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Determinants of Plasma Methylmalonic Acid in a Large Population: Implications for Assessment of Vitamin B_{12} Status

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BACKGROUND: Methylmalonic acid (MMA) in plasma or serum is widely used for assessment of vitamin B_{12} status. However, data are sparse regarding factors, besides renal function, that may influence MMA concentrations. We searched for important determinants of plasma MMA in the general population.

METHODS: In 6946 middle-aged (47–49 years) and elderly (71–74 years) individuals from the Hordaland Homocysteine Study in Norway, we collected anthropometric measurements, lifestyle data, and plasma MMA, vitamin B_{12}, and creatinine measurements. For 5820 individuals, we also collected dietary data.

RESULTS: Age and plasma creatinine were positively associated with plasma MMA, whereas plasma vitamin B_{12} was negatively associated. These variables together with sex were the strongest determinants of plasma MMA, accounting for 16% of the variation ($R^2 = 0.16$). Addition of anthropometric measures and lifestyle and dietary factors only gave slight improvement (total $R^2 = 0.167$). Increased plasma MMA was seen when plasma vitamin B_{12} was <400 pmol/L. In individuals with vitamin B_{12} ≥400 μmol/L (vitamin B_{12}–replete), the 2.5th–97.5th percentile reference limits for MMA were 0.10–0.28 μmol/L (middle-aged) and 0.10–0.36 μmol/L (elderly). When plotted against creatinine (nomograms), the 97.5th percentile of MMA was similar in men and women but approximately 0.15 μmol/L higher in elderly than middle-aged individuals. Vitamin B_{12}–replete participants had MMA upper limits approximately 0.1 μmol/L (elderly) and 0.04 μmol/L (middle-aged) below those of the unselected population at all creatinine concentrations.

CONCLUSIONS: Identified determinants accounted for <17% of the overall variation in plasma MMA. The difference in MMA between middle-aged and elderly individuals is only partly explained by creatinine and vitamin B_{12} concentrations.

Vitamin B_{12} is an essential cofactor for L-methylmalonyl-CoA mutase, which converts methylmalonyl-CoA to succinyl-CoA *(1)*. Impaired activity of L-methylmalonyl-CoA mutase results in the conversion of methylmalonyl-CoA to methylmalonic acid (MMA)[5], which accumulates in blood *(1)*. Increased concentrations of MMA due to low or low-normal vitamin B_{12} concentrations are common in the elderly *(2–5)* and more prevalent than decreased concentrations of vitamin B_{12} *(3–5)*. Normalization or significant reduction of high MMA upon vitamin B_{12} supplementation provides strong evidence for cellular vitamin B_{12} deficiency *(5, 6)*. Thus, increased MMA is considered a specific metabolic marker *(6, 7)* and a more sensitive indicator of functional vitamin B_{12} status than vitamin B_{12} concentration itself *(6, 8, 9)*.

A limitation of MMA as a specific marker of vitamin B_{12} deficiency is that MMA increases in renal dysfunction *(6, 10)*. Previous studies report a strong, independent positive association between plasma MMA and creatinine *(4, 10)*, even within the reference interval for creatinine *(10)*. In the presence of renal failure, vitamin B_{12} supplementation reduces but does not normalize MMA concentrations *(11)*. However, moderate renal dysfunction in the absence of renal failure does not affect MMA as strongly as inadequate vitamin B_{12} status *(3)*. Data on determinants of MMA in the general population are sparse *(2, 10, 12, 13)*. An age-related decline in renal function may compromise the use of MMA for the assessment of vitamin B_{12} status *(14)*. Most laboratories use 1 set of reference intervals,

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[5] Nonstandard abbreviations: MMA, methylmalonic acid; HHS, Hordaland Homocysteine Study; tHcy, total homocysteine; LC-MS/MS, liquid chromatography–tandem mass spectrometry; GAM, generalized additive model.

based on a healthy young reference population without renal impairment. In a small study of vitamin-replete elderly, Lewerin et al. *(15)* established nomograms for MMA according to serum creatinine. In the present study on approximately 7000 individuals, we used the population-based Hordaland Homocysteine Study (HHS) to search for important determinants of MMA and to further evaluate its relation to age and creatinine.

Materials and Methods

STUDY POPULATION

The HHS is a population-based study of 18 043 individuals, recruited from the general population in the county of Hordaland in Western Norway in 1992–93 *(16)*. In 1997–99, 7074 participants from the first HHS were included in the second round of the HHS, which is the basis for the current study. The participants included men and women in 2 age groups: middle-aged (47–49 years) and elderly (71–74 years). All participants underwent a brief health examination and provided a nonfasting blood sample. Information on diet, lifestyle, and medical history was collected via self-administered questionnaires. In this study, we have used 3 data sets: The first data set included participants with plasma MMA, vitamin B_{12}, total homocysteine (tHcy), and creatinine measurements available but excluded participants who had received recent vitamin B_{12} injection, those with plasma vitamin B_{12} ≥3200 pmol/L, or those with tHcy ≥40 μmol/L in the first HHS, leaving 6946 participants. This data set was used in all analyses except those requiring dietary data. A total of 6119 individuals completed a valid food frequency questionnaire *(17)* and were the basis for the second data set, which was restricted to those with plasma variables available as defined above and excluded those with a very low (<3000 kJ for women; <3300 kJ for men) or very high (>15 000 kJ for women; >17 500 kJ for men) daily energy intake, leaving 5820 participants. The third data set included elderly individuals from the second data set who also had holotranscobalamin measurements available. The 3 data sets are described in further detail in Supplemental Table 1S, which accompanies the online version of this article at http://www.clinchem.org/content/vol55/issue12. The study protocol was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate. All individuals gave written consent to participate.

BIOCHEMICAL MEASUREMENTS

Nonfasting plasma samples were collected in tubes containing EDTA and were stored at −80°C. We analyzed plasma concentration of MMA using a modified gas chromatography–mass spectrometry method based on ethylchloroformate derivatization *(18)*, with a between-day CV of <5%. We measured tHcy by automated HPLC with fluorescence detection *(19)* and vitamin B_{12} and holotranscobalamin by *Lactobacillus leichmannii* microbiological assays as described *(20)*. The CVs for tHcy, vitamin B_{12}, and holotranscobalamin measurements were 3%, 7%, and 8%, respectively. We measured creatinine by a modification of a liquid chromatography–tandem mass spectrometry (LC-MS/MS) procedure *(21)*; this method yields creatinine values that are lower than those obtained with the Jaffe photometric assay. The correlation between the Jaffe and LC-MS/MS measurements in our population is given by the following equation: $\text{creatinine}_{\text{Jaffe}} = 36.85\ \mu\text{mol/L} + 0.724 \times \text{creatinine}_{\text{LC-MS/MS}}$ (see online Supplemental Fig. 1S). The *TCN2* (transcobalamin 2) 776C>G polymorphism was determined by real-time PCR *(22)*.

REFERENCE POPULATIONS

We investigated 3 subgroups with different vitamin B_{12} status: the unselected population, reflecting a general population without vitamin B_{12} fortification of foods; a subgroup excluding individuals with low or low-normal vitamin B_{12}, i.e., including only those with vitamin B_{12} ≥200 pmol/L; and a vitamin B_{12}–replete subgroup, defined as individuals with vitamin B_{12} ≥400 pmol/L.

STATISTICAL METHODS

Distributions of plasma MMA, creatinine, and vitamin B_{12} were skewed, and we performed statistical analyses using either nonparametric tests or $\log_{10}$-transformed values. Geometric means are presented with their 95% CIs. All tests were 2-tailed, and $P < 0.05$ was considered significant. Medians and 2.5th–97.5th percentile reference intervals for MMA and creatinine are presented. We used Spearman rank correlation coefficients to assess simple correlations between plasma MMA and characteristics of the population. We used stepwise multiple linear regression analysis to assess the contribution of different variables to plasma MMA. All variables used in regression models were continuous apart from age (middle-aged/elderly) and sex (men/women).

To estimate reference limits for MMA plotted against creatinine, we used the Box–Cox *t*-distribution as a model for MMA explained by creatinine. This distribution has 4 parameters: mean, scale, skewness, and kurtosis. Each parameter is modeled as a smooth nonparametric function of the determining variable creatinine. The Box–Cox *t*-distribution provides a flexible model for skewness and kurtosis and has a formula for centiles. The model was fitted using the Generalized Additive Model for Location Scale and Shape Library *(23, 24)*, as incorporated into the statistical analysis program R *(25)*. We used segmented regression as implemented in R *(25)* to estimate the breakpoints in 2 seg-

Table 1. Characteristics of the study population.[a]

	Men		Women		
	Middle aged	Elderly	Middle aged	Elderly	P^b
n	1641	1434	2043	1828	
Characteristics					
Mean body mass index, kg/m^2 (95% CI)	26.1 (25.9–26.3)[c,d]	26.0 (25.8–26.2)[c]	24.9 (24.7–25.1)[d]	26.3 (26.1–26.5)	<0.001
Current smokers, %	33.1[d]	16.1	33.4[d]	13.6	<0.001
Geometric mean vitamin B_{12} intake, μg/d (95% CI)[e]	7.3 (7.1–7.5)[c,d]	6.8 (6.6–7.0)[c]	5.4 (5.3–5.5)[d]	5.0 (4.9–5.1)	<0.001
Plasma variables					
Geometric mean methylmalonic acid, μmol/L (95% CI)	0.16 (0.16–0.17)[d]	0.20 (0.19–0.20)	0.16 (0.16–0.17)[d]	0.20 (0.20–0.21)	<0.001
Mean creatinine, μmol/L (95% CI)	80 (79–80)[c,d]	86 (85–87)[c]	65 (64–65)[d]	69 (68–69)	<0.001
Geometric mean total homocysteine, μmol/L (95% CI)	10.4 (10.3–10.5)[c,d]	12.5 (12.3–12.7)[c]	8.8 (8.7–8.9)[d]	10.8 (10.7–11.0)	<0.001
Geometric mean vitamin B_{12}, pmol/L (95% CI)	353 (348–358)[c]	335 (328–342)[c]	358 (353–363)	352 (345–358)	<0.001
Geometric mean holotranscobalamin, pmol/L (95% CI)[f]		86 (84–89)[c]		93 (91–95)	<0.001

[a] Analyses are based on data set 1, except vitamin B_{12} intake (data set 2). Middle-aged is defined as 47–49 years old, and elderly, as 71–74 years old.
[b] ANOVA or χ^2 test followed by pairwise comparison with Bonferroni corrections.
[c] $P < 0.001$ between sexes within age groups.
[d] $P < 0.001$ between age groups within sexes.
[e] From natural dietary sources and supplements.
[f] Data available only in elderly men (n = 1331) and elderly women (n = 1742).

mented linear models with vitamin B_{12} as the independent and MMA or tHcy as the dependent variables.

We used gaussian generalized additive model (GAM) or logistic regression model implemented in S-PLUS software (version 8.0; Insightful Corporation) to generate graphic representations of the concentration–response relations between plasma vitamin B_{12}, MMA, and tHcy, after adjustment for age, sex, and creatinine.

Results

SELECTED CHARACTERISTICS

Selected characteristics are listed in Table 1. The geometric means of plasma MMA in the 2 age groups were significantly different but did not differ between sexes, whereas for vitamin B_{12}, significant differences were observed between the sexes but not the age groups. Geometric means for creatinine and tHcy differed between all 4 age–sex groups, being highest in elderly men and lowest in middle-aged women.

UNIVARIATE ASSOCIATIONS

We first investigated whether anthropometric measures, lifestyle, or dietary factors were associated with plasma MMA (online Supplemental Table 2S). Energy, protein, fat, and carbohydrate intake were negatively associated with plasma MMA in the total population but not in the subgroups. Vitamin B_{12} intake and dietary intakes of meat and fish were negatively associated with MMA, whereas dairy products, one of the strongest determinants of plasma vitamin B_{12} in this population *(17)*, were not (data not shown). In all participants and in both age groups, MMA was positively correlated with plasma creatinine and tHcy, although there was a negative association with plasma vitamin B_{12}. Plasma holotranscobalamin, available only in elderly individuals, was negatively associated with MMA, whereas the *TCN2* 776C>G polymorphism did not influence MMA concentrations.

Fig. 1 shows the association between plasma vitamin B_{12} and MMA (A) and tHcy (B). Plasma MMA and tHcy started to increase at vitamin B_{12} concentrations <400 pmol/L in both age groups, but this concentration–response relationship for MMA was more marked in the elderly. The visual threshold observed in the GAM models confirmed the breakpoints found for vitamin B_{12} by segmented regression analyses: 334 (SE 33) pmol/L for MMA and 393 (11) pmol/L for tHcy. Based on these findings, the vitamin B_{12}–replete subgroup was defined as individuals with vitamin B_{12} ≥400 pmol/L.

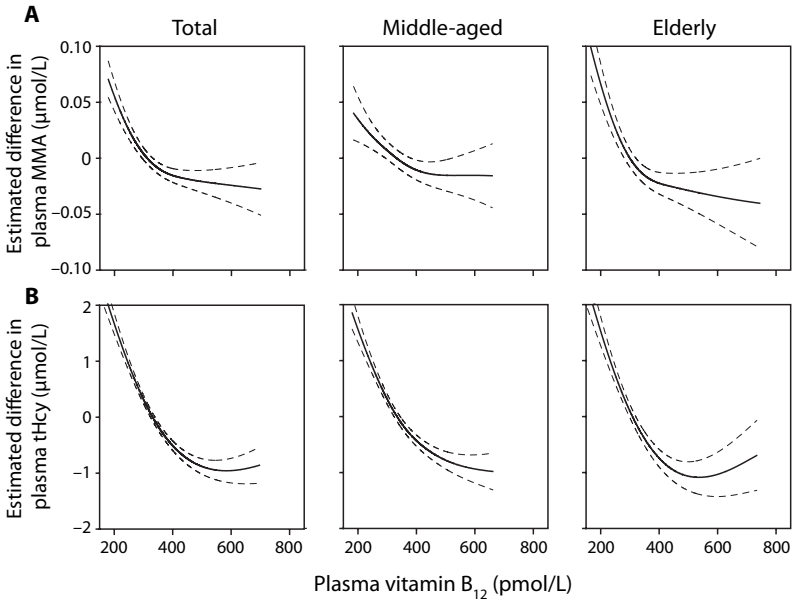

Fig. 1. Concentration–response curves for plasma MMA and tHcy according to plasma vitamin B_{12}.

The unselected population of data set 1 was used. Estimated mean (95% CI) of plasma MMA concentrations according to plasma vitamin B_{12} (A) and plasma tHcy concentrations according to plasma vitamin B_{12} (B). Solid lines show the mean estimated concentration–response curves and the dashed lines the limits of 95% CIs. The lowest and highest 2.5th percentiles of plasma vitamin B_{12} are not included.

We also investigated whether there was a threshold for MMA where the likelihood of finding a low vitamin B_{12} or high tHcy increased. When MMA was examined in relation to the odds of low vitamin B_{12} or raised tHcy as outcome variables, the associations were linear without any apparent thresholds (see online Supplemental Fig. 2S).

MULTIVARIATE ANALYSES

We evaluated the effect of variables significantly associated with MMA in multivariate analyses using a stepwise linear regression model. In the total population, age, plasma vitamin B_{12}, creatinine, and sex were associated with plasma MMA ($P < 0.001$) and explained 16.0% of the variation (R^2), whereas in the elderly subgroup the overall association with the same variables except age was weaker ($R^2 = 12.1\%$, $P < 0.05$). In the middle-aged subgroup, sex contributed less to the total variation of MMA (0.2%), with creatinine and vitamin B_{12} being the strongest determinants ($R^2 = 4.6\%$). On the whole, dietary intake of food items, such as meat and fish, contributed minimally but significantly to plasma MMA, giving a total R^2 of 16.7% for the total population. Additional characteristics, such as smoking and blood characteristics, i.e., total cholesterol and nonfasting glucose, contributed marginally to the variation of MMA. Hence, the 4 important factors that contributed consistently to plasma MMA were age, plasma vitamin B_{12}, creatinine, and sex, in that order. We repeated the analyses on 2774 elderly individuals for whom plasma holotranscobalamin was available (data set 3). Holotranscobalamin was a slightly stronger determinant of plasma MMA than vitamin B_{12} (partial $r = -0.39$, $R^2 = 13.6\%$ for holotranscobalamin; partial $r = -0.30$, $R^2 = 12.1\%$ for vitamin B_{12}). The low R^2 related to the major population determinants for MMA contrasts with plasma tHcy, where age, plasma creatinine, vitamin B_{12}, and folate accounted for 35.7% of the variation in the total population and 24.6% and 30.0% in the middle-aged and elderly, respectively.

PLASMA CONCENTRATION OF MMA IN THE 3 REFERENCE POPULATIONS

When comparing the unselected population with the 2 subgroups with better vitamin B_{12} status, the greatest differences observed were in the highest percentiles of MMA (Table 2). There was a consistent difference between vitamin B_{12}–replete participants and the unselected population in the 97.5th but not the 2.5th percentile. In the elderly, the 97.5th percentile was 26% lower in the vitamin B_{12}–replete subgroup than in the unselected population. A more modest 15% difference was observed in the middle-aged. Furthermore, the 97.5th percentile for plasma MMA differed markedly

Table 2. Median and reference intervals of MMA and creatinine plasma concentrations in different reference populations.[a]

	Unselected population	Vitamin B_{12} ≥200 pmol/L	Vitamin B_{12} ≥400 μmol/L
Middle-aged			
n	3684	3568	1306
MMA, μmol/L	0.16 (0.10–0.32)	0.16 (0.10–0.30)	0.15 (0.10–0.28)
Creatinine, μmol/L	70 (48–101)	70 (49–101)	69 (47–100)
Elderly			
n	3262	3043	1058
MMA, μmol/L	0.19 (0.11–0.49)	0.19 (0.11–0.41)	0.18 (0.10–0.36)
Creatinine, μmol/L	74 (49–121)	74 (49–120)	72 (48–126)

[a] Data are median (2.5th–97.5th percentile). All analyses are based on data set 1.

between middle-aged and elderly participants, even in vitamin B_{12}–replete individuals (0.28 and 0.36 μmol/L, respectively). Plasma creatinine was very similar in the 3 reference populations within each age group.

NOMOGRAMS FOR MMA ACCORDING TO CREATININE

We examined the creatinine–MMA association using nomograms (Fig. 2 and online Supplemental Figs. 3S and 4S). Each panel shows the creatinine–MMA association from the 2.5th to 97.5th percentiles of plasma creatinine, i.e., reflecting the reference interval of creatinine for the general population. For MMA, the curves represent the 2.5th, 50th, and 97.5th percentiles in the 3 defined reference populations. It should be noted that no formal statistical significance can be assigned to the nomograms, because the models do not allow formal testing.

SEX EFFECT

A consistent finding was that in both sexes plasma MMA increased with increasing creatinine, in all 3 reference populations (see online Supplemental Fig. 3S). In general, women had only modestly higher (0.01–0.02 μmol/L) MMA concentrations than men at

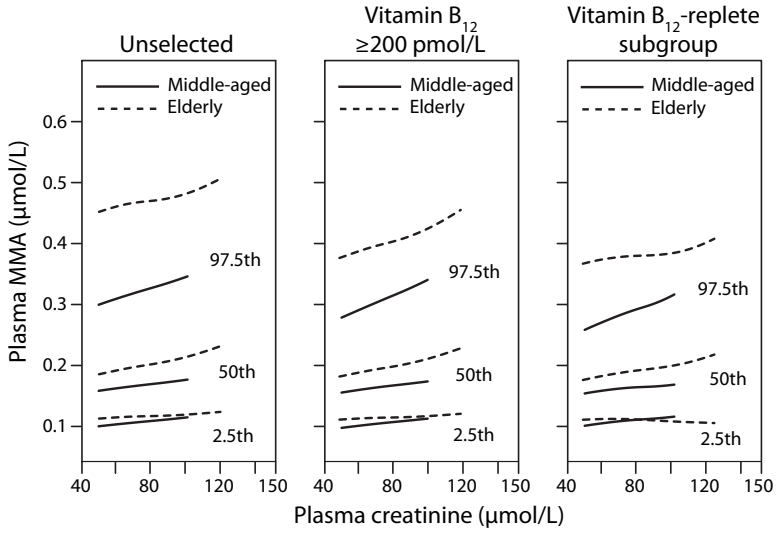

Fig. 2. Nomograms showing plasma MMA vs creatinine according to age.

Data set 1 was used. Plasma MMA 2.5th, 50th, and 97.5th percentiles plotted against creatinine (2.5th–97.5th percentiles). Groups presented are the unselected population (n = 6946), individuals with plasma vitamin B_{12} ≥200 pmol/L (n = 6611), and the vitamin B_{12}–replete subgroup (n = 2364) (individuals with plasma vitamin B_{12} ≥400 pmol/L).

a given creatinine concentration. In subsequent analyses, we have therefore combined men and women to have larger groups.

AGE EFFECT

Fig. 2 shows nomograms for middle-aged and elderly participants from the 3 reference populations. The 97.5th percentile for creatinine was higher in elderly compared to middle-aged participants, and this age-related difference in creatinine may partly explain the higher MMA in the elderly. In all 3 groups, however, the 97.5th percentile of MMA was substantially higher in elderly than in middle-aged participants at a given creatinine concentration, by approximately 0.15 μmol/L in the unselected population and 0.07–0.1 μmol/L in the vitamin B_{12}–replete population. In contrast, the medians of MMA differed by a modest 0.03 μmol/L between the middle-aged and elderly in all 3 subgroups, and the 2.5th percentiles of MMA were almost identical.

EFFECTS OF VITAMIN B_{12} CONCENTRATION

We compared nomograms for the 3 reference populations in the same panel (see online Supplemental Fig. 4S). The 2.5th centile of MMA did not differ among the 3 groups in either age cohort, whereas the vitamin B_{12}–replete group had a marginally lower median compared to the 2 others. The 97.5th percentile for the middle-aged showed an average difference in MMA of approximately 0.04 μmol/L between the unselected and the vitamin B_{12}–replete subgroups at a given creatinine concentration. For elderly individuals, the corresponding difference in MMA was approximately 0.10 μmol/L.

RAISED PLASMA MMA AND IMPAIRED VITAMIN B_{12} STATUS ACCORDING TO DIFFERENT CRITERIA

Table 3 shows the prevalence of low vitamin B_{12} concentrations according to the commonly used cutoffs of 150 and 200 pmol/L and according to the concentration above which MMA and tHcy concentrations start rising in our population, i.e., 400 pmol/L (Fig. 1). The prevalence of increased MMA using the 97.5th percentiles in our vitamin B_{12}–replete subpopulation (Table 2), and in relation to published cutoff values *(3, 26)*, is also presented. In the latter populations, MMA upper limits were not age defined, and we have therefore listed the prevalence before and after excluding individuals with increased creatinine. Table 3 also shows the prevalence of impaired vitamin B_{12} function, defined as increased MMA with low vitamin B_{12}. In online Supplemental Table 3S, we give the geometric means for MMA, vitamin B_{12}, and tHcy in the groups listed in Table 3.

Overall, few participants had low plasma vitamin B_{12}, e.g., 0.4% of the middle-aged and 1.7% of the elderly had vitamin B_{12} <150 pmol/L. Consistent with this find-

Table 3. Prevalence of low plasma vitamin B_{12}, elevated plasma MMA, or impaired vitamin B_{12} status (low vitamin B_{12} and elevated MMA) according to different criteria.[a]

	Middle aged (n = 3684)		Elderly (n = 3262)	
	n	%	n	%
Vitamin B_{12} <150 pmol/L[b]	15	0.4	55	1.7
Vitamin B_{12} <200 pmol/L[b]	116	3.1	219	6.7
Vitamin B_{12} <400 pmol/L[c]	2378	64.5	2204	67.6
MMA >0.21 μmol/L[d]	542	14.7	1197	36.7
excluding high creatinine[e]	512	13.9	1003	30.8
and B_{12} <150 pmol/L	6	0.2	45	1.4
and B_{12} <200 pmol/L	47	1.3	136	4.2
and B_{12} <400 pmol/L	384	10.4	756	23.2
MMA >0.26 μmol/L[f]	197	5.3	578	17.7
excluding high creatinine	184	5.0	470	14.4
and B_{12} <150 pmol/L	4	0.1	40	1.2
and B_{12} <200 pmol/L	28	0.8	100	3.1
and B_{12} <400 pmol/L	150	4.1	370	11.3
MMA >0.37 μmol/L[g]	45	1.2	168	5.2
excluding high creatinine	44	1.2	143	4.3
and B_{12} <150 pmol/L	3	0.1	32	1.0
and B_{12} <200 pmol/L	14	0.3	64	2.0
and B_{12} <400 pmol/L	36	1.0	127	3.9
MMA >0.28 μmol/L (middle-aged) or MMA >0.36 μmol/L (elderly)[h]	144	3.9	187	5.7
and B_{12} <150 pmol/L	5	0.1	34	1.0
and B_{12} <200 pmol/L	28	0.8	69	2.1
and B_{12} <400 pmol/L	119	3.2	162	5.0
MMA >0.75 μmol/L[i]	5	0.1	28	0.9
excluding high creatinine	5	0.1	27	0.8
and B_{12} <150 pmol/L	1	<0.1	16	0.5
and B_{12} <200 pmol/L	2	0.1	19	0.6
and B_{12} <400 pmol/L	4	0.1	22	0.7

[a] All analyses are based on data set 1.
[b] Low vitamin B_{12} concentration, as commonly defined.
[c] The concentration where MMA and tHcy start rising in this population (Fig. 1) (used to identify vitamin B_{12}–replete individuals and not for defining low plasma vitamin B_{12}).
[d] High MMA defined using the CDC's threshold [Pfeiffer et al. *(26)*].
[e] High creatinine, defined as >97.5th percentile in the middle-aged participants (>106 μmol/L for men and >87 μmol/L for women), i.e., a population not expected to suffer from renal impairment.
[f] High MMA defined using the threshold in the local laboratory [Schneede et al. *(12)*].
[g] High MMA as defined in previous US populations [Lindenbaum et al. *(3)*].
[h] High MMA defined using the age-specific 97.5th percentile in the vitamin B_{12}–replete population in the current study (Table 2).
[i] High MMA defined as definite or diagnostic of vitamin B_{12} deficiency [Hvas and Nexo *(6)*, Clarke et al. *(27)*].

ing, markedly increased MMA >0.75 μmol/L (the upper limit used to define definite vitamin B_{12} deficiency *(6, 27)*) was <1% in both age groups. In contrast, with the MMA threshold of 0.21 μmol/L *(26, 28, 29)* after excluding those with increased creatinine, approximately 30% of elderly individuals had increased MMA.

According to the criterion selected, the prevalence of impaired vitamin B_{12} status, i.e., high MMA combined with low vitamin B_{12}, differed just as much: in the elderly it varied from 23% to <1% (Table 3). Using the age-related MMA cutoffs in our study combined with vitamin B_{12} <200 pmol/L, only 0.8% of middle-aged and 2.1% of elderly individuals had impaired vitamin B_{12} status.

Using the 97.5th percentile for the middle-aged as the upper limit for plasma creatinine, increased creatinine concentrations were found in 10% of elderly and 2.4% of middle-aged participants. Among those with markedly increased MMA, i.e., >0.75 μmol/L, high creatinine did not contribute substantially.

Fig. 3 shows the prevalence of increased MMA according to different cutoff points. Increased creatinine became less common as a cause of raised MMA, whereas low vitamin B_{12} concentrations became a relatively more important cause as the MMA cutoff increased. The most striking observation is that a large proportion of increased MMA remained unexplained, except at the highest MMA cutoff.

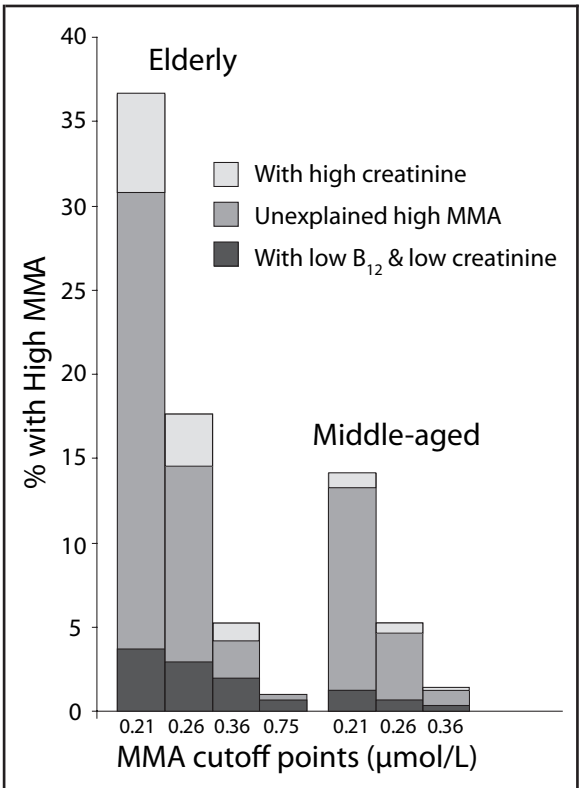

Fig. 3. Prevalence of increased MMA according to different MMA cutoff points.

Data set 1 was used. High plasma creatinine was defined as >97.5th percentile in the middle-aged individuals (>106 μmol/L for men and >87 μmol/L for women), i.e., a population not expected to suffer from renal impairment. Low plasma vitamin B_{12} was defined as <200 pmol/L.

Discussion

In this study of approximately 7000 individuals, we confirmed a positive correlation of plasma MMA with creatinine and older age, whereas high plasma vitamin B_{12} was associated with lower concentrations of MMA. We did not find other factors that had major effects on MMA concentrations. Our study shows that differences in plasma creatinine or vitamin B_{12} cannot fully explain the difference in MMA between the age groups. Furthermore, the determinants that we have been able to identify explained <17% of MMA variation in the total population, but markedly less in the middle-aged. Replacing plasma vitamin B_{12} with plasma holotranscobalamin measurements in the elderly only modestly changed the results.

The geometric mean of plasma MMA and the prevalence of raised MMA in our population were low compared to those in other published studies *(3, 10, 30, 31)*. One probable explanation is the good vitamin B_{12} status in our participants, even among the elderly. This may be related to their high dietary intake of milk and fish, i.e., products where vitamin B_{12} seems to be particularly bioavailable *(17)*.

The variation of MMA with creatinine confirms that it is important to take creatinine concentrations into account when interpreting MMA values close to the upper reference limits. Several studies have excluded people with increased creatinine *(4, 5, 30, 31)* and thus use a fairly low MMA cutoff. Rather than exclude the proportion with increased creatinine (approximately 10% among our elderly), we examined how creatinine within the reference interval influenced the mean and distribution of MMA. The upper limit of plasma MMA increased by approximately 0.05 μmol/L in the elderly and approximately 0.1 μmol/L in the middle-aged between the 2.5th and 97.5th percentiles of creatinine in vitamin B_{12}–replete individuals. Thus, the upper limit is not a single value, but a range that depends on creatinine. One important finding is that the age-related increase in creatinine explains only a modest part of high MMA in elderly. The relation between creatinine and MMA is steeper in the middle-aged than in the elderly, at least for the 97.5th percentile in the vitamin B_{12}–replete population (Fig. 2).

When comparing the unselected population with the vitamin B_{12}–replete subgroup, we observed the

largest differences for plasma MMA in the highest percentiles and in the elderly; the 97.5th percentile was approximately 26% lower in the vitamin B_{12}–replete subgroup compared to the unselected population. Thus, estimation of the upper reference limits for MMA without excluding those with low vitamin B_{12} concentrations may result in falsely high threshold values due to a high prevalence of low vitamin B_{12} status in the elderly *(32)*. On the basis of the Fig.-1 concentration-response curves, which relate plasma vitamin B_{12} to plasma MMA and tHcy, we suggest that the reference population could be confined to individuals with vitamin B_{12} concentrations ≥400 pmol/L. However, this threshold cannot be used to define vitamin B_{12} deficiency, since more than 60% of our population with good vitamin B_{12} status would then be defined as deficient.

Our findings indicate that a higher cutoff point for plasma MMA exists in vitamin B_{12}–replete elderly individuals, compared with middle-aged individuals at a given creatinine concentration. Thus, when creatinine and vitamin B_{12} are taken into account, age still affects the upper limit. The use of cutoff values established on younger populations, for instance the CDC's cutoff of 0.21 μmol/L *(26)*, will probably result in an overestimation of the prevalence of vitamin B_{12} deficiency in the elderly. Among our elderly participants, such a cutoff defined approximately 30% as having high MMA concentrations, whereas only 3.9% of these had vitamin B_{12} concentrations <200 pmol/L, a concentration often used to indicate impaired vitamin B_{12} status, leaving the vast majority with unexplained high MMA (Fig. 3).

We could not identify factors other than age, creatinine, vitamin B_{12}, and sex that substantially influenced plasma MMA. Plasma creatinine alone cannot account for the age-related increase in MMA. It is possible that creatinine may underestimate impaired renal function in elderly individuals since it is not a particularly good marker of glomerular filtration rate *(33)*. It can be debated whether reduction in glomerular filtration rate is a likely explanation for higher MMA with age, given that the age effect is present even at very low creatinine concentrations (Fig. 2).

Other factors—such as intravascular volume depletion *(34)*, inherited methylmalonic aciduria, and increased production of propionic acid from bacterial overgrowth in the intestine or increased catabolism of MMA precursors like cholesterol, branched-chain amino acids, and odd-chain fatty acids *(35)*—may also influence MMA concentrations. Equally, antibiotic treatment suppresses anaerobic gut flora and lowers MMA *(8)*. Common polymorphisms influencing MMA concentrations without changing plasma vitamin B_{12} so far have not been reported.

The 2.5th–97.5th percentiles in vitamin B_{12}–replete individuals were 0.10–0.28 μmol/L in middle-aged and 0.10–0.36 μmol/L in elderly participants. The reference intervals for the elderly agree with the findings of previous investigations *(36, 37)*. However, there is no consensus regarding appropriate cutoff points for increased MMA for the diagnosis of vitamin B_{12} deficiency; reported figures range from 0.21 to 0.75 μmol/L *(6, 26, 27, 38)*. Reference intervals estimated after supplementation with vitamin B_{12}, thus ensuring adequate vitamin B_{12} status *(36, 39, 40)*, have yielded cutoff points close to those observed in our vitamin B_{12}–replete population. This wide range of cutoffs for MMA is probably related to differences in the selection of reference populations and criteria for defining vitamin B_{12} deficiency. Some studies have not made a distinction between age groups or have used younger populations as the reference population *(5, 26)* and, thus, have found significantly lower reference intervals for MMA than observed in our study. Our findings suggest that independent of identified cutpoints for MMA, it is necessary to take into account factors other than just vitamin B_{12} status, creatinine, and age.

Conclusions

In our population, plasma MMA was strongly associated with age, plasma vitamin B_{12} status, and creatinine. Overall, <17% of the variation in MMA was explained by identified variables in this cohort. If these results apply to other general adult populations, then caution is required in interpreting MMA values, particularly in younger people.

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