of CHD. Studies have shown that supplementation with folic acid has the strongest tHcy lowering effect [7]. This can be explained by a widespread poor folate status in the general population. Furthermore, in elderly subjects, impaired vitamin B_6 [8, 9] and vitamin B_2 status [10, 11] seem to be common.

With increasing age the incidence of atrophic gastritis is more frequent, resulting in reduced vitamin B_{12} absorption [12, 13] and elevated tHcy levels. Methylmalonic acid (MMA) may be used to discriminate between folate and cobalamin deficiency. Its serum concentration increases in cobalamin but not in folate deficiency [14]. MMA levels are already elevated in mild, preclinical cobalamin deficiency [12, 15]. Various studies indicate a widespread insufficient B vitamin status as well as elevated tHcy and MMA among the elderly over 65 to 70 years of age [16–18]. Due to the constantly growing percentage of elderly people in the Western society, the issue of recommending vitamin supplements to this specific age group is of special interest to health-care systems. Supplementation could ensure an optimal vitamin intake and thereby lower tHcy concentration and CHD incidence.

Intervention studies with elderly subjects are rare. In published studies the intervention period is mostly very short (3–8 weeks) and frequently only the effect of a monosupplement (e. g. solely folic acid) is investigated.

The aim of this double blind randomized intervention trial was to investigate whether a 6 month multivitamin and mineral supplementation involving physiological dosages of vitamins B_6, B_{12}, and folic acid had an effect on the tHcy and MMA concentration in healthy women older than 60 years of age who did not use supplements or enriched foods.

Materials and methods

Study population

The volunteers were recruited via local newspaper announcements, and posters in various locations in and around the study region of Hanover. The criteria for participation were (1) the volunteer is female since the use of supplements is more common among women [19, 20] and a highly homogenous sample was sought-after, (2) the volunteer is at least 60 years of age at the time of the first blood sampling, (3) the volunteer is a resident of Hanover or environs, (4) the volunteer had not taken vitamin and/or mineral supplements in the 2 months prior to the first blood sampling, (5) drugs that influence vitamin and mineral metabolism are not in use by the volunteer, (6) chronic diseases (e. g. cancer, diabetes) have not been diagnosed. Of the 367 women originally interested, 252 (69 %) completed and returned the first questionnaire. Of these women, 11 had to be excluded since they did not fulfill all study criteria. All subjects gave written informed consent and the study was conducted in accord with the Helsinki Declaration of 1964 as amended in 1996.

Baseline blood samples were taken from 241 women. Due to various reasons 21 volunteers either dropped out or were excluded from the study during the following 6 months prior to the second blood sampling. Hence, the data of 220 women were available for evaluation. Of these, 109 women had been randomly assigned to the placebo group and 111 women to the vitamin group by a lottery. The subjects were instructed to take one capsule per day at breakfast. In order to measure compliance, each woman received a diary in which she had to mark the day she had forgotten to take the capsule. We collected these diaries at the end of the intervention and checked the number of days the subjects had not taken a capsule.

Composition of the vitamin capsule

After the baseline blood samples were drawn, each volunteer received enough capsules for the time period of 6 months. Vitamin capsules (Nobilin Q10®) and placebo capsules were donated by Medicom Pharma AG, Springe (Germany). The vitamin capsule contained 150 mg of vitamin C (calcium ascorbate), 50 mg of magnesium (magnesium carbonate), 36 mg of vitamin E (d-α-tocopherol acetate), 34 mg of niacin (nicotinamide), 16 mg of pantothenic acid, 9 mg of β-carotene, 3.4 mg of pyridoxine (pyridoxine hydrochloride), 3.2 mg of riboflavin, 2.4 mg of thiamine (thiamine mononitrate), 400 µg of folic acid (pteroyl glutamic acid), 200 µg of biotin, 60 µg of selenium (selenium-enriched yeast) and 9 µg of cobalamin (cyanocobalamin). All quantities quoted represent the amount of free vitamin or free mineral. We used this vitamin capsule as representative for this type of product because its content is like many other multivitamin products on the market.

Both capsules, the multivitamin and the placebo, were soft gelatine capsules filled with soy oil. The filling of the placebo capsule was colored for identical appearance.

3-Day dietary record

Prior to (T0) and after 6 months (T6) of intervention the volunteers completed a 3-day dietary record consisting of 142 items, which had been validated for 7-d use [21]. It was slightly modified because of the older age of the study group. In order to record average nutrition, the dietary record was completed from Sunday to Tuesday at T0 and from Thursday to Saturday at T6. Before the study began 7 women over the age of 60 years tested the comprehensibility and handling of the dietary record. The energy and nutrient values were calculated with the software program FOODOPT® (Albat & Wirsam, Linden, Germany), which is based on the German Food Code and Nutrient Data Base (BLS II.2) [22]. For evaluating the correctness of the documented data we calculated the ratio of the reported energy intake (EI) to the estimated basal metabolic rate (BMR) on the basis of individual measured body weight. The FAO/WHO/UNO equation for women of 60–74 years was used to calculate BMR [23].

Questionnaires

The volunteers were asked to complete 3 different questionnaires: (1) a questionnaire covering personal, socioeconomic data as well as questions about health status, (2) a brief questionnaire prior to each blood drawing, asking about recent blood donations and illnesses as well as intake of medicine, (3) a final questionnaire after the intervention period, covering possible changes in the last 6 months.

Blood sampling, analytical methods, and cut-off values

The blood samples (85 ml) were drawn after an overnight fast at baseline and after 6 months of intervention. Anthropometric data were determined on the day of blood sampling as well. For serum preparation blood samples were centrifuged after 20 minutes, when blood was coagulated. For determination of erythrocyte folate EDTA blood was used. Heparin blood was taken for the determination of the activity coefficient of erythrocyte aspartic aminotransferase (EAST) and heparin plasma for the homocysteine measurement. Blood samples were centrifuged at 2665 × g for 10 min at 19 °C. Serum and plasma aliquots for vitamin analysis were stored at –4 °C and transported to the laboratory (Department of Clinical Chemistry of the University of Giessen, Germany) within 5 hours. Serum aliquots for determination of MMA were stored at –20 °C and transported to the Medical Diagnostic Institute of the City Hospital of Karlsruhe, Germany.

Serum and erythrocyte folate concentrations as well as serum cobalamin were measured by automated chemiluminescence system (ACS:180, Chiron Diagnostics, Fernwald, Germany) [24–26]. The stimulation of erythrocyte alpha-EAST by pyridoxal-5'-phosphate (PLP) has been determined as a variable of pyridoxine status. The alpha-EAST activity coefficient increases with vitamin B_6 depletion whereas a low value is indicative of a higher status [27]. THcy was measured in heparin plasma by the IMx assay [28], which is based on fluorescence polarization immunoassay (FPIA) technology [29]. Serum methylmalonic acid was determined by gas chromatography and mass spectrometry (GC-MS) as described previously [30]. All methods used had a CV below 10 %.

Table 1 shows the reference ranges of the laboratory and the cut-off values from literature used in this study to determine insufficient status.

Statistical analysis

Data were analyzed using SPSS 10.0.7 (SPSS Inc., Chicago, Illinois, USA). All food and nutrient intake data have also been transferred from FOODOPT© to SPSS. Except for the nutrient intake, which is shown as mean

Table 1 Cut-off values for the different blood variables

Blood variable	Reference ranges of the laboratory	Literature cut-off values	Reference
alpha-EAST	< 1.8	≥ 1.60	Sauberlich et al. 1972 [27]
Serum cobalamin (pmol/L)	110–664	< 220	Rajan et al. 2002 [17]
Serum folate (nmol/L)	6.8–45.3	< 7	Blount et al. 1997 [56], Sauberlich et al. 1987 [57]
Erythrocyte folate (nmol/L)	340–1020	< 320	Lindenbaum et al. 1994 [52]
tHcy (µmol/L)	< 15	> 10	Ubbink et al. 1995 [58], Jacques et al. 1999 [59], Lucock et al. 1996 [60], Boushey et al. 1995 [4]
MMA (nmol/L)	–	> 271	Omenn et al. 1998 [34]

± standard deviation, data are shown as median and 5–95 percentiles, since most values showed a skewed distribution. Normal distribution of data was checked using the Kolmogorov-Smirnov test. Given normal distribution, the independent-sample t test was used to reveal significant differences between supplement and placebo group. In case of skewness the Mann-Whitney-U test was applied. In order to detect significant differences in the same group at two different times the t test for dependent variables was used in case of normal distribution and the Wilcoxon test, if data were skewed. To identify associations among normally distributed variables, correlations were analyzed with the Pearson method while Spearman correlation coefficients were calculated in case of a skewed distribution.

In order to analyze the weighting of the individual factors influencing the change in tHcy concentrations, a linear regression model was used. Skew-distributed variables were log-transformed and independent variables were included by the stepwise method. *P* values < 0.05 were considered statistically significant.

Results

Anthropometric measurements and 3-day food record

The characteristics of the study population are shown in Table 2. Since there was no significant difference of the documented energy and nutrient intake between the two assessment periods (3-d dietary record at baseline and 3-d after the intervention), the intake values of both dietary records were combined to calculate an average consumption for a total of six days (Table 3). The placebo and supplement group did not differ significantly in the intake of vitamins B_6, B_{12} or folate. According to the calculations from the diet records, more than 50 % of the participating women did not meet the estimated average requirement (EAR) for folate [31].

Table 2 Characteristics of the study population[1]

	Supplement group (n = 111)	Placebo group (n = 109)
Age (y)	63 (60–74)	64 (60–76)
Height (cm)	163 (154–174)	164 (155–174)
Weight (kg)	68.0 (52.2–88.2)	69.0 (54.5–89.5)
BMI (kg/m^2)	25.0 (20.0–33.1)	25.5 (20.4–33.0)

[1] Median (5–95 percentiles)

Table 3 Daily energy intake, EI/BMR, and daily dietary intake of macronutrients and methionine, vitamins B_6, B_{12} and dietary folate equivalents in the study population

Nutrient	Supplement group (n = 105)		Placebo group (n = 108)	
	Mean	SD	Mean	SD
Energy (MJ)	8.09[1]	1.73	8.57	1.78
(kcal)	(1934)[1]	(414)	(2048)	(426)
Energy intake/BMR	1.46	0.29	1.54	0.33
Protein (g)	76.6 (17 energy%)	20.8	80.1 (17 energy%)	18.6
Fat (g)	77.0[1] (35 energy%)	22.6	83.8 (36 energy%)	21.6
Carbohydrate (g)	207 (44 energy%)	46.5	217 (43 energy%)	50.4
Alcohol (g)	11.1 (4 energy%)	11.1	11.0 (4 energy%)	11.8
Methionine (g)	1.6	0.47	1.6	0.55
Vitamin B_6 (mg)	2.0	0.53	2.0	0.60
(µg vitamin B_6/g protein)	26.8	7.1	25.9	5.5
Vitamin B_{12} (µg)	4.9	2.7	5.3	2.7
DFE (µg)	317	86.2	331	93.4

EI energy intake; *BMR* basal metabolic rate; *SD* standard deviation; *DFE* Dietary Folate Equivalents
[1] significantly different from placebo group, $p < 0.05$ (t test)

Vitamin status, tHcy, and MMA prior to and after the intervention period

The baseline and T6 values of both subgroups are shown in Table 4. At baseline, 61.5 % of the study population had an alpha-EAST value less than 1.6. Thus, a poor vitamin B_6 status can be assumed in 84 volunteers (38.5 %). After the intervention the median alpha-EAST value in the supplementation group was significantly lower than at baseline, indicating an improved pyridoxine status. After the intervention period, 19 % of the women in the supplement group had an alpha-EAST value equal to or above 1.6, whereas 59 % of the placebo group had a poor pyridoxine status.

The two subgroups had statistically significant different serum folate concentrations at both baseline and T6 blood sampling. On average, the supplement group already had a 1.9 nmol/L higher serum folate concentration prior to the intervention. Investigating the change in serum folate concentration in each subgroup separately, the placebo group showed no significant change, whereas the average serum folate concentration of the vitamin group was elevated by 38.6 nmol/L (190 %) after the intervention period.

At baseline, 52 (24 %) of the study participants had vitamin B_{12} serum concentrations below the desirable value of 220 pmol/L.

Table 4 alpha-EAST activity coefficients, concentrations of erythrocyte folate, serum folate, serum cobalamin, plasma tHcy as well as serum MMA concentration of both groups prior to (T0) and after intervention (T6)[1]

Blood variable	Supplement group		Placebo group	
	T0	T6	T0	T6
alpha-EAST activity coefficient	1.51 (1.25–2.05) n = 110	1.48[2, 3] (1.32–1.77) n = 111	1.56 (1.21–1.93) n = 108	1.63[4] (1.38–1.98) n = 107
Serum folate (nmol/L)	20.3[6] (12.5–34.3) n = 108	58.9[5, 7] (35.0–89.5) n = 92	18.4 (10.5–30.1) n = 104	18.5 (9.59–43.2) n = 108
Erythrocyte folate (nmol/L)	608 (354–1033) n = 110	1298[2, 7] (952–1761) n = 111	612 (353–961) n = 107	686[7] (392–981) n = 107
Serum cobalamin (pmol/L)	277 (173–494) n = 110	328[5, 7] (205–545) n = 111	264 (182–433) n = 107	253[8] (163–391) n = 108
tHcy (µmol/L)	9.65 (6.86–14.1) n = 110	8.20[5, 7] (6.32–11.6) n = 111	9.40 (6.30–15.9) n = 109	10.1[3] (7.15–16.8) n = 109
Serum MMA (nmol/L)	164 (93.1–313) n = 109	169[9] (104–247) n = 108	159 (102–391) n = 107	174[3] (100–353) n = 107

[1] Values are medians (5–95 percentiles)
[2] Significantly different from placebo group, $p < 0.001$ (t test)
[3] Significantly different from T0, $p < 0.01$ (Wilcoxon test)
[4] Significantly different from T0, $p < 0.01$ (paired t test)
[5] Significantly different from placebo group, $p < 0.001$ (Mann-Whitney-U test)
[6] Significantly different from placebo group, $p < 0.05$ (Mann-Whitney-U test)
[7] Significantly different from T0, $p < 0.001$ (paired t test)
[8] Significantly different from T0, $p < 0.001$ (Wilcoxon test)
[9] Significantly different from placebo group, if data were log-transformated, $p < 0.05$ (t test)

THcy concentrations prior to and after the intervention period and factors associated to the tHcy reduction in the vitamin group

The total study population had a median tHcy concentration of 9.5 µmol/L at baseline. Nine women (4 %) had tHcy ≥ 15 µmol/L and 95 women (43 %) had tHcy values above the desirable limit of 10 µmol/L (Table 4). When evaluating the decrease in tHcy within the supplement group, it became evident that the degree of tHcy reduction was dependent on the baseline concentrations of all three vitamins (vitamins B_6, B_{12} and folate). We found that vitamin supplementation had a greater decreasing effect on the tHcy concentration of volunteers with lower vitamin concentration at baseline. The bivariate correlation coefficients between the change in tHcy concentration (tHcy T6 minus T0) and baseline values of serum folate, erythrocyte folate, cobalamin, and alpha-EAST were $r = 0.547$ ($p < 0.001$) (Fig. 1), $r = 0.302$ ($p = 0.001$), $r = 0.209$ ($p = 0.029$), and $r = -0.263$ ($p = 0.006$), respectively. In a linear regression model, we assigned the change in tHcy concentration (tHcy T0 minus T6) as the dependent variable and baseline values of tHcy, serum folate, serum cobalamin, alpha-EAST activity coefficient, average dietary intake of the three vitamins and methionine as well as the age as independent variables (method stepwise). We determined that baseline tHcy ($p < 0.001$), serum folate ($p = 0.001$), age

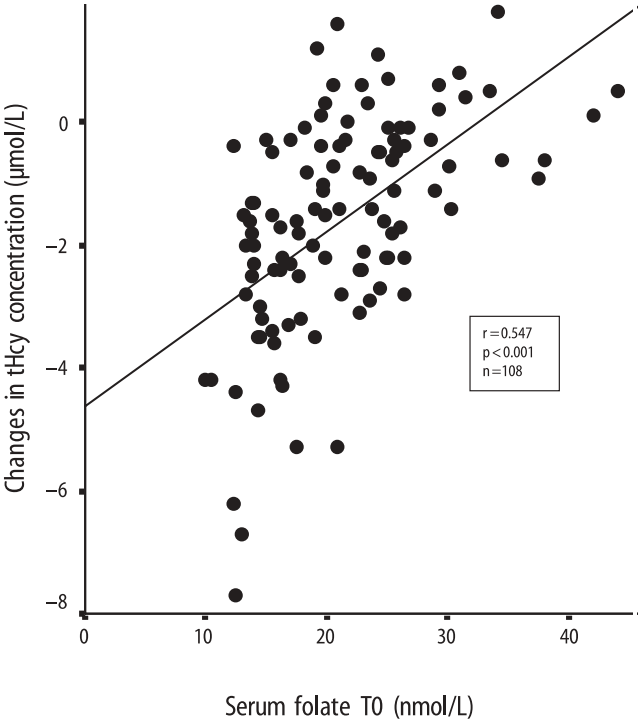

Fig. 1 Association between baseline (T0) serum folate concentration and changes in tHcy concentration (T6 minus T0) in the supplement group

($p = 0.002$) and alpha-EAST activity coefficient ($p = 0.019$) correlated significantly with the change in tHcy ($R^2 = 0.683$, $p < 0.001$).

The degree to which the tHcy concentration changed after the intervention period was dependent on individual tHcy concentration prior to supplementation (Fig. 2). The strongest reduction of the tHcy concentration was seen in the women with the highest baseline tHcy. In the vitamin group, 41 participants had a desirable status of vitamin B_6, vitamin B_{12} and folate (alpha-EAST < 1.6; $B_{12} \geq 220$ pmol/L; MMA ≥ 271 nmol/L; serum folate ≥ 7 nmol/L, erythrocyte folate ≤ 320 nmol/L) at baseline. At this time, the analyzed median tHcy concentration of this particular subgroup was 9.5 µmol/L. After the intervention the tHcy concentration was further reduced by 12.6 % to 8.3 µmol/l ($p < 0.001$).

MMA concentrations and associated variables at baseline and after the intervention period

Baseline MMA concentrations exceeded the cut-off value that we adopted from the literature in 23 women (10.6 %) in the total sample. Out of the 52 study participants who had vitamin B_{12} serum concentrations below 220 pmol/L, only 17 women had MMA concentrations above the cut-off value. On the other hand, out of 23 women exceeding the MMA cut-off value, only 10 had vitamin B_{12} values below 220 pmol/L. The baseline and T6 MMA concentrations were significantly inversely correlated with the corresponding cobalamin concentrations ($r = -0.201$, $p = 0.003$ and $r = -0.161$, $p = 0.018$, respectively). There was a significant positive correlation between baseline MMA concentrations and age ($r = 0.249$, $p < 0.001$) as well as tHcy ($r = 0.229$, $p = 0.001$).

A significant difference in the analyzed T6 MMA concentrations between supplement and placebo group could only be shown if the data were log-transformed and normally distributed (t test). There was a highly significant correlation between the change in MMA concentration (MMA T6 minus T0) and the baseline MMA values in the supplement group ($r = -0.729$, $p < 0.001$) (Fig. 3).

Discussion

Our results show that a combination of physiological dosages of B vitamins can improve the functional status of these vitamins, as judged by tHcy, MMA and alpha-EAST, in presumed healthy elderly women. Since the content of the vitamin capsule used in the present study is like many other multivitamin products on the market, we used it as a representative for this type of products.

The observed decreased vitamin status of the placebo group in the course of the observation period of 6 months may indicate that some participants used supplements until shortly before the intervention trial, and

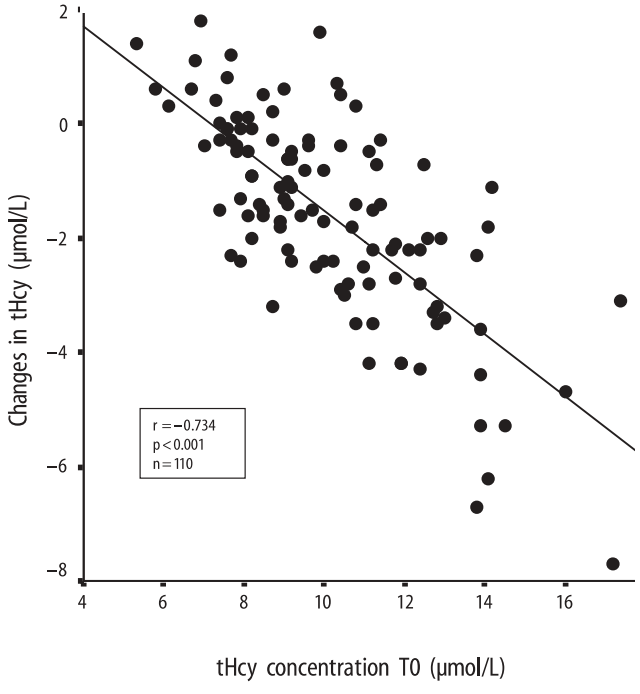

Fig. 2 Association between baseline (T0) tHcy status and changes in tHcy concentration (T6 minus T0) in the supplement group

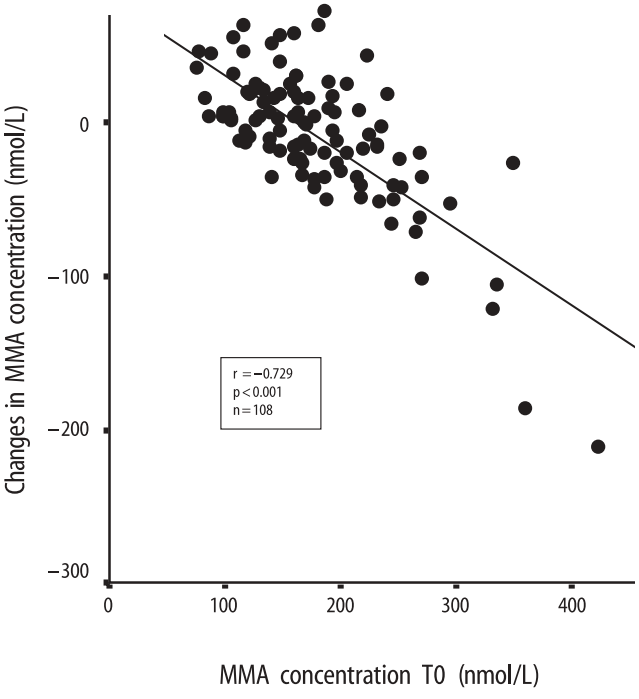

Fig. 3 Association between baseline (T0) MMA status and changes in MMA concentration (T6 minus T0) in the supplement group

thus had a reduced intake during the study period. It may also show that the period of 8 weeks was too short to reduce the vitamin status of those women who used supplements before commencing study participation.

The indicated cut-off values of cobalamin, tHcy and MMA are desirable values (Table 1). Based on results with MMA and tHcy as indicators of cobalamin deficiency, a serum cobalamin cut-off value of < 220 pmol/L (< 300 pg/mL) has been proposed [17, 32]. The comparatively low tHcy limit has been chosen because studies have shown that the risk of cardiovascular disease rises continuously with the tHcy concentrations and may become appreciable at levels > 10 µmol/L [33]. Some authors assumed a relative risk of coronary heart disease mortality of 1.4 for the difference between tHcy levels of > 15 µmol/L compared with levels of < 10 µmol/L after adjustment for other cardiovascular risk factors [34].

The present study shows that 6 month supplementation of a multivitamin preparation containing physiological dosages of vitamins B_6, B_{12} and folic acid can lead to a significant reduction of plasma tHcy in presumed healthy elderly women. In accordance with previous results [7, 35, 36], we observed that the degree to which tHcy concentration decreased depended on individual vitamin status and tHcy level prior to vitamin supplementation. The percentage of tHcy reduction after standardization of the pretreatment blood concentrations of homocysteine of 12 µmol/L and of folate of 12 nmol/L was 26 % (CI 23–29 %) in studies with folic acid dosages of less than 1 mg [36]. In our sample, tHcy was only reduced by 15 % after the intervention. The lower tHcy reduction in our sample compared to the 26 % reduction in the above mentioned study is probably due to the fact that our sample had relatively high pretreatment serum folate and comparatively low tHcy concentrations. The same tHcy reduction of 15 % as in our study was observed in a trial with supplemental 400 µg of folic acid and 6 µg of cobalamin for four weeks in young women aged 20–34 y [37].

In our supplement group, the baseline values of tHcy, serum folate, age and alpha-EAST activity coefficient explained 68 % of the variance in the reduction of tHcy after or during vitamin supplementation. If the additional vitamin B_{12} contributed to the tHcy reduction, it was a small effect and might have been masked by folic acid supplementation. As described previously, with increasing folic acid dosage the dependency of tHcy on folate diminished and cobalamin became the main determinant of plasma tHcy concentration [38].

In contrast to other findings [7], the vitamin B_6 status prior to the study period seems to affect the extent of tHcy reduction in elderly women. This might be explained by the relatively high prevalence of a low B_6 status (38.5 %) in our sample at baseline. In elderly subjects, low vitamin B_6 status is highly prevalent [8, 9] and might be important in risk for CHD. In a prospective cohort study, the lowest CHD risk was found in women with the highest intake of both folate and vitamin B_6 [39].

A reduction of tHcy concentration to 10 µmol/L or lower was achieved in most but not all women of our supplement group. This could have been due to the following: (i) The vitamin B_{12} content of the supplement might have been too low for subjects with poor cobalamin status due to impaired absorption. However, most women in our supplement group had MMA and serum cobalamin concentrations within the normal range after supplementation. (ii) In subjects who are homozygous for the thermolabile MTHFR allele higher folate dosages than in our supplement are required to normalize elevated tHcy [40, 41]. (iii) The content of 3.4 mg of vitamin B_6 in our supplement was shown to normalize the pyridoxine status of most, but not all, women. The fact that nearly 20 % of the supplement group still had a low vitamin B_6 status after the intervention period might be another explanation for the remaining elevated tHcy in some (16 %) women.

Compared to previous investigations in Americans [42, 43], the MMA concentrations of our sample were quite low at both measurements. However, the MMA concentrations in our study population were in the same range as reported for German seniors aged 65–75 y with a median of 186.1 nmol/L [44]. Our findings suggest that MMA was not a reliable predictor of baseline cobalamin status. A correlation coefficient of $r = 0.2$ does not indicate a strong association. Furthermore, subjects with serum cobalamin concentrations below the desirable value were only for a small part identical with subjects exhibiting elevated MMA and vice versa. Carmel and coworkers found elevated MMA in 55.1 % of elderly subjects with cobalamin concentrations below 140 pmol/L, but only in 12.4 % of those with serum cobalamin between 140 and 258 pmol/L [45]. This may indicate that, if already a comparatively high cobalamin cut-off value is used as in our study, the value of MMA as a measurement is lower. Some cases of high MMA concentrations in our sample might have been attributable to impaired kidney function [46], which was not assessed in our study.

In our sample supplementation seemed to have only a weak effect on MMA concentrations. Within the supplement group the median blood concentration did not significantly differ between T0 and T6 measurements. On the contrary, the placebo group showed a significant increase in median MMA concentration after the study period. Whether this increase was prevented in the supplement group by the additional cobalamin or if the increase was due to supplement intake by the placebo group prior to the investigation, leading to a reduced status during the study period, is unclear. The increase in median MMA concentration in the placebo group was

accompanied by a significant decrease in serum cobalamin. The correlation between MMA baseline values and changes in MMA in the supplement group (Fig. 3) showed that in women with high baseline concentrations MMA could be reduced, whereas in women with low baseline values the MMA response seemed to vary at random. This result indicates that (i) the vitamin B_{12} dose was not sufficient to reduce MMA in vitamin B_{12} replete subjects. (ii) Opposite to tHcy, there seems to be a stable low MMA level in vitamin replete subjects which cannot be influenced by vitamin supplementation in physiological doses. This has been shown previously by Rasmussen et al. [47]. (iii) The vitamin B_{12} dosage in the preparation was possibly too low to safely treat all subjects with marginal vitamin B_{12} malabsorption. According to the findings of Seal and coworkers, a dose of 50 or possibly 100 µg/d seems to be more adequate [18].

In a Swedish sample of persons 70 y or older, serum levels of cobalamin, folate, MMA and tHcy were correlated to supplementation of cobalamin and folic acid as well as to additional intake via multivitamin treatment [48]. As observed in our sample serum cobalamin was inversely correlated to MMA. We found only a weak effect of supplementation on MMA values compared with those previously reported for elderly Americans [42, 43].

In the supplement subgroup of our sample, median cobalamin concentration was improved by 18.4 % after intervention. The concentration in the placebo group declined significantly by 4.2 % after the 6 month study period. Elderly people are often subject to poor cobalamin status as a consequence of impaired absorption of the vitamin from food sources due to chronic atrophic gastritis [12, 49]. This explains the absence of any association between cobalamin status and dietary intake as described previously in elderly people [50].

In case of malabsorption, MMA (and other B_{12} deficiency symptoms) can be reduced by intramuscular or oral supplementation of high dosages of cobalamin (about 1 mg) [51, 52]. In a supplementation study with elderly people with poor cobalamin status, 100 µg of vitamin B_{12}/d were additionally consumed for the duration of one month. The serum concentrations were in a normal range in most patients after this time period [53].

Some limitations of the study design have to be considered. Due to the multivitamin preparation it is almost impossible to sort out the effects of single vitamins on tHcy or MMA status or to make dose-finding studies for a specific vitamin. Further other micronutrients of the multivitamin preparation might have influenced tHcy and MMA concentrations. Riboflavin, for example, in the form of flavin adenine dinucleotide (FAD), takes part in homocysteine metabolism as a cofactor of methylenetetrahydrofolate reductase (MTHFR, EC 1.7.99.5). Studies have demonstrated that low dietary intake and low plasma riboflavin concentrations are associated with elevated tHcy [35, 54, 55]. Furthermore, some confounders of tHcy like renal function and coffee were not considered in the study.

Conclusions

The six month supplementation with a multivitamin preparation containing among other micronutrients physiological amounts of vitamins B_6, B_{12}, and folate can significantly reduce the tHcy concentration in women over 60 years of age, and hence may contribute to reduce an assumed risk factor for atherosclerosis. A further tHcy reduction was also observed in subjects with sufficient vitamin B_6, vitamin B_{12} and folate status and median tHcy below the desirable value prior to the intervention. The vitamin B_{12} dose of 9 µg is most likely too low to restore the serum cobalamin concentration to normal in subjects with marginal vitamin B_{12} malabsorption. Our results indicate that in addition to folate and cobalamin, vitamin B_6 may also significantly contribute to lowering tHcy in elderly women. Since poor vitamin B_6 status seems to be highly prevalent in elderly people, supplements can help to achieve the desirable intake of this vitamin as well.

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