

Appendix I. Inclusion and exclusion criteria for participants in the study

Inclusion criteria

- Resident within the catchment areas of the social services elderly teams.
- Over the age of 60 years in Manchester and over 65 years in east Cheshire.
- Living at home in the community, either in their own home or that of a relative.
- Experiencing any physical or mental deterioration that leads the social services care manager to consider the older person for admission to a nursing or residential care home. This might include a recent unexplained history of falling, not eating, immobility, incontinence, symptoms of depression, social withdrawal, confusion or wandering.
- Actively discussed as a potential care home admission by the care manager with their team leader.

Exclusion criteria

- Self-funding entrants to a care home, not having been assessed by a care manager under the community care legislation.
 - Emergency admissions to a care home, in whose circumstance there would have been insufficient time to mobilise a research clinician if required. However, care managers were encouraged to make referrals of individuals who they considered to be of 'emergency' status, as this was a common social services' perception of an individual's situation.
 - Given the diagnosis of a terminal illness. This would not have permitted the collation of outcome data or have been appropriate for the type of medical assessment on offer.
 - Examined by a hospital based geriatrician or old age psychiatrist within the last 14 days, either at home or as part of a period of stay or attendance at hospital.
 - Having a medical condition which was being monitored by a specialist other than a geriatrician or old age psychiatrist and which was responsible for the deterioration in health.
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Age and Ageing 2004; **33**: 34–41
DOI: 10.1093/ageing/afg109

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Vitamin B12 and folate deficiency in later life

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Abstract

Objectives: to examine the prevalence of vitamin B12 deficiency and folate deficiency in later life in representative samples of the elderly population in the United Kingdom.

Design: a population-based cross-sectional analysis of 3,511 people aged 65 years or older from three studies was used to estimate the age-specific prevalence of vitamin B12 deficiency and of folate deficiency. Vitamin B12 deficiency is

conventionally diagnosed if serum vitamin B12 <150 pmol/l ('low vitamin B12'). We defined 'metabolically significant vitamin B12 deficiency' as vitamin B12 <200 pmol/l and blood total homocysteine >20 µmol/l. Folate deficiency, which usually refers to serum folate <5 nmol/l, was defined as 'metabolically significant' if serum folate was <7 nmol/l and homocysteine >20 µmol/l.

Results: the prevalence of vitamin B12 deficiency, whether defined as low vitamin B12 or metabolically significant vitamin B12 deficiency increased with age in all three studies, from about 1 in 20 among people aged 65–74 years to 1 in 10 or even greater among people aged 75 years or greater. The prevalence of folate deficiency also increased with age, and was similar to that for vitamin B12 deficiencies, but only about 10% of people with low vitamin B12 levels also had low folate levels.

Conclusion: the high prevalence of vitamin B12 and folate deficiency observed in older people indicates a particular need for vigilance for deficiency of these vitamins. Reliable detection and treatment of vitamin deficiency could reduce the risk of deficiency-related disability in old age.

Keywords: vitamin B12, folate, age, folate deficiency in later life

Introduction

Vitamin B12 deficiency caused by intrinsic factor deficiency or hypochlorhydria chiefly affects older people [1, 2]. Vitamin B12 deficiency due to malabsorption arising from bacterial overgrowth syndrome is also common in older people. Vitamin B12 deficiency may present as macrocytic anaemia, but also causes neuropathy such as sub-acute combined degeneration of the spinal cord. The neurological symptoms may occur in the absence of anaemia in 20–30% of cases [3]. The diagnosis is complicated by the limitations of current assay techniques, as a low serum vitamin B12 level does not always indicate vitamin B12 deficiency and a normal vitamin B12 level does not always exclude it [4–6]. However, individuals with biologically significant vitamin B12 deficiency almost always have elevated plasma levels of total homocysteine and methylmalonic acid [1, 2]. Consequently, measurement of blood levels of either of these metabolites can be used to confirm the diagnosis of vitamin B12 deficiency [1–6]. Thus, individuals with low or borderline levels of vitamin B12 and elevated levels of homocysteine or methylmalonic acid can be defined as having 'metabolically significant' vitamin B12 deficiency [7].

Folate deficiency may occur at all ages, particularly in persons ingesting a poor diet or suffering from intestinal malabsorption or who have excessive alcohol intake or have excessive demands as in haemolytic anaemia, psoriasis or other medical conditions with increased cell proliferation or use of certain drugs [8]. Individuals with reduced folate status have elevated levels of homocysteine. Thus, individuals with low or borderline levels of folate and elevated levels of homocysteine can be defined as having 'metabolically significant' folate deficiency [7]. Individuals with combined folate and vitamin B12 deficiency may be defined as 'metabolically significant' combined deficiency if they have low or borderline levels of folate and vitamin B12 and elevated homocysteine.

The aims of the present study were (i) to determine the age-specific prevalence of vitamin B12 deficiency; (ii) the age-specific prevalence of folate deficiency; and (iii) to assess the prevalence of combined deficiency of both vitamin B12 and folate deficiency. Vitamin B12 deficiency is conventionally diagnosed if vitamin B12 <150 pmol/l; we

defined it as 'metabolically significant' if vitamin B12 <200 pmol/l and homocysteine >20 µmol/l (90th percentile). Folate deficiency, which usually is defined as a serum folate <5 nmol/l, was defined as 'metabolically significant' if folate <7 nmol/l and homocysteine >20 µmol/l. Combined vitamin deficiency was defined as being 'metabolically significant' if folate was <7 nmol/l and vitamin B12 was <200 pmol/l and homocysteine was >20 pmol/l.

Methods

Study populations

Data on blood concentrations of B12, folate, homocysteine, and creatinine were provided from three population-based studies of elderly people living in the UK: the Oxford Healthy Aging Project (OHAP); the National Diet and Nutrition Survey (NDNS) of people aged 65 years and over; and the Medical Research Council (MRC) nutrition study. Selected characteristics of the people eligible for each study are shown in Table 1.

The OHAP was a module of the MRC Cognitive Function and Ageing study (CFAS) [9]. This study involved a random sample of 2740 people aged 65 and over who were resident in Oxford City when first interviewed in 1991–1994. The sample was drawn from general practice registers to provide equal numbers of individuals aged 65–74 and 75 years and over. Surviving participants were approached again in 1995–1996 and invited to provide a blood specimen and blood was obtained from 68% of survivors (47% of the eligible population). Blood was collected into plain vacutainers, allowed to clot at room temperature, and the serum was separated within 2 hours and stored at –80°C. Analyses are restricted to 1549 individuals with complete data on homocysteine, vitamin B12 and folate.

The NDNS study of people aged 65 years and over was conducted on behalf of the Ministry of Agriculture, Fisheries and Food and the Department of Health. Participants were recruited using an age and sex-stratified sampling from randomly selected postcode sectors throughout Britain between 1994 and 1995 [10, 11]. The sampling strategy aimed to provide equal numbers by sex for the age groups 65–74, 75–84 and 85 years and older, although there was to

Table 1. Characteristics of people eligible for each study, those selected and those who provided a blood sample

	OHAP		NDNS		MRC	
	Selected sample	Blood sample	Selected sample	Blood sample	Selected Sample	Blood sample
Eligible sample, <i>n</i>	3555	2521	2626	1687	2959	1529
Responding sample, <i>n</i>	2740	1670	2060	1276	2040	1214
Response: % of eligible	77	47	78	49	69	41
Response: % of responders	–	66	–	76	–	79
No. with biochemical data	–	1549	–	956	–	1006
Year of survey	1991/1994	1995/1996	1994/1995	1994/1995	1995/1996	1995/1996
Age: <75 years (%)	48	43	50	51	0	0
75 + years (%)	52	57	50	49	100	100
Sex: male (%)	38	41	50	51	42	44
Female (%)	62	59	50	49	58	56
Resident in an institution (%)	7	4	21	23	0	0
Current smoker (%)	171	16	15	15	12	10

be a lower proportion of males aged 85 years or older. Among the 2626 people who were approached, 1276 (49% of the eligible population) provided a blood sample, but the analyses reported here were restricted to 956 who had complete data on homocysteine and vitamins. Plasma samples were collected into Sarstedt monovettes containing heparin and kept chilled at 4–8°C during transport to a local laboratory where the plasma was separated. The plasma aliquots were frozen and transferred to Cambridge for vitamin assays and subsequently to Stavanger, Norway for homocysteine determinations.

The MRC nutrition study was a sub-study of the MRC trial of assessment and management of elderly people who were living in the community throughout Britain [12]. The MRC nutrition study was carried out in 51 general practices from the MRC General Practice Framework, selected to be representative of the mortality and deprivation levels of practices in the UK between 1995 and 1999 [12]. A sample of 2959 trial participants aged 75 years or older were randomly selected to take part in the nutrition study (i.e. interview plus blood sample) and 2040 (68%) agreed, and blood samples with sufficient amount for analysis were obtained from 1214 people (41% of those eligible). A few patients were older than 84 years by the time the blood sample was taken. Forty-four per cent of the samples came from men and 56% from women. Plasma samples were collected in a monovette containing EDTA and sent to a local hospital within 4 hours of collection, where they were centrifuged and frozen. The samples were stored at a local hospital laboratory at –70°C, if possible, otherwise at –40°C or at –20°C for 2 months. The samples were then sent on dry ice to the Rowett Institute, Aberdeen (where they were stored at –80°C) for the vitamin analyses to be carried out. Subsequently, the samples were transferred to Oxford on dry ice for homocysteine analyses. The analyses presented here were restricted to 1006 people with complete data on homocysteine, vitamin B12 and folate.

Laboratory methods

In the OHAP study, vitamin B12 assays were determined in Aarhus, Denmark, by a competitive protein-binding radioimmunoassay on an ACS Centaur™ using an automated chemiluminescence system (Bayer A/S, New York, USA),

that had an intra-assay coefficient of variation (CV) of 8%. Blood total homocysteine concentrations were measured in serum samples at the University Department of Pharmacology in Oxford using a fluorescence polarisation immunoassay (FPIA: AXIS-Shield, Oslo, Norway) on an Abbott IMx auto-analyser [13, 14]. The CV for the homocysteine assays was <3.5% (*n* = 600). The Oxford laboratory participated in an external quality control scheme for homocysteine determinations involving 50 laboratories and the results (*n* = 12/year) at Oxford were within 95% of the mean [15]. A sample of 400 individuals had repeat homocysteine determinations carried out by gas chromatography mass spectrometry (GCMS) in the University of Bergen [16] and the Bland and Altman 95% confidence intervals for the agreement between these two homocysteine assays was ±0.09 μmol/l. Serum folate assays were carried out by a microbiological method, that had an intra-assay CV of 6% [17].

In the NDNS study, vitamin B12 and folate were measured within a few days of blood collection in Addenbrooke's Hospital, Cambridge. Vitamin B12 was measured by a radioimmunoassay using human intrinsic factor as a binder and folate was measured by a microbiological assay. The B12 assay used in NDNS was included in a National External Quality Control scheme and the deviation index (number of standard deviations from the mean) for low, medium and high values were consistently <1. Plasma homocysteine concentrations were measured by HPLC in Stavanger, Norway [18].

In the MRC study, plasma vitamin B12 and folate concentrations were measured at the Rowett laboratory, Aberdeen using a Becton Dickinson Simultrac kit for B12 and folate which had an analytical imprecision of 12–15% [19]. Homocysteine was measured at University Department of Pharmacology in Oxford using the same FPIA assay as in the OHAP study.

Statistical methods

Associations of vitamin B12, folate and homocysteine concentrations with age in each study were assessed using linear regression. Differences in the prevalence of biochemical evidence of B12 or folate deficiency were examined by age, domicile (whether free-living or resident in an institution) and study population. Associations between B12 and

homocysteine concentrations in each study were also compared by plotting mean homocysteine concentrations for deciles of vitamin B12. To control for possible confounding by age and sex, the odds ratio of having elevated homocysteine concentrations (>20 µmol/l) for each level of serum B12 was assessed using regression splines [20]. The splines were estimated using the generalised additive logistic regression models in S-Plus 2000 (Professional Release 2), with four degrees of freedom, and adjustment for age, sex, folate and creatinine concentration. Serum B12 and age were specified as continuous variables for the regression splines, while the other covariates were categorical (with folate and creatinine divided into quartiles).

Results

Selected characteristics of study populations

The three populations were chosen to be representative of the UK elderly population, but blood samples were collected from each study in the latter half of the 1990s. Table 1 shows that all studies were designed to provide approximately similar numbers of men and women. The OHAP and NDNS studies were designed to provide approximately similar numbers of those aged 65–74 years and 75 years or older, but the MRC study was designed to be restricted to those aged 75 years or older. The NDNS study was designed to include 20% who were resident in an institution and the remaining 80% were to be free-living individuals. In contrast, about 7% of the participants in OHAP and 1% of MRC (which were both selected from General Practice Registers) were resident in an institution. The prevalence of current cigarette smokers was similar in NDNS to that for OHAP, but the prevalence of current smokers was somewhat lower in the MRC studies. The response rate for the actual individuals with complete data who have been used in the analyses varied from 57% for OHAP, to 46% for NDNS and 49% for the MRC study. The lower proportion of available samples in the MRC and NDNS may reflect the problems inherent in multi-centre studies compared with OHAP study which was restricted to Oxford city rather than reflecting any other reasons for non-response bias. Thus, apart from differences in the age distribution and domicile, the

three studies were otherwise similar and constituted a nationally representative sample of people aged 65 years and older.

Age-specific concentrations of vitamin B12 and folate by study

The mean concentrations of vitamin B12, folate and homocysteine in age-specific groups are shown separately for each of the three studies in Table 2. The mean homocysteine concentrations increased with age in all studies and mean concentrations of folate and vitamin B12 declined with increasing age in OHAP and MRC study, but not in NDNS. The mean folate concentration was higher in the MRC study that used a radio-immunoassay than in either of the other two studies, which used a microbiological method to determine folate concentrations. The mean vitamin B12 concentrations were lower and the homocysteine concentrations were higher in the NDNS than in either of the other two studies. The lower absolute mean concentration of vitamin B12 in NDNS (about 15%) may reflect differences in the vitamin B12 assay method used in NDNS which differed from those used in the other studies. This difference of 15% in vitamin B12 concentrations is compatible with being <1 SD (i.e. <50 pmol/l) of the mean values for those with low, medium or high values.

Figure 1 demonstrates the distribution of vitamin B12 concentrations and the relationship of mean homocysteine to deciles of vitamin B12 in each of the three populations studied. There was a similar proportional relationship of vitamin B12 concentrations with homocysteine in all three studies, which provides support for the validity of the assays in each study. Figure 2 shows for each of the three studies, plots of the fitted odds ratio (95% CI) of having elevated homocysteine concentration (>20 µmol/l), in relation to vitamin B12 level, after adjustment for age, sex, folate and creatinine, which excludes confounding by differences in the other covariates studied. The odds ratio of having an elevated homocysteine increased sharply at vitamin B12 concentrations below 200 pmol/l and the pattern was similar in all three studies, confirming the proportional validity of the assays used in the three studies.

Table 2. Mean concentrations of vitamin B12, folate and homocysteine by age and by study

Age (years)	OHAP			MRC			NDNS					
	<i>n</i>	Vitamin B12 (pmol/l)	Folate (nmol/l)	Homocysteine (µmol/l)	<i>n</i>	Vitamin B12 (pmol/l)	Folate (nmol/l)	Homocysteine (µmol/l)	<i>n</i>	Vitamin B12 (pmol/l)	Folate (nmol/l)	Homocysteine (µmol/l)
65–69	138	284	16.8	12.7	–	–	–	–	171	226	16.5	13.6
70–74	517	282	16.0	12.6	8*	–	–	–	165	246	15.3	15.3
75–79	325	268	15.2	14.1	633	287	23.0	14.4	216	222	13.3	17.5
80–84	332	266	16.2	15.8	356	252	21.1	15.7	150	247	15.5	17.1
85–89	178	253	12.0	18.1	9*	–	–	–	175	235	15.2	18.4
90+	59	269	13.5	19.8	–	–	–	–	79	222	15.3	20.3
All	1549	272	15.6	14.5	1006	275	23.5	14.9	956	233	15.1	16.8
<i>P</i> for trend with age		0.010	0.032	<0.001		0.005	0.022	<0.001		0.90	0.50	<0.001

*Excluded because of insufficient numbers.

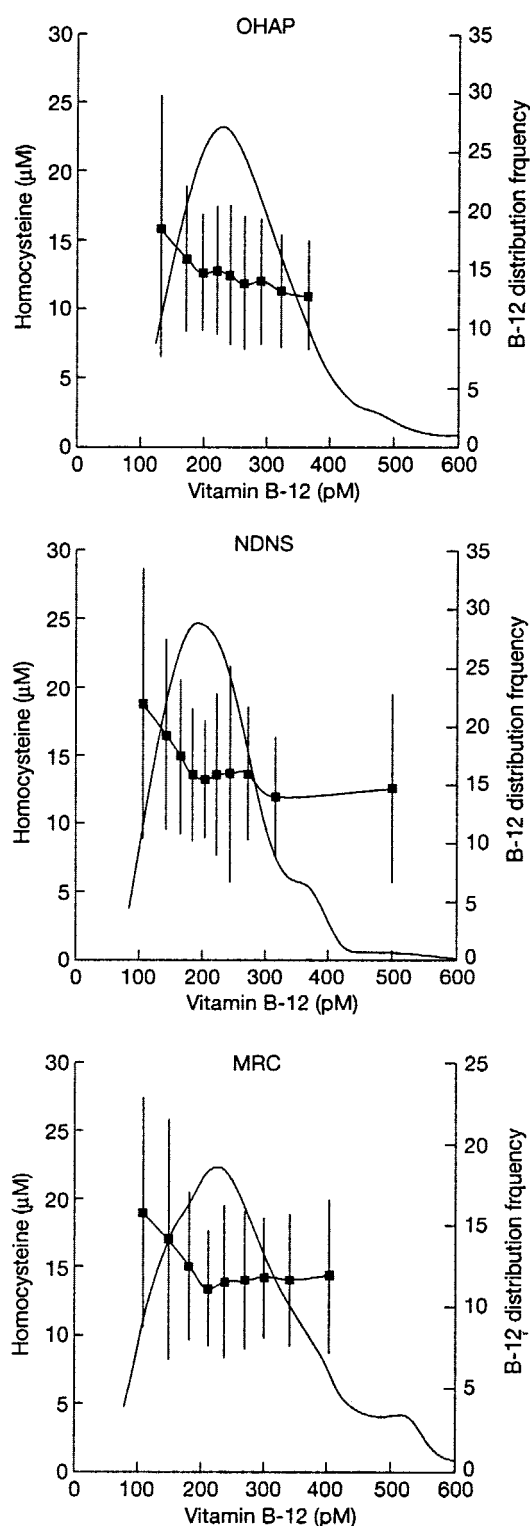


Figure 1. Distribution of blood total homocysteine concentrations in relation to vitamin B12 concentrations in the three population-based studies. Distribution of vitamin B12 concentrations and mean (95% CI) of total homocysteine according to deciles of vitamin B12 in the Oxford Healthy Aging Project (OHAP: top panel), the National Diet and Nutrition Study (NDNS: middle panel) and the Medical Research Council nutrition study (MRC: bottom panel).

Vitamin B12 deficiency in institutionalised and free-living individuals

In the NDNS study, 30 (9%) of 336 people aged <75 years and 187 of 620 people (30%) aged 75 or older were resident in an institution. However, there was no difference in the prevalence of those with low vitamin B12 levels (<150 pmol/l) among those who were free-living compared with those who were institutionalised, respectively in the younger group (12% versus 13%; $\chi^2=0.04$, $P=0.84$) or in the older group (26% versus 22%; $\chi^2=1.2$, $P=0.27$). In the OHAP study, 6 of 657 people (1%) aged 65–74 years and 55 of 905 (6%) people aged 75 years or older were resident in institutions at the time a blood sample was collected. None of the younger age group in OHAP who were resident in an institution had low vitamin B12 levels (<150 pmol/l). Among those aged 75 years or older in OHAP, there was no difference in the prevalence of vitamin B12 deficiency among those who were free-living compared with those who were institutionalised (9% versus 7%; $\chi^2=0.16$, $P=0.69$), respectively. Hence, the remaining analyses combined data from free-living people with those from people who were resident in institutions.

Age-specific prevalence of vitamin B12 deficiency

The age-specific prevalence of vitamin B12 deficiency using the two diagnostic criteria is shown separately for each study in Table 3. The prevalence of vitamin B12 deficiency increased with age in all three studies, whether estimated as low B12 levels or as metabolically significant B12 deficiency. On average, about 1 in 20 people aged 65–74 had low vitamin B12 concentration levels or had metabolically significant vitamin B12 deficiency. About 1 in 10 people aged 75 years or older had low vitamin B12 levels or metabolically significant B12 deficiency. While similar numbers of individuals had low vitamin B12 concentrations and also had elevated homocysteine, only about half of those with metabolically significant vitamin B12 deficiency also had vitamin B12 concentrations <150 pmol/l. Irrespective of which diagnostic criteria were used, the NDNS had a higher proportion with vitamin B12 deficiency than was found in the other two studies. This is probably not too surprising as both criteria for vitamin B12 deficiency are dependent on serum vitamin B12, and the NDNS generally measured lower values and we used an identical cut-off for vitamin B12 levels for all studies.

Age-specific prevalence of folate deficiency

The age-specific prevalence of folate deficiency using the two diagnostic criteria is shown separately for each study in Table 4. The prevalence of low folate concentrations increased with age in the MRC and NDNS studies. The prevalence of metabolically significant folate deficiency increased with age in all three studies ($P<0.05$). On average, about 1 in 20 people aged 65–74 years, and almost 1 in 10 people aged 75 years or greater had metabolically significant folate deficiency. The prevalence of folate deficiency was much lower in the MRC Study compared with the other two studies, and this difference may reflect differences in the folate assays used in the MRC study. As with vitamin B12 deficiency, only about half of those who

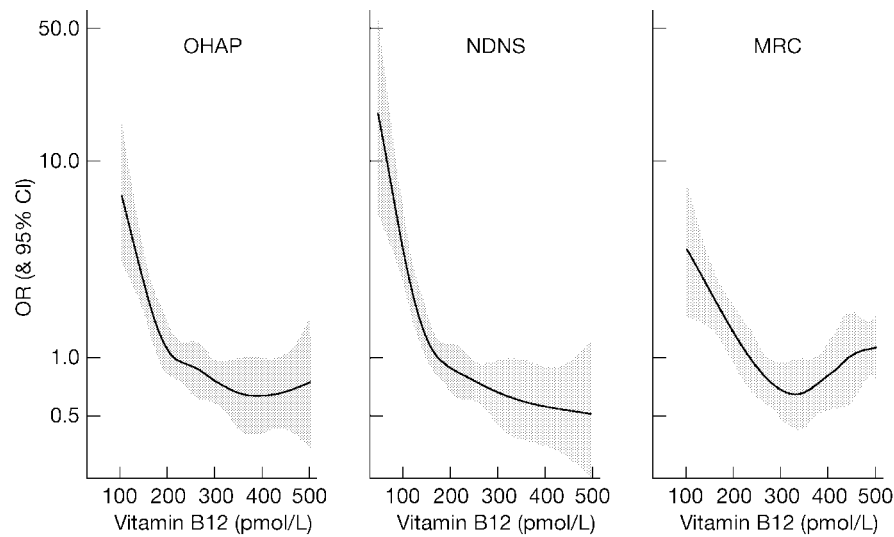


Figure 2. Dose–response relation between homocysteine and vitamin B-12 in the three population-based studies. The solid lines are the odds ratios of having elevated tHcy levels (>20 μmol/l), after adjustment for age, sex, and blood levels of folate and creatinine. The shaded areas are 95% confidence bands.

had metabolically significant folate deficiency had folate levels <5 nmol/l. Only 11% of those with vitamin B12 <150 pmol/l also had folate <7 nmol/l, which suggests that deficiency of vitamin B12 is largely independent of deficiency of folate and vice versa. But 37% of those with metabolically significant vitamin B12 deficiency also had metabolically significant folate deficiency, indicating the prevalence of combined vitamin B12 and folate deficiency that is metabolically significant may be quite high anyway.

Discussion

The prevalence of both vitamin B12 and folate deficiency increases with age. A generally accepted definition of these vitamin deficiencies is hampered by the limitations of current assays for vitamin B12 and folate, whereby serum vitamin concentration does not always indicate deficiency and a normal concentration does not always exclude it. Moreover, there is considerable inter-assay variability in folate and vitamin B12 making application of uniform cut-off values almost impossible [21–23]. Hence, use of homocysteine

assays, whose results are much more closely correlated between individuals [24] with low or borderline concentrations of vitamin B12 and folate may help to determine those who are most likely to benefit from treatment and avoid over-treatment among individuals for whom it is not indicated. Whichever diagnostic criteria were used, about 1 in 20 people aged 65–74 and 1 in 10 people aged 75 years or greater had vitamin B12 deficiency. The prevalence of folate deficiency was similar to that for vitamin B12 deficiency. Furthermore, deficiency of either vitamin is largely independent of the other using traditional criteria. However, using the definition of metabolically significant vitamin deficiency, a considerable proportion of individuals have combined vitamin deficiency.

The prevalence of vitamin B12 deficiency and of folate deficiency differed between studies, and it is likely that assay differences were responsible for this. For example, the method used to measure vitamin B12 in NDNS [10] differed from either of the other two studies [7, 12]. The difference in prevalence of vitamin B12 deficiency in NDNS was not explained by differences in age distribution or by

Table 3. Prevalence (95% CI) of low folate and metabolically significant folate deficiency by age and by study

Age (Years)	Low folate (folate <5 nmol/l)			Metabolically significant folate deficiency (folate <7 nmol/l and homocysteine >20 μmol/l)		
	OHAP (%)	MRC (%)	NDNS (%)	OHAP (%)	MRC (%)	NDNS (%)
65–69	9 (6–11)	–	6 (5–8)	4 (2–5)	–	5 (4–7)
70–74	6 (5–7)	–	9 (7–11)	2 (1–2)	–	5 (3–6)
75–79	10 (9–12)	1 (1–11)	10 (8–12)	6 (4–7)	4 (3–5)	13 (11–15)
80–84	6 (4–7)	3 (2–4)	11 (9–14)	6 (5–7)	11 (6–22)	10 (8–13)
85–89	11 (9–14)	–	11 (9–13)	11 (9–14)	–	15 (12–18)
90+	7 (4–10)	–	18 (13–22)	14 (9–18)	–	22 (17–26)
<i>P</i> for trend with age	0.45	0.024	0.0026	<0.0001	0.019	<0.0001

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Table 4. Prevalence (95% CI) of low vitamin B12 and metabolically significant vitamin B12 deficiency by age and by study

Age (years)	Low vitamin B12 (B12 < 150 pmol/l)			Metabolically significant vitamin B12 deficiency (B12 < 200 pmol/l and homocysteine >20 µmol/l)		
	OHAP (%)	MRC (%)	NDNS (%)	OHAP (%)	MRC (%)	NDNS (%)
65–69	3 (2–4)	–	12 (10–15)	3 (2–4)	–	4 (3–6)
70–74	7 (6–8)	–	12 (10–15)	3 (2–4)	–	9 (6–11)
75–79	9 (7–10)	14 (12–15)	25 (19–28)	4 (3–5)	5 (4–6)	19 (17–22)
80–84	9 (8–11)	20 (18–22)	27 (24–31)	9 (8–11)	12 (10–13)	13 (10–15)
85–89	10 (7–12)	–	22 (19–25)	14 (11–16)	–	18 (15–21)
90+	10 (6–14)	–	28 (23–33)	10 (6–14)	–	28 (23–33)
<i>P</i> for trend with age	0.004	0.052	<0.0001	<0.0001	<0.0001	<0.0001

differences in the proportions institutionalised between the populations studied. The lower response rate for the provision of a blood sample in the NDNS is unlikely to account for the higher prevalence of B12 deficiency observed in the NDNS study. The higher prevalence of vitamin B12 deficiency in the NDNS study is more likely to reflect differences in vitamin B12 assays used in the NDNS study compared with the other two studies. Similarly, the method used to measure folate in the MRC study differed from that used in either of the other two studies. It is well known that there is considerable inter-laboratory and inter-assay variation for the different methods for folate and vitamin B12 determination [21–23].

The prevalence of biochemical evidence of vitamin B12 and folate deficiency is greater than the prevalence of macrocytic anaemia and vitamin B12 deficiency-related neurological disease as commonly found in clinical practice. There are a number of possible explanations for this apparent discrepancy. The usual textbook description of the clinical correlates of vitamin B12 deficiency may be less frequent than widely believed, or the typical symptoms are attributed to ageing rather than to B12 deficiency. Furthermore, improvements in the national diet over the last half-century or so may have modified the natural history of vitamin B12 deficiency and of folate deficiency towards a milder or more incomplete history. As increased metabolite values are considered as an early sign of vitamin B12 or folate deficiency, it is to be expected that most persons with biochemical evidence of vitamin deficiency have not yet developed relevant symptoms or signs [8]. However, longitudinal studies are needed to ascertain the proportion of such persons who may subsequently develop typical symptoms and signs of vitamin B12 deficiency.

Treatment with vitamin B12 and folic acid supplementation is safe, inexpensive and effective if administered before the onset of symptoms [1–3]. In particular, in relation to diagnosis and treatment of affected individuals with vitamin B12 deficiency, early detection should lead to a reduction in vitamin B12 related neurological disability [3]. This study highlights the need for increased clinical vigilance for vitamin B12 and folate deficiency in older people. The findings may be particularly relevant for countries, such as the UK, that are considering mandatory fortification of flour with folic acid for the prevention of neural tube defects, and North America where this has already been introduced [25, 26]. The administration of folic acid to people with vitamin B12

deficiency may prevent the anaemia, but not the neurological sequelae associated with vitamin B12 deficiency. A greater awareness of the high prevalence of vitamin B12 and folate deficiency in older people could reduce the risk of deficiency related disability in old age. The relevance of treatment of people with vitamin B12 deficiency in the absence of symptoms, requires evidence on the efficacy of treatment, cost and the number of people likely to develop neurological syndromes.

Elevated homocysteine levels have been also associated with increased risk of coronary heart disease, stroke and dementia [27, 28]. The results of ongoing large-scale trials of folic acid-based vitamin supplements are required to assess the relevance of lowering homocysteine levels for the prevention of cardiovascular disease [29]. Almost all of these trials include high-dose oral vitamin B12 supplements and measure B12 and folate at enrolment. Such trials should provide evidence about the relevance of lowering homocysteine levels for vascular and non-vascular outcomes in people with varying levels of B12 and of folate prior to starting treatment.

Key points

- Vitamin B12 deficiency affects about 5% of people aged 65–74 years and over 10% of people aged 75 years or older.
- Folate deficiency is also common in older people, but only 10% of people with low vitamin B12 levels have low folate levels.
- Clinicians should be vigilant for vitamin B12 and folate deficiency in older people.
- Detection and treatment of vitamin deficiency could reduce the risk of deficiency-related disability in old age.
- Using metabolic criteria, a considerable number of individuals may have combined vitamin B12 and folate deficiency.

Acknowledgements

We would like to thank Dr Azam Mansoor, Department of Clinical Chemistry, Central Hospital in Rogaland, Stavanger, Norway for the homocysteine analyses that were included in the NDNS study.

Funding

British Heart Foundation and Medical Research Council, EU Commission (Demonstration Project Contract No BMH 4-98-3549), Medical Research Council, Clothworkers' Foundation, NHS R&D Directorate, and Department of Health and Food Standards Agency, London.

Conflict of interest

The views expressed are those of the authors and not necessarily those agencies who supported the three studies.

References

- Carmel R. Current concepts in cobalamin deficiency. *Annu Rev Med* 2000; 51: 357–75.
- Stabler SP. B12 and nutrition. In Banerjee R ed. *Chemistry and Biochemistry of B12*. New York: John Wiley & Sons, Inc., 2000.
- Lindenbaum J, Healton EB, Savage DE, Brust JCM, Garrett TJ, Podell ER. Neuropsychiatric diseases caused by cobalamin deficiency in the absence of anemia or macrocytosis. *N Engl J Med* 1988; 318: 1720–8.
- Holleland G, Schneede J, Ueland PM, Lund PK, Refsum H, Sandberg S. Cobalamin deficiency in general practice. Assessment of the diagnostic ability and cost benefit analysis of methylmalonic acid determination in relation to current diagnostic strategies. *Clin Chem* 1998; 45: 189–98.
- Hvas AM, Juul S, Gerdes LU, Nexo E. The marker of cobalamin deficiency, plasma methylmalonic acid, correlates to plasma creatinine. *J Int Med* 2000; 247: 507–12.
- Klee GG. Cobalamin and folate evaluation: measurement of methylmalonic acid and homocysteine vs vitamin B(12) and folate. *Clin Chem* 2000; 46: 1277–83.
- Clarke R, Refsum H, Birks J *et al*. Screening for vitamin B12 and folate deficiency in older people. *Am J Clin Nutr* 2003; 77: 1241–7.
- Lindenbaum J, Allen RH. Clinical spectrum and diagnosis of folate deficiency. In Bailey LB. *Folate in Health and Disease*. New York: Marcel Dekker, 1995: 43–73.
- Medical Research Council Cognitive Function and Ageing Study (MRC CFAS). Cognitive function and dementia in six areas of England and Wales: the distribution of MMSE and prevalence of GMS organicity level in the MRC CFA study. *Psychological Med* 1998; 28: 319–35.
- Finch S, Doyle W, Lowe C *et al*. National Diet and Nutrition Survey: People Aged 65 Years and Over. Vol. 1: Report of the Diet and Nutrition Survey. London: The Stationery Office, 1998.
- Bates CJ, Mansoor MA, vd Pols J, Prentice A, Cole TJ, Finch S. Plasma total homocysteine in a representative sample of 972 British men and women aged 65 and over. *Eur J Clin Nutr* 1997; 51: 1–7.
- Smeeth L, Fletcher AE, Stirling S *et al*. Randomised comparison of three methods of administering a screening questionnaire to elderly people: findings from the MRC trial of the assessment and management of older people in the community. *Br Med J* 2001; 323: 1403–7.
- Shipchandler MT, Moore EG. Rapid, fully automated measurement of plasma homocysteine with the Abbott IMX analyzer. *Clin Chem* 1995; 41: 991–4.
- Nexo E, Engbaek F, Ueland PM *et al*. Evaluation of novel assays in clinical chemistry: quantification of plasma total homocysteine. *Clin Chem* 2000; 46: 1150–56.
- Moller J, Rasmussen K, Christensen L. External quality assessment of methylmalonic acid and total homocysteine. *Clin Chem* 1999; 45: 1536–42.
- Ueland PM, Refsum H, Stabler SP, Malinow MR, Andersson A, Allen RH. Total homocysteine in plasma or serum: methods and clinical applications. *Clin Chem* 1993; 39: 764–79.
- Molloy AM, Scott JM. Microbiological assay for serum, plasma and red-cell folate using cryopreserved, microtiter plate method. *Methods Enzymol* 1997; 281: 43–53.
- Mansoor AM, Bergmark C, Svardal AM, Lonning PE, Ueland PM. Redox status and protein binding of plasma homocysteine and other aminothiols in patients with early-onset peripheral vascular disease. *Arterioscler Thromb Vasc Biol* 1995; 15: 232–40.
- Nexo E, Gimsing P. Insolubilized pure human intrinsic factor used for quantitation of cobalamin in serum. *Scand J Lab Invest* 1981; 41: 465–68.
- Hastie TJ, Tibshirani RJ. *Generalized Additive Models*. London: Chapman and Hall, 1990.
- Gunter E, Bowman BA, Caudill SP, Twite DB, Adams MJ, Sampson EJ. Results of an international round robin for serum and whole-blood folate. *Clin Chem* 1996; 42: 1689–94.
- van den Berg H, Finglas PM, Bates C. International comparisons on serum and red cell folate. *Int J Vitam Nutr Res* 1994; 64: 288–93.
- Mollin DL, Hoffbrand AV, Ward PG, Lewis SM. Interlaboratory comparison of serum vitamin B12 assay. *J Clin Pathol* 1980; 33: 243–8.
- Clarke R, Woodhouse P, Ulvik A *et al*. Variability and determinants of total homocysteine concentrations in plasma in an elderly population. *Clin Chem* 1998; 44: 102–7.
- Food and Drug Administration. Food standards: amendment of standards of identity for enriched grain products to require the addition of folic acid. *Fed Regist* 1996; 61: 8781–97.
- Department of Health. Report on Health and Social Subjects. 50. Folic Acid and the Prevention of Disease: Report of the Committee on Medical Aspects of Food and Nutrition Policy. London: Department of Health, 2000.
- Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM. Folate, vitamin 12, and serum total homocysteine levels in confirmed Alzheimer's disease. *Arch Neurol* 1998; 55: 1449–55.
- Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA* 2002; 288: 2015–22.
- Clarke R, Collins R. Can dietary supplements with folic acid or vitamin B-6 reduce cardiovascular risk? Design of clinical trials to test the homocysteine hypothesis of vascular disease. *J Cardiovasc Risk* 1998; 5: 249–55.

Received 7 May 2002; accepted in revised form 11 June 2003