Screening for vitamin B-12 and folate deficiency in older persons[1–3]

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ABSTRACT

Background: Vitamin B-12 deficiency is usually accompanied by elevated concentrations of serum total homocysteine (tHcy) and methylmalonic acid (MMA). Folate deficiency also results in elevated tHcy. Measurement of these metabolites can be used to screen for functional vitamin B-12 or folate deficiency.

Objective: We assessed the prevalence of vitamin B-12 and folate deficiency in a population-based study (n = 1562) of older persons living in Oxford City, United Kingdom.

Design: We postulated that, as vitamin B-12 or folate concentrations declined from adequate to impaired levels, tHcy (or MMA) concentrations would increase. Individuals were classified as being at high risk of vitamin B-12 deficiency if they had low vitamin B-12 (< 150 pmol/L) or borderline vitamin B-12 (150–200 pmol/L) accompanied by elevated MMA (> 0.35 μmol/L) or tHcy (> 15.0 μmol/L). Individuals were classified as being at high risk of folate deficiency if they had low folate (< 5 nmol/L) or borderline folate (5–7 nmol/L) accompanied by elevated tHcy (> 15 μmol/L).

Results: Cutoffs of 15.0 μmol/L for tHcy and 0.35 μmol/L for MMA identified persons with normal or elevated concentrations. Among persons aged 65–74 and ≥75 y, respectively, ≈10% and 20% were at high risk of vitamin B-12 deficiency. About 10% and 20%, respectively, were also at high risk of folate deficiency. About 10% of persons with vitamin B-12 deficiency also had folate deficiency.

Conclusion: Use of tHcy or MMA among older persons with borderline vitamin concentrations may identify those at high risk of vitamin B-12 deficiency who should be considered for treatment. *Am J Clin Nutr* 2003;77:1241–7.

KEY WORDS Screening, folate, vitamin B-12, homocysteine, methylmalonic acid, elderly

INTRODUCTION

The clinical presentation of vitamin B-12 deficiency varies considerably and rarely includes all the classic features, such as macrocytic anemia, peripheral neuropathy, and subacute combined degeneration of the spinal cord (1). More typically, vitamin B-12 deficiency presents as nonspecific symptoms of fatigue, lassitude, malaise, vertigo, and cognitive impairment that could be attributed to old age. Moreover, the clinical severity of vitamin B-12 deficiency is unrelated to vitamin B-12 concentrations, reflecting the limitations of standard vitamin B-12 assays (1, 2).

Folate deficiency also causes macrocytic anemia but may have neurologic features that differ from those of vitamin B-12 deficiency. Accurate identification of vitamin B-12 deficiency is important because inappropriate treatment with folic acid will correct the hematologic signs of vitamin B-12 deficiency but leave the neurologic symptoms unaltered (3).

Both vitamin B-12 and folate are involved in a common metabolic pathway supplying essential methyl groups for DNA and protein synthesis (2). Vitamin B-12 acts as a cofactor for methionine synthase, the enzyme that remethylates homocysteine to methionine by using 5methyltetrahydrofolate as a methyl donor. Deficiency of either folate or vitamin B-12 results in increased serum total homocysteine (tHcy) concentrations. Vitamin B-12 also acts as a cofactor for methylmalonyl-CoA mutase, which converts methylmalonyl-CoA to succinyl-CoA; hence, deficiency of vitamin B-12 results in elevated serum concentrations of methylmalonic acid (MMA). Consequently, elevated concentrations of MMA have been suggested to indicate vitamin B-12 deficiency, whereas elevated concentrations of tHcy may indicate either vitamin B-12 or folate deficiency (2, 4–7). There is no consensus, however, on the cutoffs of tHcy or MMA to use to define vitamin B-12 deficiency in an elderly population in which impaired renal function can be an important confounding factor. The aims of this study were *1)* to determine the prevalence of individuals at high risk of vitamin B-12 deficiency, *2)* to determine the prevalence of individuals at high risk of folate deficiency, and *3)* to compare the utility of tHcy with that of MMA when used together with vitamin assays to identify persons at high risk of vitamin B-12 deficiency defined by borderline serum concentrations of vitamin B-12 and elevated metabolite concentrations.

SUBJECTS AND METHODS

Study population

The Oxford Healthy Aging Project was a component of the Medical Research Council Cognitive Function and Aging Study

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TABLE 1
Selected characteristics of the study population by age and sex[1]

	Men		Women		*P* value		
	<75 y (*n* = 285)	≥75 y (*n* = 333)	<75 y (*n* = 354)	≥75 y (*n* = 530)	Sex	Age	Interaction
Age (y)	71.8 ± 2.0	81.8 ± 4.6	71.9 ± 2.0	82.0 ± 4.9	—	—	—
Folate (nmol/L)	16.6 ± 14.5	14.5 ± 13.2	16.2 ± 13.2	15.9 ± 15.1	0.48	0.11	0.26
Vitamin B-12 (pmol/L)	262 ± 92	255 ± 161	298 ± 119	272 ± 130	0.0001	0.02	0.15
Homocysteine (μmol/L)	13.1 ± 4.2	16.4 ± 7.4	12.2 ± 4.6	15.6 ± 7.0	0.1	<0.0001	0.90
MMA (μmol/L)	0.30 ± 0.21	0.42 ± 0.41	0.29 ± 0.18	0.40 ± 0.33	0.37	<0.0001	0.79
Creatinine (μmol/L)	102.2 ± 17.9	110.3 ± 24.0	91.7 ± 18.0	96.7 ± 22.5	<0.0001	<0.0001	0.15
Hemoglobin (g/L)	141 ± 13	137 ± 13	132 ± 11	127 ± 13	<0.0001	<0.0001	0.39
MCV (fL)	91.0 ± 5.1	90.1 ± 5.1	89.6 ± 5.0	89.3 ± 5.9	<0.0001	0.37	0.97

[1] $\bar{x}$ ± SD. MMA, methylmalonic acid; MCV, mean corpuscular volume.

carried out in 6 centers throughout Great Britain (8). The study population involved a random sample of 2740 persons who were residents of Oxford City and were aged ≥65 y when first examined between 1991 and 1994. The sample was drawn from general practice registers to provide equal numbers of individuals aged 65–74 and ≥75 y. All participants took part in a structured interview in their own homes. The data collected included information on medical history (including pernicious anemia), smoking habits, use of multivitamin supplements, and other aspects of health and lifestyle. Blood samples were collected from 1670 persons (68% of the survivors; 219 subjects died before blood samples were obtained) ≈6–12 mo after the interview. A blood sample was collected into a plain evacuated serum tube for vitamin and metabolite assays and into an additional evacuated tube containing EDTA for a full blood count. Laboratory data were incomplete for 108 persons, leaving valid data for 1562. The blood samples were transported to the laboratory in an insulated box, in which samples were stored at −20 °C for 0.5–2 h before centrifugation. Whole blood samples were centrifuged immediately (3000 × *g*, 10 min, 24 °C) on arrival in the laboratory, and serum samples were stored at −80 °C. All participants provided their written informed consent to take part in the study, and the Central Oxford Research Ethics Committee approved the study protocol, including the blood collection.

Laboratory methods

Serum tHcy concentrations were measured by using a fluorescence polarization immunoassay (FPIA; AXIS-Shield, Oslo) and an Abbott IMx autoanalyzer (Abbott Laboratories, Chicago) in Oxford (9). The CV for the FPIA tHcy assays (10) was <3.5%. The laboratory participated in an external quality-control scheme, and the tHcy determinations for the Oxford laboratory were consistently within 95% of the mean (11). Repeat tHcy determinations were made for a sample of 400 individuals by gas chromatography–mass spectrometry (GCMS) at the University of Bergen (12), and the correlation coefficient between the GCMS and FPIA tHcy assays was 0.99. MMA assays were carried out by GCMS at the University of Bergen (12). Serum folate was measured by a microbiological method at Trinity College Dublin (13). Vitamin B-12 determinations were made by a competitive protein binding assay at Aarhus University Hospital, Denmark, on an ACS Centaur with an automated chemiluminescence detection system (Bayer A/S, Tarrytown, NY) that had an analytic imprecision of <10%.

Individuals were classified as being at high risk of vitamin B-12 deficiency if they had low vitamin B-12 (<150 pmol/L) or borderline vitamin B-12 (150–200 pmol/L) concentrations accompanied by elevated MMA (>0.35 μmol/L) or elevated tHcy (>15 μmol/L) concentrations. Individuals were classified as being at high risk of folate deficiency if they had low folate (<5.0 nmol/L) or borderline folate (5–7 nmol/L) concentrations accompanied by elevated tHcy concentrations (>15 μmol/L; *see* Discussion). Anemia was defined as hemoglobin concentrations <130 g/L in men and <120 g/L in women.

Statistical analysis

Continuous variables are presented as means and SDs. Differences in the mean values of biochemical indexes were compared by analysis of covariance or tests for linear trend across groups. Associations of tHcy or MMA with age, sex, folate, vitamin B-12, and creatinine were assessed by analysis of covariance with the general linear models in SAS for PC, version 8.1 (SAS Institute Inc, Cary, NC).

RESULTS

Blood concentrations of vitamins and metabolites

Among the 1562 persons examined, 33 (2%) who had self-reported pernicious anemia were excluded. Only 17 persons reported having vitamin B-12 injections at the time of blood collection, including 12 with pernicious anemia, and these subjects were excluded. Four persons with extreme elevations of serum vitamin B-12 ranging from 10 000 to 40 000 pmol/L were also excluded from the analyses. Selected characteristics of the study population are shown separately by age and sex in **Table 1**. The mean serum concentrations of tHcy, MMA, and creatinine were higher in the older age group than in the younger age group ($P < 0.001$). Mean vitamin B-12 concentrations were lower in men than in women ($P < 0.001$), and mean creatinine and hemoglobin concentrations were higher in men than in women ($P < 0.001$). There were no significant age-by-sex interactions for vitamin or metabolite concentrations.

tHcy and MMA according to folate, vitamin B-12, and creatinine concentrations

Shown in **Table 2** are the mean serum values of folate, vitamin B-12, tHcy, MMA, and creatinine according to quintiles of folate, vitamin B-12, and creatinine in men and women separately. The absolute differences in mean tHcy concentrations for the highest (quintile 5) and lowest (quintile 1) quintiles of folate were of

TABLE 2
Distribution by sex of mean concentrations of serum folate, vitamin B-12, total homocysteine (tHcy), methylmalonic acid (MMA), and creatinine by quintiles of serum folate, vitamin B-12, and creatinine[1]

	Folate (nmol/L)		Vitamin B-12 (pmol/L)		tHcy (μmol/L)		MMA (μmol/L)		Creatinine (μmol/L)	
	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women
Quintiles of folate										
1	4.9 ± 1.3	5.1 ± 1.3	246 ± 89	264 ± 107	18.0 ± 8.9	16.1 ± 6.4	0.39 ± 0.35	0.36 ± 0.28	104 ± 23	93 ± 23
2	8.0 ± 0.7	8.2 ± 0.8	282 ± 235	273 ± 114	15.3 ± 6.8	15.6 ± 8.3	0.36 ± 0.33	0.37 ± 0.34	107 ± 24	95 ± 24
3	11.2 ± 1.2	11.4 ± 1.1	263 ± 105	283 ± 100	14.2 ± 4.0	14.0 ± 5.3	0.39 ± 0.48	0.31 ± 0.18	106 ± 18	95 ± 19
4	16.2 ± 2.0	17.1 ± 2.3	246 ± 73	271 ± 87	14.0 ± 4.5	13.3 ± 5.6	0.34 ± 0.22	0.35 ± 0.26	107 ± 21	95 ± 20
5	36.8 ± 18	39.5 ± 22.1	260 ± 89	326 ± 186	12.8 ± 4.7	12.4 ± 4.9	0.32 ± 0.23	0.37 ± 0.31	108 ± 21	95 ± 19
Difference (5 − 1)	31.9	34.4	14	62	−5.2	−3.7	−0.07	0.01	4	2
P for trend	—	—	0.10	<0.001	<0.001	<0.001	0.03	0.36	0.17	0.14
Quintiles of vitamin B-12										
1	14.8 ± 13	14.0 ± 11.1	148 ± 23	156 ± 29	18.2 ± 9.7	17.0 ± 8.6	0.53 ± 0.50	0.51 ± 0.44	106 ± 24	93 ± 25
2	14.4 ± 15.3	15.2 ± 16.7	200 ± 11	215 ± 12	14.4 ± 4.4	14.9 ± 5.8	0.32 ± 0.17	0.35 ± 0.21	102 ± 58	94 ± 18
3	14.9 ± 11.9	14.7 ± 10.3	240 ± 12	260 ± 15	14.6 ± 4.9	14.4 ± 6.6	0.37 ± 0.36	0.33 ± 0.25	108 ± 19	96 ± 22
4	16.8 ± 12.8	16.4 ± 13.1	285 ± 17	317 ± 19	13.8 ± 4.9	12.9 ± 4.4	0.29 ± 0.16	0.30 ± 0.22	108 ± 23	96 ± 20
5	16.1 ± 15.4	20.1 ± 22.9	424 ± 207	467 ± 145	13.4 ± 4.6	12.2 ± 4.3	0.29 ± 0.30	0.27 ± 0.10	108 ± 23	94 ± 19
Difference (5 − 1)	1.3	6.1	276	311	−4.8	−4.8	−0.24	−0.24	2	1
P for trend	0.15	<0.001	—	—	<0.001	<0.001	<0.001	<0.001	0.16	0.62
Quintiles of creatinine										
1	15.0 ± 15.3	13.3 ± 10.4	252 ± 98	280 ± 122	12.3 ± 4.6	11.3 ± 4.3	0.33 ± 0.33	0.26 ± 0.14	80 ± 8	71 ± 9
2	15.9 ± 14.6	19.0 ± 19.3	245 ± 77	279 ± 106	13.4 ± 4.5	12.9 ± 4.6	0.33 ± 0.34	0.33 ± 0.22	94 ± 3	84 ± 2
3	15.0 ± 12.2	16.0 ± 14.7	252 ± 79	281 ± 122	13.9 ± 4.1	13.5 ± 6.4	0.34 ± 0.36	0.33 ± 0.27	104 ± 3	92 ± 2
4	16.1 ± 11.8	17.3 ± 16.6	262 ± 112	300 ± 140	16.1 ± 6.2	14.7 ± 4.2	0.34 ± 0.18	0.36 ± 0.19	114 ± 3	101 ± 3
5	15.0 ± 12.0	15.7 ± 12.1	286 ± 219	275 ± 105	18.7 ± 7.9	19.0 ± 7.7	0.46 ± 0.35	0.48 ± 0.41	139 ± 18	125 ± 20
Difference (5 − 1)	0	2.4	34	−5	6.4	7.7	0.13	0.22	59	54
P for trend	0.5	0.08	0.21	0.74	<0.001	<0.001	<0.001	<0.001	—	—

[1] $\bar{x}$ ± SD. *n* = 631 men and 903 women.

approximately similar magnitude as the corresponding absolute difference between the highest (quintile 5) and lowest (quintile 1) quintiles of vitamin B-12. The absolute differences in mean tHcy concentrations between the highest and lowest quintiles of creatinine were greater than those for either of the vitamins. The absolute differences in mean MMA concentrations between the highest and lowest quintiles were greater for vitamin B-12 than for creatinine.

Cutoffs for elevated tHcy and MMA

The distribution of both tHcy and MMA in the overall population and in the subset with serum creatinine concentrations < 100 μmol/L (*n* = 842) is shown in **Figure 1**. The distribution of both metabolites was shifted down in the subset with creatinine concentrations < 100 μmol/L. In addition, the 50th, 75th, and 95th percentiles are shown for tHcy and for MMA in the overall population (*n* = 1502) and in a subset with normal renal

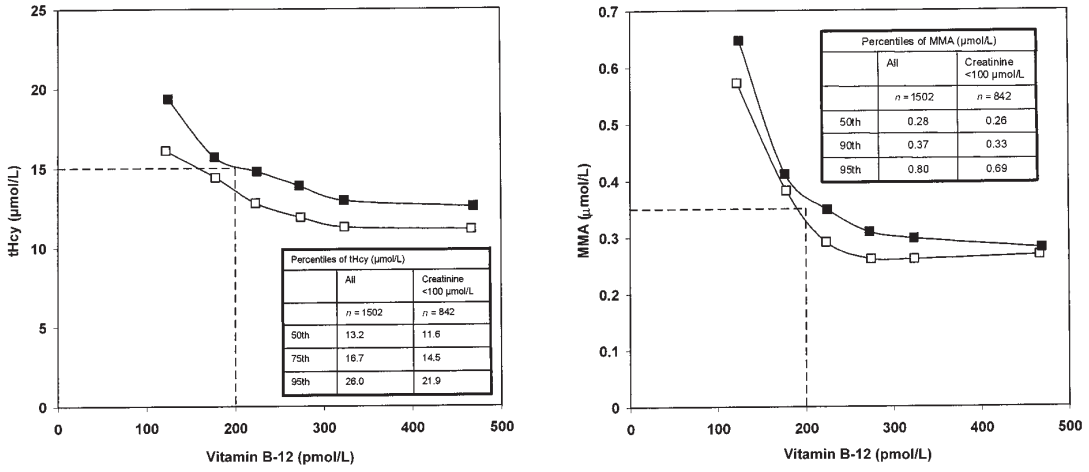

FIGURE 1. Distribution of total homocysteine (tHcy) and of methylmalonic acid (MMA) for the overall population (■) and for the subset with serum creatinine concentrations < 100 μmol/L (□). The vitamin B-12 categories for each metabolite are < 150, 150 to < 200, 200 to < 250, 250 to < 300, 300 to < 350, and ≥ 350 pmol/L. The percentile cutoffs for tHcy and MMA in the overall population and in the subset with normal renal function are shown in the text box. The cutoffs used to define elevated tHcy and MMA concentrations are represented by the dotted lines.

TABLE 3
Distribution by age and sex of vitamins and metabolites according to vitamin B-12 status[1]

	Men				Women			
		Vitamin B-12 150 to <200 pmol/L				Vitamin B-12 150 to <200 pmol/L		
Age and variable	Vitamin B-12 ≥200 pmol/L	MMA <0.35 μmol/L	MMA ≥0.35 μmol/L	Vitamin B-12 <150 pmol/L	Vitamin B-12 ≥200 pmol/L	MMA <0.35 μmol/L	MMA ≥0.35 μmol/L	Vitamin B-12 <150 pmol/L
65–74 y								
[*n* (%)]	213 (75)	41 (14)	13 (5)	18 (6)	290 (82)	32 (9)	14 (4)	18 (5)
Age (y)	71.7 ± 2.0[2]	71.4 ± 2.1	72.6 ± 1.8	72.2 ± 1.5	71.8 ± 2.0	71.9 ± 1.7	72.4 ± 2.0	72.6 ± 1.6
Vitamin B-12 (pmol/L)	295 ± 83	177 ± 15	169 ± 14	130 ± 15	328 ± 110	180 ± 13	174 ± 15	125 ± 24
Folate (nmol/L)	17.1 ± 15.5	14.9 ± 10.3	16.5 ± 16.2	14.2 ± 10.3	16.6 ± 13.7	13.6 ± 8.3	12.6 ± 7.9	18.3 ± 14.5
MMA (μmol/L)	0.27 ± 0.16	0.25 ± 0.06	0.62 ± 0.46	0.47 ± 0.40	0.26 ± 0.11	0.24 ± 0.05	0.59 ± 0.24	0.52 ± 0.52
Homocysteine (μmol/L)	12.8 ± 4.0	12.5 ± 4.7	16.6 ± 5.5	14.4 ± 4.3	11.8 ± 3.8	12.2 ± 3.8	18.7 ± 7.2	14.5 ± 8.5
Creatinine (μmol/L)	103 ± 18	99 ± 15	106 ± 26	100 ± 14	92 ± 18	89 ± 16	97 ± 24	86 ± 18
Elevated MMA (%)	15	0	100	39	11	0	100	39
Elevated tHcy (%)	23	12	62	33	14	22	64	28
Anemia (%)	14	7	15	33	10	6	14	11
≥75 y								
[*n* (%)]	216 (65)	36 (11)	41 (12)	40 (12)	390 (74)	48 (9)	54 (10)	38 (7)
Age (y)	81.6 ± 4.7	81.4 ± 3.8	82.4 ± 4.1	83.1 ± 4.8	82.4 ± 5.0	82.3 ± 4.6	83.6 ± 4.7	82.1 ± 4.5
Vitamin B-12 (pmol/L)	307 ± 179	181 ± 14	177 ± 16	127 ± 18	312 ± 129	176 ± 16	174 ± 14	120 ± 28
Folate (nmol/L)	14.3 ± 11.4	13.0 ± 9.5	19.0 ± 23.0	12.2 ± 10.9	16.6 ± 16.2	13.7 ± 10.0	13.8 ± 12.7	13.7 ± 10.1
MMA (μmol/L)	0.36 ± 0.36	0.26 ± 0.05	0.52 ± 0.19	0.76 ± 0.73	0.35 ± 0.25	0.28 ± 0.06	0.68 ± 0.46	0.66 ± 0.56
Homocysteine (μmol/L)	15.1 ± 5.2	14.9 ± 5.8	17.6 ± 4.7	23.5 ± 14.0	14.8 ± 6.1	14.3 ± 4.7	19.5 ± 6.7	19.7 ± 13.2
Creatinine (μmol/L)	112 ± 24	101 ± 17	116 ± 29	104 ± 25	98 ± 21	91 ± 20.0	96 ± 18	96 ± 39
Elevated MMA (%)	29	0	100	60	28	0	100	68
Elevated tHcy (%)	38	42	71	75	37	35	72	68
Anemia (%)	25	8	27	45	20	21	31	34

[1] MMA, methylmalonic acid; tHcy, total homocysteine.
[2] $\bar{x}$ ± SD.

function. These data show that as vitamin B-12 concentrations declined from adequate to impaired status, the concentrations of tHcy and of MMA transitioned from a stable low level to an elevated level. A cutoff of >15.0 μmol/L (80th percentile in the subset with normal renal function) was used to distinguish persons with elevated tHcy concentrations from those with normal concentrations. A cutoff of >0.35 μmol/L (80th percentile in the subset with normal renal function) was used to define elevated MMA concentrations. In individuals with low or borderline vitamin B-12 concentrations, elevated concentrations of MMA or of tHcy can be used to identify persons with functional or metabolically significant vitamin B-12 deficiency. Hence, persons were defined as being at high risk of vitamin B-12 deficiency if they had either vitamin B-12 concentrations of <150 pmol/L or vitamin B-12 concentrations of <200 pmol/L accompanied by a tHcy concentration >15 μmol/L or an MMA concentration >0.35 μmol/L.

As shown in Table 2, the pattern for folate was similar to that for vitamin B-12. Hence, persons were also defined as being at high risk of folate deficiency if they either had folate concentrations <5.0 nmol/L or folate concentrations <7.0 nmol/L accompanied by a tHcy concentration >15.0 μmol/L.

Prevalence of vitamin B-12 deficiency

The distribution of vitamin B-12 concentrations for each age- and sex-specific category separately is shown in **Table 3**. In younger men, the prevalences of normal (≥200 pmol/L), borderline (150 < 200 pmol/L), or low (<150 pmol/L) vitamin B-12 concentrations were 75%, 19%, and 6%, respectively. Among these categories, the proportion with elevated concentrations of MMA and elevated concentrations of tHcy was inversely related to the vitamin B-12 concentration. In older men, there was a downward shift in the distribution of vitamin B-12 values in the respective vitamin B-12 categories (65%, 23%, and 12%) and higher proportions in each category with elevated concentrations of tHcy and MMA. Thus, among men aged 65–74 and ≥75 y, ≈10% and 20%, respectively, were at high risk of vitamin B-12 deficiency. The prevalence in the corresponding vitamin B-12 categories was 82%, 13%, and 5% in the younger women and 74%, 19%, and 7% in the older women. Thus, 9% of the women aged 65–74 y and 17% of the women aged ≥75 y were at high risk of vitamin B-12 deficiency.

The proportion with anemia increased with increasing severity of vitamin B-12 deficiency, although whether these associations were causally related to vitamin B-12 cannot be established from these analyses. Individuals classified as being at high risk of vitamin B-12 deficiency on the basis of elevated MMA (>0.35 μmol/L) had a 2- to 3-fold higher prevalence of anemia than did individuals with borderline vitamin B-12 concentrations. About 10% of persons with low vitamin B-12 or at high risk of vitamin B-12 deficiency also had low folate concentrations.

tHcy compared with MMA for identifying persons at high risk of vitamin B-12 deficiency

As shown in **Figure 2**, the proportion of persons with elevated tHcy and elevated MMA increased with decreasing concentrations of vitamin B-12. The associations were approximately linear and graded, and the pattern was similar for both metabolites. MMA concentrations were closely correlated with tHcy ($r = 0.49$) in the overall population, but the agreement between these was

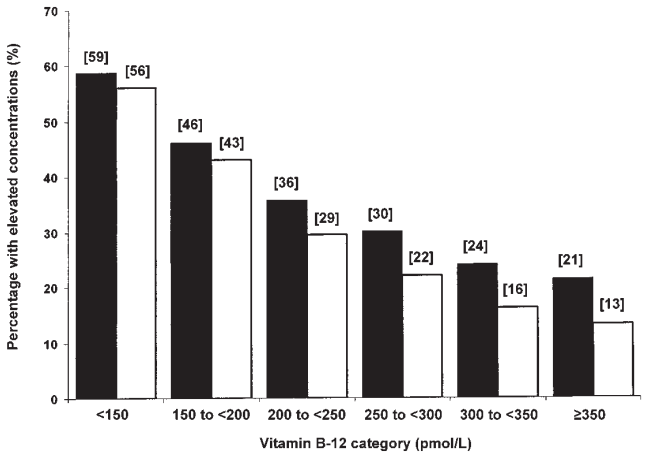

FIGURE 2. Distribution of individuals with elevated concentrations of total homocysteine (> 15 μmol/L; ■) and of methylmalonic acid (≥ 0.35 μmol/L; □) among groups classified by their vitamin B-12 concentration. *n* in brackets.

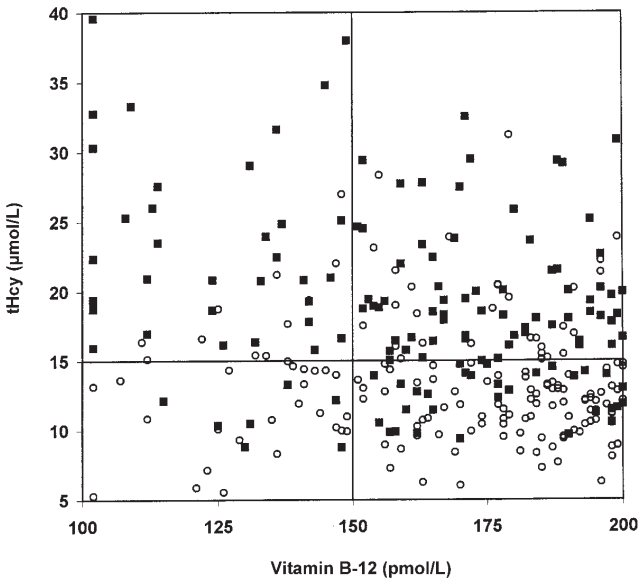

FIGURE 3. Distribution of individuals with normal (< 0.35 μmol/L; ○) and elevated (≥ 0.35 μmol/L; ■) serum methylmalonic acid concentrations according to their serum vitamin B-12 and total homocysteine (tHcy) concentrations.

greater ($r = 0.61$) among those with vitamin B-12 concentrations < 150 pmol/L.

The proportion of persons with elevated concentrations of tHcy and of MMA for age- and sex-specific categories separately is shown in Table 3. In both the younger men and the younger women, about one-third of those with low vitamin B-12 concentrations had elevated tHcy or elevated MMA concentrations. Among the older persons, about two-thirds of those with low vitamin B-12 concentrations had elevated tHcy or elevated MMA concentrations. Almost two-thirds of the younger population and three-quarters of the older population with elevated concentrations of MMA also had elevated concentrations of tHcy.

Shown in **Figure 3** are the tHcy results plotted by each individual's corresponding vitamin B-12 concentration in the subset with vitamin B-12 concentrations < 200 pmol/L and according to the presence or absence of elevated MMA. As shown in the figure, some misclassification of functional vitamin B-12 deficiency for folate deficiency would persist between those defined by elevated MMA or tHcy concentrations, irrespective of which cutoffs were used to classify elevated tHcy concentrations. The conventional criteria used to detect low vitamin B-12 concentrations (< 150 pmol/L) would identify those individuals in the left 2 quadrants, and the use of elevated tHcy (> 15.0 μmol/L) among those with vitamin B-12 concentrations < 200 pmol/L would identify those individuals in the top 2 quadrants. However, use of this cutoff for tHcy would still misclassify those individuals in the lower right quadrant as not having vitamin B-12 deficiency even though they had elevated MMA concentrations (*see* Discussion).

Prevalence of folate deficiency

The distribution of folate concentrations for each age- and sex-specific category separately is shown in **Table 4**. The prevalence of those with borderline folate concentrations are combined for some age- and sex-specific analyses. In the younger men, the prevalences of those with normal (≥ 7.0 nmol/L), borderline (5.0 < 7.0 nmol/L), or low (< 5.0 nmol/L) folate concentrations were 80%, 13%, and 7% of the population, respectively; among these, 22%, 24%, and 40% had elevated tHcy concentrations. In the older men, there was a downward shift in the distribution of folate concentrations, with 77%, 12%, and 11%, respectively, in the corresponding folate categories and a higher proportion in each category with elevated tHcy concentrations. Among the women, the prevalence of folate deficiency was similar to that in the men. The proportions with anemia were inversely related to the severity of folate deficiency, although whether these associations were causally related to folate cannot be established from these analyses. Among those with borderline folate concentrations, the prevalence of anemia was more than two-fold higher among individuals with elevated tHcy than in those without it, but the difference was less pronounced in those aged ≥ 75 y. Thus, among persons aged 65–74 and ≥ 75 y, ≈10% and 20%, respectively, were at high risk of folate deficiency. Overall, ≈10% of the younger age group with low folate and 20% of the older age group with low folate also had low vitamin B-12 concentrations (vitamin B-12 < 150 pmol/L).

DISCUSSION

Functional vitamin B-12 deficiency

Deficiencies of both vitamin B-12 and folate are common in older people, and the prevalence of both vitamin deficiencies increases with age. The results of the present study showed that the prevalence of vitamin B-12 deficiency was higher in men than in women, which is consistent with previous reports (14, 15). Concentrations of tHcy and MMA were elevated among those who had the lowest vitamin B-12 concentrations. Hence, use of either tHcy or MMA among those with borderline vitamin concentrations may identify those with functional deficiency and those for whom treatment is indicated. Among persons aged 65–74 and ≥ 75 y, respectively, ≈5% and 10% had low vitamin B-12 concentrations and 10% and 20% were at high risk of vitamin B-12

TABLE 4
Distribution by age and sex of vitamins and metabolites according to folate status[1]

	Men				Women			
		Folate 5.0 to <7.0 nmol/L				Folate 5.0 to <7.0 nmol/L		
Age and variable	Folate ≥7.0 nmol/L	tHcy <15.0 μmol/L	tHcy ≥15.0 μmol/L	Folate <5.0 nmol/L	Folate ≥7.0 nmol/L	tHcy <15.0 μmol/L	tHcy ≥15.0 μmol/L	Folate <5.0 nmol/L
65–74 y								
[*n* (%)]	228 (80)	28 (10)	9 (3)	20 (7)	290 (82)	35 (10)	8 (2)	21 (6)
Age (y)	71.7 ± 2.0[2]	72.2 ± 1.8	70.6 ± 1.5	71.7 ± 2.1	72.0 ± 1.9	71.5 ± 2.3	72.2 ± 1.9	71.2 ± 1.9
Vitamin B-12 (pmol/L)	264 ± 93	243 ± 72	248 ± 79	273 ± 117	301 ± 119	292 ± 110	354 ± 185	255 ± 97
Folate (nmol/L)	19.4 ± 15.0	6.0 ± 0.7	6.0 ± 0.7	3.8 ± 0.9	18.7 ± 13.4	6.0 ± 0.6	5.9 ± 0.5	4.0 ± 0.9
MMA (μmol/L)	0.29 ± 0.22	0.26 ± 0.09	0.43 ± 0.21	0.31 ± 0.26	0.29 ± 0.19	0.24 ± 0.07	0.35 ± 0.13	0.35 ± 0.20
Homocysteine (μmol/L)	12.8 ± 3.9	11.9 ± 2.0	21.6 ± 6.4	14.4 ± 5.2	12.1 ± 4.6	10.8 ± 2.1	19.2 ± 1.6	13.1 ± 5.5
Creatinine (μmol/L)	103 ± 18	99 ± 17	105 ± 23	99 ± 20	92 ± 17	87 ± 14	112 ± 37	91 ± 27
Elevated MMA (%)	18	11	44	15	13	9	38	33
Elevated tHcy (%)	22	0	100	40	18	0	100	19
Anemia (%)	14	4	22	25	10	6	12	14
≥75 y								
[*n* (%)]	256 (77)	12 (4)	30 (9)	35 (11)	407 (77)	39 (7)	46 (9)	38 (7)
Age (y)	82.0 ± 4.5	80.0 ± 3.8	81.1 ± 4.5	82.3 ± 5.4	82.4 ± 4.9	82.2 ± 5.4	84.4 ± 4.6	81.7 ± 4.8
Vitamin B-12 (pmol/L)	263 ± 178	236 ± 69	220 ± 70	240 ± 91	280 ± 138	243 ± 90	227 ± 77	272 ± 115
Folate (nmol/L)	17.4 ± 13.8	6.0 ± 0.6	5.8 ± 0.5	3.7 ± 1.0	19.1 ± 15.8	6.0 ± 0.6	5.9 ± 0.5	3.5 ± 1.1
MMA (μmol/L)	0.41 ± 0.41	0.25 ± 0.07	0.48 ± 0.29	0.49 ± 0.55	0.40 ± 0.33	0.31 ± 0.15	0.43 ± 0.19	0.48 ± 0.50
Homocysteine (μmol/L)	15.2 ± 5.9	11.4 ± 1.9	22.5 ± 8.7	21.7 ± 11.0	15.0 ± 7.1	12.7 ± 1.8	20.3 ± 4.9	19.4 ± 8.3
Creatinine (μmol/L)	111 ± 23	91 ± 15	116 ± 29	104 ± 23	97 ± 23	92 ± 17	99 ± 21	94 ± 26
Elevated MMA (%)	38	8	47	46	34	15	61	45
Elevated tHcy (%)	39	0	100	77	39	0	100	58
Anemia (%)	26	25	30	23	20	23	33	34

[1] tHcy, total homocysteine; MMA, methylmalonic acid.
[2] $\bar{x}$ ± SD.

deficiency. The present study was carried out in a population without folic acid fortification of flour. Among persons in this population aged 65–74 and ≥75 y, respectively, ≈10% and 20% were at high risk of folate deficiency.

Selection of cutoffs for MMA and tHcy

There is no consensus on appropriate cutoffs for elevated tHcy or elevated MMA for the diagnosis of vitamin B-12 deficiency. Previous studies defined cutoffs by using means or medians or as being >2 SDs (the 95th percentile) or >3 SDs (the 99th percentile) above the mean in younger populations and applied these cutoffs to older populations. The present study adopted cutoffs that took account of both age and renal function. The study showed an inverse association that was approximately linear and graded in the proportions with elevated tHcy and elevated MMA with decreasing concentrations of vitamin B-12. However, there was an inflexion point of >15.0 μmol/L for tHcy and >0.35 μmol/L for MMA (ie, about the 80th percentile for tHcy and for MMA in the subset with normal creatinine concentrations) that could be used to distinguish individuals with elevated concentrations from those with normal concentrations. Thus, these cutoffs for tHcy or MMA were used among those with vitamin B-12 <200 pmol/L to define individuals as being at high risk of vitamin B-12 deficiency. In clinical practice, measurement of either tHcy or MMA (or both) in individuals with vitamin B-12 concentrations <200 pmol/L on a single measurement at screening would exclude concerns about false-positive low vitamin B-12 concentrations. However, it is recommended that tHcy and creatinine be measured in all patients with borderline vitamin B-12 concentrations. If tHcy concentrations are elevated, it would be prudent to measure vitamin B-12 again in a subsequently collected blood sample before the start of treatment.

MMA compared with tHcy

The present study showed that the severity of vitamin B-12 deficiency is correlated with both elevated MMA and elevated tHcy concentrations. About three-quarters of those aged ≥75 y who had elevated MMA concentrations also had elevated tHcy concentrations. Analysis of the tHcy results for individuals showed that some misclassification is likely to persist between these metabolites, irrespective of which cutoffs are used for tHcy or MMA. Measurement error involving either metabolite may account for some of this misclassification of functional vitamin B-12 deficiency. It is not possible to compare the predictive value of elevated tHcy or elevated MMA for vitamin B-12 deficiency in the absence of some gold standard for diagnosis of vitamin B-12 deficiency. It is not appropriate to regard elevated MMA as a gold standard, because ≈15–30% of persons with high vitamin B-12 concentrations also had elevated MMA concentrations, which may reflect renal impairment rather than true vitamin B-12 deficiency. The ultimate gold standard for vitamin B-12 deficiency may be the reduction in tHcy or MMA concentrations and improvement in clinical symptoms or signs in response to vitamin B-12 treatment.

The present study suggests a possible algorithm that could be adopted to screen for vitamin B-12 deficiency, commencing with measurement of vitamin B-12 and folate. If individuals have a vitamin B-12 concentration <150 pmol/L, more detailed investigation is required to find an underlying cause and treatment. If individuals have a vitamin B-12 concentration between 150 and 200 pmol/L, then use of tHcy or MMA may help to identify those

who require more detailed investigation and treatment. If MMA assays are too expensive or unavailable, the results of the present study suggest that elevated tHcy concentrations would detect about three-quarters of those identified by elevated MMA.

Implications for public health

Previously, screening for vitamin B-12 deficiency was indicated only for the evaluation of those with relevant symptoms and signs, such as anemia, neuropathy, or cognitive impairment. However, in view of the high prevalence of vitamin B-12 and folate deficiency reported in this population without folic acid fortification of flour, routine screening of older persons (particularly those aged ≥ 75 y) for vitamin B-12 or folate deficiency may be indicated. In populations where all cereal grain products are fortified with folic acid (16–18) or where this is currently being considered (19), screening for vitamin B-12 deficiency may be particularly relevant. Concerns about the effect of folic acid fortification on neurologic complications among individuals with vitamin B-12 deficiency persist, and range from a delay in diagnosis of vitamin B-12 deficiency to an exacerbation of either peripheral neuropathy (20–22) or neuropsychiatric complications associated with vitamin B-12 deficiency (23).

Elevated homocysteine concentrations have been also associated with increased risk of coronary heart disease, stroke, and dementia (23, 24). The results of ongoing large-scale trials of folic acid–based vitamin supplements are required to assess the relevance of lowering tHcy concentrations for the prevention of cardiovascular disease (25). Almost all of these trials include high-dose oral vitamin B-12 supplements and measure vitamin B-12 and folate at enrollment. Such trials should provide evidence about the relevance of lowering tHcy concentrations for vascular and nonvascular outcomes in persons with various concentrations of vitamin B-12 and folate before the start of treatment.

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