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ORIGINAL CONTRIBUTION

Factors affecting the distribution of folate forms in the serum of elderly German adults

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Abstract
Purpose We investigated the roles of age, vitamin B_{12} markers, and the 5,10-methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism as determinants of folate forms in serum.
Methods We measured the serum concentrations of (6*S*)-5-CH_3–H_4folate, (6*S*)-H_4folate, (6*S*)-5-HCO–H_4folate, (6*R*)-5,10-CH^+–H_4folate, and folic acid in 146 non-supplemented older participants (median age 74 years). The concentrations of total vitamin B_{12}, holotranscobalamin (holoTC), methylmalonic acid (MMA), and total homocysteine (tHcy) were also measured.
Results Elevated metabolites (MMA > 271 nmol/L and tHcy > 12.0 μmol/L) were found in 24.0 and 63.0 % of the participants, respectively. We found a significant age-dependent decrease (participants with a median age of 87 years compared with participants with a median age of 60 years) in the sum of serum folate levels, the (6*S*)-5-CH_3–H_4folate concentration, and the (6*S*)-5-CH_3–H_4folate proportion. In addition, participants with elevated metabolite levels were older, had lower concentrations of the sum of folates and (6*S*)-5-CH_3–H_4folate, and had higher concentrations of (6*S*)-5-CHO–H_4folate and creatinine but had a comparable holoTC/total vitamin B_{12} ratio. No association was found between the MTHFR C677T genotype and serum folate forms.
Conclusion Low serum (6*S*)-5-CH_3–H_4folate concentrations and the proportion of (6*S*)-5-CH_3–H_4folate (percentage of the sum of folate forms) are related to older age and elevated MMA and tHcy levels.

Keywords Folate · Folic acid · Age · Elderly · Vitamin B_{12}

Abbreviations

holoTC	Holotranscobalamin
MMA	Methylmalonic acid
MTHFR	5,10-Methylenetetrahydrofolate reductase
tHcy	Total homocysteine
UPLC–MS/MS	Ultra-performance liquid chromatography/tandem mass spectrometry

Introduction

Folates are essential coenzymes in one-carbon metabolism. (6*S*)-5-CH_3–H_4folate is the predominant folate form in blood, comprising 82–93 % of the total folate in human serum [1, 2]. (6*S*)-5-CH_3–H_4folate delivers methyl groups for the methylation of homocysteine (Hcy) to form methionine and (6*S*)-H_4folate. Methionine is the precursor of *S*-adenosylmethionine, the universal methyl group donor in many biological pathways. Folates are also required for the de novo synthesis of purines and thymidylate. The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) converts (6*R*)-5,10-CH_2–H_4folate into (6*S*)-5-CH_3–H_4folate and thus plays a key role in the availability of (6*S*)-5-CH_3–H_4folate, especially when the folate supply is limited. A common

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polymorphism in MTHFR (C677T) might be associated with lower levels of serum (6*S*)-5-CH_3–H_4folate, serum (6*S*)-H_4folate, or (6*S*)-5-CH_3–H_4folate in red blood cells compared with the CC genotype [2, 3].

Folate deficiency can cause neural tube defects [4] and hyperhomocysteinemia [5]. Moreover, folate deficiency is associated with cardiovascular diseases [6, 7] and cancer [8, 9]. Elevated concentrations of total Hcy (tHcy) might be a risk factor or a marker for age-associated diseases [5, 10–12]. The plasma and serum concentrations of total folate and tHcy depend on age. The age-related decrease in the concentration of folate [13] and the physiological decline in renal function explain the increase in tHcy levels in the elderly [13, 14]. However, it is not known whether the methylated folate form that is the direct methyl donor for Hcy is present at a lower concentration in the elderly.

The fortification of staple foods with folic acid is not mandatory in Germany, and the median daily intake of folate from natural foods appears to be below the recommended 400 μg/day (229 μg for women and 276 μg for men) [15]. In the European Prospective Investigation into Cancer and Nutrition (EPIC)–Potsdam study of more than 22,200 apparently healthy participants aged between 33 and 64 years, 50 % of them had an intake of folate below 100 μg/day [16]. Deficiencies in folate and vitamin B_{12} are common in the elderly [13, 17–19], and these deficiencies might be related to a lower intake or bioavailability or to a reduced intestinal absorption of the vitamins. Furthermore, folate metabolism and vitamin B_{12} metabolism are interrelated because vitamin B_{12} is required for converting (6*S*)-5-CH_3–H_4folate into (6*S*)-H_4folate. It has been shown that the supplementation of folic acid plus vitamin B_{12} has a better Hcy-lowering effect than folic acid alone [20]. However, there are no studies showing the distribution of folate forms in the serum of non-supplemented individuals who have elevated tHcy and methylmalonic acid (MMA) levels, which may indicate the vitamin B_{12} and folate status.

The aim of this study was to determine the concentration and the distribution of key folate forms ((6*S*)-5-CH_3–H_4folate, (6*S*)-H_4folate, (6*S*)-5-HCO–H_4folate, (6*R*)-5,10-CH^+–H_4folate, and folic acid) in the serum of older adults in relation to age, the MTHFR C677T genotype, and the concentrations of MMA and tHcy.

Materials and methods

Participants

The study included 146 older adults (median (10th–90th percentiles) age = 74 (58–87) years, 50 males). Study participants were recruited between 2009 and 2010 during a stay in the Geriatric Health Center St. Ingbert, Germany. The inclusion criteria were age >50 years and no acute disease condition requiring hospitalization. The exclusion criteria were a history of renal dysfunction or creatinine >115 μmol/L for women and >133 μmol/L for men [21], a recent stroke or coronary event in the last 3 months, a history of cancer, methotrexate treatment, a history of ileum resection, and current B-vitamin supplementation. The study was approved by the local ethics commission; all of the participants signed informed consent documents. The original trial was registered at clinicaltrials.gov as NCT01105351.

Blood sampling and measurements of metabolites

Fasting blood samples (peripheral venous blood) were collected in tubes containing no anticoagulant. The blood was allowed to clot and was centrifuged within 30 min at 2,000×*g* for 10 min at 4 °C. The serum was separated and immediately frozen at −70 °C until analysis. Blood samples that were collected in tubes containing K^+-EDTA as an anticoagulant were available from 120 participants for genotype analysis. EDTA whole blood was frozen at −70 °C until the isolation of genomic DNA.

The serum concentrations of tHcy and MMA were measured with gas chromatography/tandem mass spectrometry as described by Stabler et al. [22]. The serum vitamin B_{12} concentration was measured with a chemiluminescence immunoassay (ADVIA Centaur System, Bayer, Germany). The level of holoTC in the serum was determined by utilizing a specific monoclonal antibody against holoTC, followed by detection using alkaline phosphatase-labeled anti-transcobalamin (AxSYM, Abbott, Germany) [23]. The concentrations of the folate forms were determined using a previously described ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS) method [2]. In brief, 250 μL of serum was incubated with 50 μL of internal standard solution mix (0.4 μmol/L (6*S*)-5-CH_3–H_4Pte[$^{13}C_5$]Glu, 0.2 μmol/L (6*S*)-5-CHO–H_4Pte[$^{13}C_5$]Glu, 0.4 μmol/L (6*R*)-5,10-CH^+–H_4Pte[$^{13}C_5$]Glu, 0.4 μmol/L (6*S*)-H_4Pte[$^{13}C_5$]Glu, and 0.6 μmol/L Pte[$^{13}C_5$]Glu (Merck Eprova AG, Schaffhausen, Switzerland) and 700 μL of 200 mmol/L ammonium acetate buffer containing 10 g/L ascorbic acid, pH = 10. A sample cleanup was performed using solid phase extraction with OASIS Max columns (Waters Corporation, Milford, MA, USA). The columns were conditioned with methanol and ammonium acetate buffer before sample loading. The wash steps were performed with 5 % NH_4OH solution and methanol. Elution was performed with methanol containing 1 % formic acid. The samples were dried and dissolved in 100 μL of H_2O/methanol (60:40, v/v) containing 0.1 % formic acid and 1 g/L ascorbic acid. The limits of detection

were 0.09 nmol/L for (6*S*)-5-HCO–H_4folate, 0.10 nmol/L for (6*S*)-5-CH_3–H_4folate, 0.15 nmol/L for (6*R*)-5,10-CH^+–H_4folate, 0.20 nmol/L for folic acid, and 0.90 nmol/L for (6*S*)-H_4folate. The recovery rates of the folate forms were between 82.3 and 110.8 %. The between-day coefficient of variation for (6*S*)-5-CH_3–H_4folate was 2.8 % in pooled serum samples. Quality control samples at three different concentrations (80, 25, and 2.5 nmol/L of each folate form) and one pooled serum sample were included in each batch of samples.

The determination of MTHFR C677T genotype was performed by polymerase chain reaction amplification of genomic DNA and pyrosequencing (PSQ 96MA instrument; Biotage AB, Uppsala, Sweden) using primers as previously described [24, 25].

In participants without overt renal dysfunction, concentrations of MMA > 271 nmol/L can indicate a vitamin B_{12} deficiency even when the serum vitamin B_{12} and holoTC levels are normal [26]. A level of tHcy > 12.0 µmol/L might be associated with low vitamin B_{12} or folate [17]. Therefore, we considered a combination of MMA > 271 nmol/L and tHcy > 12.0 µmol/L as a metabolic marker for vitamin B_{12} deficiency or a combination of folate and vitamin B_{12} deficiency.

The data analyses were performed with SPSS (version 18.0; SPSS, Chicago, IL, USA). The data are presented as medians (10th–90th percentiles). A oneway ANOVA and the Tamhane-*t* test were used to test for possible differences in the means of continuous variables between several groups. The differences in continuous variables between two independent groups were tested with the Mann–Whitney-*U* test, and the differences in categorical variables were tested with the χ^2 test. The correlations between the variables were tested with the Spearman-Rho test. All of the tests were 2-sided and p values <0.05 were considered to be statistically significant.

Results

The main characteristics of the study population are presented in Table 1. The median fasting serum concentration of (6*S*)-5-CH_3–H_4folate in this study was in the range found in our earlier study on older adults from Germany [27]. (6*S*)-5-CH_3–H_4folate constituted 87.5 % of the sum of folate forms in the current study. The study population had a median tHcy concentration of 14.1 µmol/L and a median MMA concentration of 221 nmol/L. Elevated concentrations of MMA (>271 nmol/L) were found in 35 participants (24.0 %), and levels of tHcy > 12.0 µmol/L were found in 92 participants (63.0 %). In total, 38 participants had elevated MMA and tHcy levels, and 35 had levels of both markers that were in the normal range.

Table 1 Characteristic and main metabolite levels of the study population in the serum (n = 146)

Variables[a]	
Age, years	74 (58–87)
Creatinine, µmol/L	70.7 (44.2–114.9)
tHcy, µmol/L	14.1 (9.0–28.4)
tHcy > 12.0 µmol/L, *n* (%)	92 (63.0 %)
MMA, nmol/L	221 (133–424)
MMA > 271 nmol/L, *n* (%)	35 (24.0 %)
holoTC, pmol/L[b]	50 (28–117)
Vitamin B_{12}, pmol/L[b]	284 (164–486)
Sum of folate forms, nmol/L	11.87 (4.69–36.90)
(6*S*)-5-CH_3–H_4folate, nmol/L	10.04 (3.42–35.32)
(6*S*)-5-CH_3–H_4folate, % of the sum of folate forms	87.5 % (70.2–96.0 %)
(6*S*)-H_4folate, nmol/L	1.03 (<LOD–3.47)
(6*S*)-H_4folate ≥ 0.91 nmol/L, *n* (%)	88 (60.3 %)
(6*S*)-5-HCO–H_4folate, nmol/L	0.14 (<LOD–0.51)
(6*S*)-5-HCO–H_4folate ≥ 0.10 nmol/L, *n* (%)	122 (83.6 %)
(6*R*)-5,10-CH^+–H_4folate, nmol/L	0.08 (<LOD–0.25)
(6*R*)-5,10-CH^+–H_4folate ≥ 0.16 nmol/L, *n* (%)	70 (47.9 %)
Folic acid, nmol/L	<LOD (<LOD–0.22)
Folic acid ≥ 0.21 nmol/L, *n* (%)	17 (11.6 %)
(6*S*)-5-CH_3–H_4folate/(6*S*)-H_4folate ratio	8.97 (2.84–40.92)

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

[a] The data are medians (10th–90th percentiles) unless otherwise specified. The limits of detection (LOD) were 0.09 nmol/L for (6*S*)-5-HCO–H_4folate, 0.10 nmol/L for (6*S*)-5-CH_3–H_4folate, 0.15 nmol/L for (6*R*)-5,10-CH^+–H_4folate, 0.20 nmol/L for folic acid, and 0.90 nmol/L for (6*S*)-H_4folate

[b] holoTC and vitamin B_{12} concentrations were available from n = 131 participants

Seventeen (11.6 %) of the older adults had detectable concentrations of folic acid (≥0.21 nmol/L) in their serum (Table 1). Compared with older adults with levels of serum folic acid <0.21 nmol/L, adults with serum folic acid ≥0.21 nmol/L showed no significant differences in the median (6*S*)-5-CH_3–H_4folate level (12.70 vs. 9.53 nmol/L; p = 0.357) or the sum of individual folate forms (13.62 vs. 11.62 nmol/L; p = 0.276) (data not shown).

No significant differences in folate forms were observed between males and females (data not shown). We further tested the folate forms distribution in relation to age. The folate forms and metabolite concentrations according to quartiles of age are presented in Table 2. We found significant differences in the concentration of tHcy (p < 0.001), the sum of folate forms (p = 0.003), and the (6*S*)-5-CH_3–H_4folate level (p = 0.001) and its percentage (p = 0.001) among the age quartiles. The youngest group

Table 2 Concentrations of serum folate forms and metabolites in relation to age

Variable[a]	Quartile of age				p	p^b
	1 (lowest)	2	3	4 (highest)		
Number	38	36	35	37	–	–
Age, years	60 (52–64)	71 (68–74)	80 (76–83)	87 (84–91)	–	–
Creatinine, μmol/L	70.7 (53.0–106.1)	70.7 (32.7–126.4)	79.6 (26.5–123.8)	70.7 (47.7–144.9)	0.718	–
tHcy, μmol/L	11.9 (8.0–15.1)	12.6 (9.0–23.2)	18.4 (11.4–39.6)	17.5 (10.2–37.3)	<0.001	<0.001
MMA, nmol/L	209 (120–300)	202 (115–501)	277 (139–511)	242 (138–472)	0.141	0.126
holoTC, pmol/L	50 (26–66)	48 (31–88)	47 (26–128)	61 (28–128)	0.093	0.037
Vitamin B_{12}, pmol/L	306 (189–436)	280 (184–436)	265 (136–641)	266 (165–529)	0.459	0.447
holoTC × 100/vitamin B_{12} ratio	16.7 (11.2–24.8)	17.8 (11.4–29.2)	19.0 (9.9–31.8)	22.0 (12.2–32.5)	0.032	0.041
Sum of folate forms, nmol/L	18.22 (8.55–51.67)	14.85 (6.41–38.89)	8.85 (4.50–22.26)	7.75 (3.28–17.65)	0.003	0.004
(6*S*)-5-CH_3–H_4folate, nmol/L	16.50 (7.63–49.08)	13.72 (4.03–36.26)	7.13 (3.27–19.27)	6.25 (2.62–16.08)	0.001	0.127
(6*S*)-5-CH_3–H_4folate, % of the sum of folate forms	91.8 % (82.4–96.7 %)	89.1 % (72.7–96.7 %)	82.7 % (60.9–92.7 %)	84.5 % (60.6–92.5 %)	0.001	0.002
(6*S*)-H_4folate, nmol/L	1.14 (<LOD–3.12)	1.06 (<LOD–3.60)	1.27 (<LOD–3.86)	0.96 (<LOD–3.02)	0.851	0.460
(6*S*)-H_4folate ≥ 0.91 nmol/L, *n* (%)	23 (60.5 %)	19 (52.8 %)	21 (60.0 %)	25 (75.7 %)	–	–
(6*S*)-5-HCO–H_4folate, nmol/L	0.18 (<LOD–0.50)	0.23 (<LOD–0.65)	0.08 (<LOD–0.47)	0.08 (<LOD–0.67)	0.189	0.093
(6*S*)-5-HCO–H_4folate ≥ 0.10 nmol/L, *n* (%)	30 (78.9 %)	31 (86.1 %)	30 (85.7 %)	31 (83.8 %)	–	–
(6*R*)-5,10-CH^+–H_4folate ≥ 0.16 nmol/L, *n* (%)	17 (44.7 %)	15 (41.7 %)	21 (60.0 %)	17 (45.9 %)	–	–
Folic acid ≥ 0.21 nmol/L, *n* (%)	4 (10.5 %)	3 (8.3 %)	7 (20.0 %)	3 (8.1 %)	–	–

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

[a] The data are medians (10th–90th percentiles) unless otherwise specified. The limits of detection (LOD) were 0.09 nmol/L for (6*S*)-5-HCO–H_4folate, 0.10 nmol/L for (6*S*)-5-CH_3–H_4folate, 0.15 nmol/L for (6*R*)-5,10-CH^+–H_4folate, 0.20 nmol/L for folic acid, and 0.90 nmol/L for (6*S*)-H_4folate. *p* values were calculated using the ANOVA test

[b] *p* values adjusted for creatinine

(median age 60 years) had a significantly higher sum of folates level (2.35 times) and a significantly higher (6*S*)-5-CH_3–H_4folate level (2.64 times) in the serum and a lower tHcy level (0.68 times) than the oldest group (median age 87 years). Participants in the lowest age quartile had a significantly higher median (6*S*)-5-CH_3–H_4folate proportion (percentage of the sum of folate forms) compared with the participants in the third and fourth age quartile (91.8 vs. 82.7 and 84.5 %, respectively: both $p < 0.01$). No significant differences were found in the concentrations of the other folate forms between the age quartiles.

Figure 1 shows the correlation between the serum MMA and tHcy concentrations according to tertiles of (6*S*)-5-CH_3–H_4folate. The majority of participants in the lowest tertile of (6*S*)-5-CH_3–H_4folate had elevated MMA and tHcy levels. Moreover, the majority of participants in the highest tertile of (6*S*)-5-CH_3–H_4folate had tHcy and MMA levels in the reference range. We then compared participants with normal MMA and tHcy levels (MMA ≤ 271 nmol/L and tHcy ≤ 12.0 μmol/L; $n = 35$) with participants with levels of MMA > 271 nmol/L and tHcy > 12.0 μmol/L ($n = 38$; 26.0 % of the study participants) (Table 3). Participants with elevated tHcy and MMA levels were significantly older and had higher creatinine levels compared with participants with normal metabolite levels. In addition, the concentrations of holoTC, total vitamin B_{12}, (6*S*)-5-CH_3–H_4folate, and the sum of folates were significantly lower in the group with elevated tHcy and MMA levels. Moreover, elevated MMA and tHcy levels were associated with lower (6*S*)-5-CH_3–H_4folate proportions (percentage of the sum of folate forms) (median

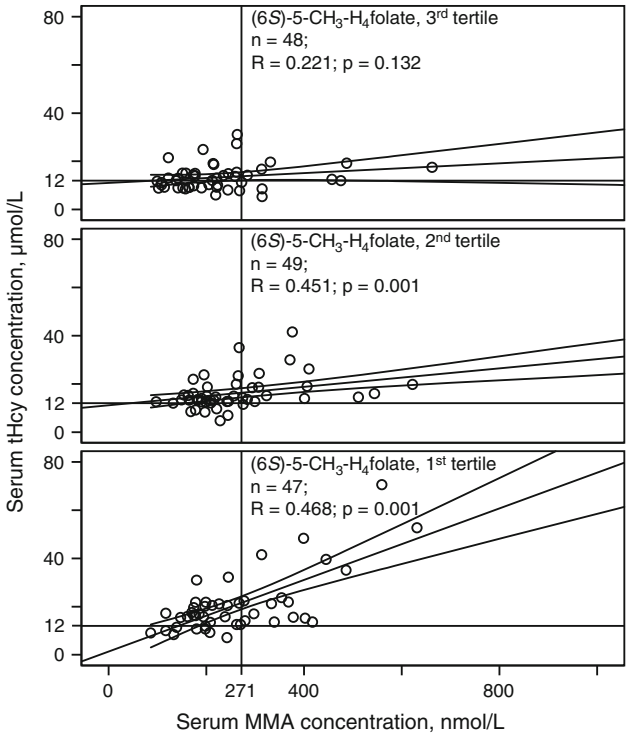

Fig. 1 Correlation of the serum MMA and tHcy concentrations with serum (6*S*)-5-CH_3–H_4folate tertiles in older adults. The means of the (6*S*)-5-CH_3–H_4folate concentrations were 6.10 nmol/L (1st tertile), 13.90 nmol/L (2nd tertile), and 27.30 nmol/L (3rd tertile). The *straight lines* at 271 nmol/L for MMA and 12.0 µmol/L for tHcy represent the cutoff values. *p* values were calculated using the Spearman-Rho test. *MMA* methylmalonic acid, *tHcy* total homocysteine

83.2 vs. 92.6 %) but comparable active vitamin B_{12} proportions (as the percentage of total vitamin B_{12}) and higher (6*S*)-5-HCO–H_4folate concentrations compared with the group with normal MMA and tHcy levels. The ratio of (6*S*)-5-CH_3–H_4folate/(6*S*)-H_4folate was also lower in participants with elevated MMA and tHcy levels compared with participants with normal metabolites (median 6.67 vs. 14.77).

Of the 120 participants with MTHFR C677T genotyping results available, 40.8 % were wild-type (CC), 48.3 % were heterozygous (CT), and 10.8 % were homozygous (TT). No differences in age or the concentrations of folate forms or metabolites in the serum were found between the CC and TT genotypes (Table 4).

Discussion

Low serum concentrations of folate can cause hyperhomocysteinemia and are associated with age-related diseases [5, 10–12]. In this study, we determined the distribution of serum folate forms according to age, MMA and tHcy concentrations, and the common MTHFR C677T polymorphism. The study participants were from Germany, where the voluntary fortification of foods with folic acid is allowed but accounts for a minor, non-significant part of the daily folate intake [15].

The median concentrations of the sum of folates in the serum were comparable to those found in our earlier study [27] but were approximately 50 % lower than the concentrations in populations that regularly consume products fortified with folic acid [28]. As expected, (6*S*)-5-CH_3–H_4folate was the predominant serum folate form in the participants in the current study, which is consistent with earlier results [2, 27]. However, compared with our earlier study [27], we found higher (6*S*)-5-CH_3–H_4folate (current study: 10.0 vs. 6.5 nmol/L) and lower (6*S*)-H_4folate (current study: 1.0 vs. 5.5 nmol/L) concentrations. Our study confirms the inverse association between folate levels and age (lower sum of folates in the fourth age quartile than in the first age quartile) [18] and shows that age is also inversely associated with the serum concentration of (6*S*)-5-CH_3–H_4folate but not with the serum concentrations of other folate forms. The reason for the lower (6*S*)-5-CH_3–H_4folate proportion (percentage of the sum of folate forms) in older individuals is not clear. The lower intake of folate or lower absorption might be associated with a lower sum of folates in older individuals. The differences of (6*S*)-5-CH_3–H_4folate levels with age were partly explained by the renal function. Other possible explanations might be decreasing enzyme activities with age, lower intestinal absorption of (6*S*)-5-CH_3–H_4folate, or vitamin B_{12} deficiency, which is common in older adults and affects the turnover rate of (6*S*)-5-CH_3–H_4folate [29].

Unmetabolized folic acid ($\geq$0.21 nmol/L) was detected in the serum of 17 of the 146 participants (11.6 %), but the presence of unmetabolized folic acid was not associated with significant differences in the concentrations of the sum of folates or (6*S*)-5-CH_3–H_4folate. Unmetabolized folic acid in the serum might be associated with consuming foods fortified with folic acid or supplements [30]. In addition, minor amounts of folic acid might have been formed during sample preparation (most likely during the drying step at 45 °C) as the result of the oxidation of other folate forms, especially (6*S*)-H_4folate [31]. Unmetabolized folic acid showed no age-dependent changes in this study of non-supplemented participants. However, this result might not exclude age-related differences in folic acid metabolism in participants using folic acid supplementation. A higher turnover rate of folate in younger than in older adults has been proposed [32]. The differences in folic acid absorption according to age might be associated with higher gastric pH, atrophic gastritis, a lower turnover rate, or changes in folate enzyme activities in the elderly [32].

Table 3 Serum folate forms and metabolites according to the concentrations of MMA and tHcy

Variable[a]	MMA ≤ 271 nmol/L and tHcy ≤ 12.0 μmol/L	MMA > 271 nmol/L and tHcy > 12.0 μmol/L	*p*	p^d
Number	35	38	–	–
Age, years	66 (56–84)	81 (62–87)	<0.001	<0.001
Creatinine, μmol/L	61.9 (40.7–88.4)	79.6 (53.0–125.5)	0.007	–
tHcy, μmol/L	9.2 (7.1–11.8)	18.8 (13.6–42.3)	<0.001	<0.001
MMA, nmol/L	174 (106–243)	374 (284–623)	<0.001	<0.001
holoTC, pmol/L[b]	54 (39–110)	39 (25–103)	0.002	0.283
Vitamin B_{12}, pmol/L[b]	322 (195–498)	221 (132–434)	0.002	0.020
holoTC × 100/vitamin B_{12} ratio	18.0 (11.4–28.5)	18.2 (10.2–33.2)	0.723	0.424
(6*S*)-5-CH_3–H_4folate, nmol/L	23.70 (6.18–47.74)	10.20 (2.80–26.31)	<0.001	0.030
(6*S*)-5-CH_3–H_4folate, % of the sum of folate forms	92.6 % (78.1–97.1 %)	83.2 % (58.1–91.2 %)	<0.001	0.001
(6*S*)-H_4folate, nmol/L	1.10 (<LOD–2.94)	1.49 (<LOD–4.29)	0.194	0.413
(6*S*)-H_4folate ≥ 0.91 nmol/L, *n* (%)	22 (62.9 %)	28 (73.9 %)	0.450[c]	–
(6*S*)-5-HCO–H_4folate, nmol/L	0.20 (<LOD–0.45)	0.33 (<LOD–0.91)	0.014	0.014
(6*S*)-5-HCO–H_4folate ≥ 0.10 nmol/L, *n* (%)	29 (82.9 %)	33 (86.8 %)	0.748[c]	–
Folic acid ≥ 0.21 nmol/L, *n* (%)	3 (8.6 %)	6 (15.8 %)	0.482[c]	–
(6*S*)-5-CH_3–H_4folate/(6*S*)-H_4folate ratio	14.77 (4.14–58.75)	6.67 (1.60–27.74)	<0.001	0.011

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

[a] The data are medians (10th–90th percentiles) unless otherwise specified. The limits of detection (LOD) were 0.09 nmol/L for (6*S*)-5-HCO–H_4folate, 0.10 nmol/L for (6*S*)-5-CH_3–H_4folate, 0.15 nmol/L for (6*R*)-5,10-CH^+–H_4folate, 0.20 nmol/L for folic acid, and 0.90 nmol/L for (6*S*)-H_4folate. *p* values were calculated using the Mann–Whitney-*U* test unless otherwise specified

[b] holoTC and vitamin B_{12} concentrations were available from $n = 32$ participants in the group with normal MMA and tHcy levels and $n = 33$ participants in the group with low MMA and tHcy levels

[c] *p* values were calculated using the χ^2 test for categorical variables

[d] *p* values adjusted for creatinine

Table 4 Concentrations of the serum folate forms and the metabolites in relation to the MTHFR C677T genotype

Variable[a]	MTHFR 677			*p* (CC vs. TT)
	CC	CT	TT	
Number	49	58	13	–
Age, years	76 (56–88)	73 (58–87)	78 (57–92)	0.508
tHcy, μmol/L	14.1 (8.7–29.6)	14.0 (9.2–24.8)	14.2 (7.2–54.4)	0.815
MMA, nmol/L	199 (123–390)	235 (145–462)	238 (126–540)	0.367
holoTC, pmol/L	49 (25–108)	49 (31–123)	54 (29–81)	0.886
Vitamin B_{12}, pmol/L	259 (163–448)	302 (162–503)	265 (164–370)	0.977
Sum of folate forms, nmol/L	10.78 (4.68–49.81)	13.63 (5.32–37.00)	12.97 (4.86–48.12)	0.306
(6*S*)-5-CH_3–H_4folate, nmol/L	14.70 (4.54–55.60)	14.15 (4.49–33.80)	13.93 (3.34–44.26)	0.949
(6*S*)-5-CH_3–H_4folate, % of the sum of folate forms	89.1 (72.9–96.1)	87.6 (69.6–96.7)	91.4 (54.8–96.1)	0.425
(6*S*)-H_4folate, nmol/L	1.27 (<LOD–4.29)	1.31 (<LOD–3.69)	0.94 (<LOD–3.31)	0.384
(6*S*)-5-HCO–H_4folate, nmol/L	0.20 (<LOD–0.70)	0.24 (<LOD–0.70)	0.40 (<LOD–0.98)	0.131
(6*S*)-5-CH_3–H_4folate/(6*S*)-H_4folate ratio	9.02 (3.26–63.07)	8.30 (2.73–37.13)	14.15 (1.76–183.68)	0.242

holoTC holotranscobalamin, *MMA* methylmalonic acid, *MTHFR* 5,10-methylenetetrahydrofolate reductase, *tHcy* total homocysteine

[a] The data are medians (10th–90th percentiles) unless otherwise specified. The limits of detection (LOD) were 0.09 nmol/L for (6*S*)-5-HCO–H_4folate, 0.10 nmol/L for (6*S*)-5-CH_3–H_4folate, 0.15 nmol/L for (6*R*)-5,10-CH^+–H_4folate, 0.20 nmol/L for folic acid, and 0.90 nmol/L for (6*S*)-H_4folate. *p* values were calculated using the ANOVA test

Our findings demonstrate that elevated tHcy and MMA levels are common in older adults and that elevated tHcy and MMA levels are related to lower serum concentrations of active vitamin B_{12} (holoTC) and (6*S*)-5-CH_3–H_4folate. The concentrations of serum MMA and tHcy were directly related, indicating a decreased amount of Hcy remethylation to methionine when vitamin B_{12} is limited. Interestingly, the proportion of the predominant folate form decreased with increasing MMA and tHcy levels (lower (6*S*)-5-CH_3–H_4folate proportion). However, a lower (6*S*)-5-CH_3–H_4folate proportion was also related to age because participants with elevated tHcy and MMA levels were older than participants with normal metabolite levels. Low vitamin B_{12} levels are common in the elderly and are most likely related to a lower dietary vitamin B_{12} intake or vitamin B_{12} malabsorption. The lower holoTC and (6*S*)-5-CH_3–H_4folate levels in participants with elevated concentrations of these metabolites might suggest a lower intake or absorption of both vitamins. However, this possibility does not explain the reduction in the (6*S*)-5-CH_3–H_4folate proportion. Our results suggest that vitamin B_{12} might play an unknown role in the absorption of (6*S*)-5-CH_3–H_4folate or in the in vivo turnover of folates, which has previously been proposed [33, 34].

Taken together, our results are the first to provide information regarding the concentration of folate forms in the serum according to age in a population of older German adults. We observed a significant decrease in the (6*S*)-5-CH_3–H_4folate concentration and in the (6*S*)-5-CH_3–H_4folate proportion in older adults. Moreover, elevated concentrations of tHcy and MMA were related to lower holoTC, vitamin B_{12}, and (6*S*)-5-CH_3–H_4folate concentrations and a lower (6*S*)-5-CH_3–H_4folate proportion. No association was found between the MTHFR C677T genotype and serum folate forms. Future studies should determine whether improving the vitamin B_{12} status can enhance the formation of the active folate forms ((6*S*)-H_4folate and (6*S*)-5-CH_3–H_4folate).

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Conflict of interest None.

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