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Folate and vitamin B_{12} status in relation to cognitive impairment and anaemia in the setting of voluntary fortification in the UK

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Concerns about risks for older people with vitamin B_{12} deficiency have delayed the introduction of mandatory folic acid fortification in the UK. We examined the risks of anaemia and cognitive impairment in older people with low B_{12} and high folate status in the setting of voluntary fortification in the UK. Data were obtained from two cross-sectional studies (*n* 2403) conducted in Oxford city and Banbury in 1995 and 2003, respectively. Associations (OR and 95 % CI) of cognitive impairment and of anaemia with low B_{12} status (holotranscobalamin <45 pmol/l) with or without high folate status (defined either as serum folate >30 nmol/l or >60 nmol/l) were estimated after adjustment for age, sex, smoking and study. Mean serum folate levels increased from 15·8 (SD 14·7) nmol/l in 1995 to 31·1 (SD 26·2) nmol/l in 2003. Serum folate levels were greater than 30 nmol/l in 9 % and greater than 60 nmol/l in 5 %. The association of cognitive impairment with low B_{12} status was unaffected by high *v.* low folate status (>30 nmol/l) (OR 1·50 (95 % CI 0·91, 2·46) *v.* 1·45 (95 % CI 1·19, 1·76)), respectively. The associations of cognitive impairment with low B_{12} status were also similar using the higher cut-off point of 60 nmol/l for folate status ((OR 2·46; 95 % CI 0·90, 6·71) *v.* (1·56; 95 % CI 1·30, 1·88)). There was no evidence of modification by high folate status of the associations of low B_{12} with anaemia or cognitive impairment in the setting of voluntary fortification, but periodic surveys are needed to monitor fortification.

Vitamin B_{12}: Folate: Cognitive impairment: Older people

Reports of adverse effects from treating individuals with pernicious anaemia with a high-dose folic acid have suggested that high-level folic acid fortification might delay the diagnosis or exacerbate the effects of vitamin B_{12} deficiency in older people. Low B_{12} status affects about 10–25 % of older people and has been associated with a higher prevalence of anaemia and cognitive impairment[1–4]. Using direct measurements of holotranscobalamin (holoTC), the active fraction of B_{12}[5], it has been estimated that almost 25 % of older people have low B_{12} status (holoTC <45 pmol/l)[4]. Concerns that high-level folic acid fortification might delay the diagnosis of B_{12} deficiency[6,7] or exacerbate the neurological complications of B_{12} deficiency (and cause colon cancer) have delayed the introduction of mandatory fortification in the UK[1]. In North America, mandatory folic acid fortification does not appear to increase the risk of anaemia[6,7], but it has been suggested that it may increase the rate of cognitive decline[8,9] and risk of cognitive impairment[10] in people with low vitamin B_{12} status. In the UK, folic acid was added to most breakfast cereals in 1987 and the amount of added folic acid was subsequently increased in 1994. In addition, folic acid was added to 'spreads' in 2000. Voluntary folic acid fortification has resulted in a substantial increase in blood folate levels in the population[1]. Data collected on vitamin status in older people in the Oxford Healthy Aging Project (OHAP) in 1995[2] and in the Banbury B_{12} study in 2003[4] provided an opportunity to assess the effects of voluntary folic acid fortification in the UK. The aim of the present study was to assess if high blood folate levels might affect the association of low B_{12} status with anaemia and with cognitive impairment in older people living in the UK.

Methods

The study sample comprises all participants with data on vitamin status in two population-based studies of older people living in Oxford City (OHAP[2]) and Oxfordshire (Banbury B_{12} study[4]). The OHAP is a longitudinal cohort study of

Abbreviations: holoTC, holotranscobalamin; MMA, methylmalonic acid; OHAP, Oxford Healthy Aging Project; tHcy, homocysteine.

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2741 randomly selected people aged 65 years and over and is a component part of the Medical Research Council Cognitive Function and Aging Study[11].

Oxford Healthy Aging Project

In 1993, we randomly selected the population sample from general practice registers for people living in Oxford City to provide equal numbers of individuals aged 65–74 years and 75 years or older. Among the 3555 people in the selected sample, 2740 (77 %) individuals agreed to participate in the study. Research nurses visited study participants in their own homes and carried out a structured interview. The collected data included medical history, smoking habits, education and use of medication (including multivitamin supplements or vitamin B_{12} injections). All surviving participants who had not previously refused to be interviewed were invited to provide a blood sample in 1995. Non-fasting blood samples were obtained from 68 % of surviving participants and collected into vacutainers that were allowed to clot at room temperature. The serum was separated within 2 h and stored at −80°C until shipped on dry ice or thawed for analysis[2].

Banbury

Participants in the Banbury B_{12} study were recruited between March 2003 and April 2004 from a random sample of people aged 75 years or older living in their own homes and registered with three general practices in Banbury, Oxfordshire[4]. Individuals who were known to have a terminal illness or were living in institutions were excluded. Eligible participants (*n* 1934) were invited to participate in the study and those who agreed (*n* 1000) were asked to provide written informed consent. Participants were visited in their own homes by a research nurse between March 2003 and April 2004 and the data collected included medical history and use of medication. All participants had their blood pressure measured. Non-fasting venous blood samples were collected and kept chilled (using a cooling box to ensure that the temperature was maintained below 4°C) until the serum was separated at the local hospital laboratory within 2 h of blood collection and stored at −40°C until analysis.

In both the OHAP and Banbury, a blood count was measured on the same day as the blood was collected. Participants also had their cognitive function assessed around the same time as their blood was collected using the Mini-Mental State Examination[12] and cognitive impairment was defined if the Mini-Mental State Examination was <25/30. Participants provided consent and the protocols (in accordance with the current version of the Helsinki Declaration) were approved by the relevant research ethics committees.

Laboratory methods

Frozen serum samples were thawed for measurements of levels of folate, holoTC, B_{12} and homocysteine (tHcy). Serum holoTC concentrations in the OHAP study were carried out at Aarhus University Hospital, Aarhus, Denmark using an ELISA method modified for use on an automated analyser[13]. Serum holoTC levels in the Banbury B_{12} study were measured at the Oxford University Clinical Trial Service Unit using a RIA (AXIS-Shield ASA, Oslo, Norway)[14] that has been shown to have a very good agreement with the ELISA assay[14]. Serum tHcy concentrations were measured on an Abbott IMx autoanalyser by means of a fluorescence polarization immunoassay in the OHAP[2] and by GC-MS in the Banbury study[4] and both assays provide good agreement[15]. Serum methylmalonic acid (MMA) levels were measured at the University of Bergen, Bergen, Norway, using stable isotope–dilution capillary GC-MS in both studies[16]. Serum vitamin B_{12} concentrations were measured on an ACS Centaur with an automated chemiluminescence system (Bayer A/S, Germany), using a competitive protein binding assay at Aarhus University Hospital, Aarhus, Denmark in both studies. Serum folate levels were measured using a microbiological method at the University of Dublin, Republic of Ireland for both the OHAP and Banbury populations[17]. Anaemia was defined if the Hb level was <120 g/l in men and <110 g/l in women (Table 1).

Statistical methods

Continuous variables were summarized as means, standard deviations and ranges. Individuals with extreme elevations of vitamin B_{12} (>1000 pmol/l) or holoTC (>400 pmol/l) or who reported use of vitamin B_{12} injections were excluded. Differences in mean values were assessed using *t* tests. OR (with 95 % CI) of anaemia and cognitive impairment were estimated using logistic regression after adjustment for age, sex, smoking and study. Since data on some covariates, such as blood pressure, prior CVD and education, were missing on some or all individuals in either population, the primary analyses were adjusted for the covariates with complete data available on all participants. Additional models were carried out in the OHAP population only (that had the relevant data) to also adjust for education. Low vitamin B_{12} status was defined as holoTC <45 pmol/l. Individuals were defined as having high folate status if serum folate >30 nmol/l for some analyses or >60 nmol/l for other analyses.

Results

Characteristics of the study sample

Among the 2559 individuals with data on vitamin status in the two studies, seventy who reported current use of vitamin B_{12} injections were excluded, as were thirteen other individuals with extreme values of vitamin B_{12} (>1000 pmol/l) or holoTC (>400 pmol/l) (possibly indicating unreported vitamin B_{12} treatment or malignancy), leaving 2476 untreated individuals for analysis. HoloTC concentrations were missing on some individuals but complete data were available on 2403 individuals (1464 studied in Oxford City in 1995 and 939 studied in Banbury in 2003). Table 1 shows that the mean age of these 2403 study participants was 79·2 (SD 6·2) years and 59 % were women. Mean serum folate levels were 15·8 (SD 14·7) nmol/l in 1995 in the OHAP and 31·1 (SD 26·2) nmol/l in 2003 in Banbury ($P<0·001$) and the median folate levels were 11·3 and 23·7 nmol/l, respectively. About 3 % of participants in the OHAP and 7 % in Banbury reported current use of folic acid supplements, but data on folic acid use was available for only 1269 (53 %) participants. In the sub-set with complete data on folic acid use and after excluding current

NS British Journal of Nutrition

Table 1. Distribution of selected characteristics of study participants in Oxford City and in Banbury (*n* 2403)*
(Mean values and standard deviations)

	OHAP							
	All		Age <75 years (*n* 617)		Age 75 + years (*n* 847)		Banbury (*n* 939)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Demographic/lifestyle								
Age (years)	79·2	6·2	71·8	4·6	82·3	4·8	81·3	4·6
Women								
n	1425		340		519		566	
%	59		55		61		60	
Cigarette smoker								
n	285		124		113		48	
%	12		20		14		5	
Laboratory variables								
Folate (nmol/l)	21·8	21·3	16·6	15·1	15·2	14·2	31·1	26·2
Vitamin B_{12} (pmol/l)	261	101	284	107	258	102	248	94
HoloTC (pmol/l)	71·5	42·4	79·3	45·1	67·5	40·6	70·0	41·5
tHcy (μmol/l)	13·3	1·3	12·6	4·4	15·9	7·2	15·4	7·7
MMA (μmol/l)	0·36	0·50	0·29	0·19	0·40	0·36	0·37	0·69
Hb (g/l)	133	13	136	12	131	13	132	13
Anaemia								
n	168		22		66		80	
%	7·0		3·6		7·9		8·6	
Cognitive function								
MMSE (maximum 30)	25·9	3·7	27·1	2·6	24·7	4·5	26·0	3·3
Cognitive impairment								
n	604		75		292		237	
%	25·1		12·5		36·3		25·9	

OHAP, Oxford Healthy Aging Project; HoloTC, holotranscobalamin; tHCY, homocysteine; MMA, methylmalonic acid; MMSE, Mini-Mental State Examination.
* For details of subjects and procedures, see Methods.

users of folic acid supplements, the median level of serum folate was 12·4 nmol/l in the OHAP in 1995 and 22·7 nmol/l in Banbury in 2003. Since the mean age was older in Banbury compared with the OHAP, the results for the OHAP are provided separately for those aged <75 years or 75 years and older. Since there were no significant differences in the mean holoTC levels between the individual studies, the data from both studies were pooled.

Associations with anaemia and cognitive impairment

Table 2 shows the associations of anaemia (involving 168 cases (7 %)) with biochemical markers of vitamin B_{12} and folate status, respectively. After adjustment for age, sex, smoking and study, individuals with holoTC levels in the bottom tertile compared with the top had a 1·87-fold higher risk of anaemia (OR 1·87; 95 % CI 1·44, 2·43) and, for folate, a 2-fold risk (OR 2·02; 95 % CI 1·46, 2·80) of anaemia, respectively. Similarly, individuals with tHcy levels in the top tertile compared with the bottom tertile had a 3-fold higher risk of anaemia (OR 3·18; 95 % CI 2·53, 3·98).

Table 2 also shows the associations of cognitive impairment (involving 578 cases (26 %)) with vitamin status. Individuals with holoTC levels in the bottom compared with the top tertile had a 1·8-fold higher risk of cognitive impairment (OR 1·80; 95 % CI 1·35, 2·12) and, for folate, a 1·55-fold higher risk (OR 1·55; 95 % CI 1·28, 1·87) of cognitive impairment, respectively. Similarly, individuals with tHcy levels in the top tertile compared with the bottom tertile had a 1·59-fold higher risk of cognitive impairment (OR 1·59; 95 % CI 1·35, 1·87). In the OHAP study (which had data on education) there were 346 cases (25 %) with cognitive impairment. After adjustment for age, sex, smoking and education, the OR for cognitive impairment for the bottom tertile compared with the top tertile were 1·39 (95 % CI 1·15, 1·67) for folate, 1·08 (95 % CI 0·86, 1·37) for B_{12} and 1·61 (95 % CI 1·29, 2·00) for holoTC and the corresponding OR for the top compared with the bottom tertile of tHcy was 1·16 (95 % CI 0·92, 1·46).

Associations with low vitamin B_{12} status and high folate status

Table 3 shows the mean levels of tHcy and of MMA classified by the presence or absence of low vitamin B_{12} and high folate status, respectively. There was no significant difference in the mean levels of tHcy or MMA among those with low vitamin B_{12}, with and without high folate status, respectively. Table 3 also shows the associations of anaemia and of cognitive impairment with low B_{12} status (25 % of population) according to high serum folate levels. About 9 % of this population had a serum folate >30 nmol/l and 5 % had serum folate >60 nmol/l. There was no difference in the risk of cognitive impairment associated with low B_{12} status in individuals with or without serum folate levels above 30 nmol/l (OR 1·50 (95 % CI 0·91, 2·46) *v.* OR 1·45 (95 % CI 1·19, 1·76), respectively). Similarly, there was no difference in the risk of anaemia associated with low B_{12} in individuals with or without high serum folate levels (Table 3). The association of cognitive impairment with low B_{12} status was also similar

Table 2. Association of B vitamins with anaemia and cognitive impairment (*n* 2403)*

Tertiles of vitamin status			Anaemia† (*n* 168 cases)				Cognitive impairment‡ (*n* 578 cases)			
Vitamin status category	Mean	*n*	*n*	%	OR	95 % CI§	*n*	%	OR	95 % CI§
Folate (nmol/l)										
III	42·1	783	39	5·2	1·0	0·70, 1·42	167	22·2	1·0	0·82, 1·22
II	15·5	783	58	7·7	1·75	1·36, 2·24	190	25·2	1·23	1·04, 1·45
I	7·3	782	56	7·4	2·02	1·46, 2·80	221	29·4	1·55	1·28, 1·87
B_{12} (pmol/l)										
III	371	749	46	6·1	1·0	0·74, 1·35	170	22·7	1·0	0·84, 1·20
II	245	753	37	4·9	0·74	0·53, 1·03	177	23·5	1·06	0·89, 1·26
I	167	755	70	9·3	1·32	1·03, 1·70	231	30·6	1·41	1·20, 1·66
HoloTC pmol/l)										
III	115	755	32	4·2	1·0	0·70, 1·43	148	19·6	1·0	0·83, 1·21
II	64	750	54	7·2	1·67	1·26, 2·20	177	23·6	1·25	1·05, 1·49
I	35	752	67	8·9	1·87	1·44, 2·43	253	33·6	1·80	1·35, 2·12
tHcy (μmol/l)										
I	9·7	752	26	3·5	1·0	0·67, 1·49	144	19·1	1·0	0·82, 1·21
II	13·4	752	34	4·5	1·13	0·80, 1·60	185	24·6	1·30	1·02, 1·45
III	21·2	753	93	12·4	3·18	2·53, 3·98	249	33·1	1·59	1·35, 1·87

HoloTC, holotranscobalamin; tHCY, homocysteine.
* For details of subjects and procedures, see Methods.
† Anaemia was defined as Hb < 120 g/l in men and < 110 g/l in women.
‡ Cognitive impairment was defined as Mini-Mental State Examination < 25/30.
§ Adjusted for age, sex, smoking and study.

at serum folate levels above compared with below the higher cut-off point of 60 nmol/l ((OR 2·46; 95 % CI 0·90, 6·71) *v.* OR 1·56 (95 % CI 1·30, 1·88)). After additional adjustment for education in the OHAP only, the association of cognitive impairment with low B_{12} status was examined and did not differ significantly among individuals with high *v.* low folate status (> 30 nmol/l) (OR 2·47 (95 % CI 0·95, 6·34) *v.* OR 1·62 (95 % CI 1·26, 2·08)), respectively.

Discussion

Serum folate levels of the UK population have increased almost 2-fold between 1995 and 2003. In the overall study population, the mean levels of serum folate increased from 15·8 (SD 14·7) in the OHAP in 1995 to 31·1 (SD 26·2) nmol/l in Banbury in 2003. The present study reported that 9 % had a serum folate > 30 nmol/l and 5 % had serum folate > 60 nmol/l and 25 % had biochemical evidence of low vitamin B_{12} status. However, the present study provided no evidence that high blood levels of folate affected the associations of low B_{12} status with anaemia or with cognitive impairment. Thus, the results of the present study differ from those of the 1999–2002 US National Health and Nutrition Examination Survey[10], involving 1459 older people, which reported direct associations of high serum folate (> 60 nmol/l) with both anaemia and cognitive impairment in individuals with low B_{12} status. Another North American population-based survey of people aged over 65 years[9], also conducted after the introduction of folic acid fortification, reported that participants with a total folate intake at baseline > 400 μg/d had a more rapid cognitive decline over 6 years of follow-up than did individuals with intakes < 201 μg/d. The discrepant results of the present UK study and the two North American studies[9,10] may reflect the fact that mean blood levels of folate in the UK population have increased relatively recently; it may need longer follow-up to assess the full impact of change in folate levels on the association of low vitamin B_{12} status with cognitive function or that the present study lacked a sufficient number of individuals with high folate levels to have statistical power to detect a difference between the groups. The present study used a similar cut-off point to define high folate status as was used in the National Health and Nutrition Examination Survey study, but differed in using holoTC measurements as indicative of vitamin B_{12} status. However, holoTC is considered to be a more sensitive and specific diagnostic test for low vitamin B_{12} status compared with standard vitamin B_{12} assays[5]. The present study had a cross-sectional design and, hence, the observed association of low serum folate levels with cognitive function could, theoretically, reflect the adverse effects of cognitive impairment on diet. However, such reverse causation could not explain any effect of high folate levels on the prevalence of cognitive impairment among individuals with low vitamin B_{12} status as observed in the North American studies[9,10]. The results of the present cross-sectional analyses are consistent with a previously published report on the longitudinal analyses of cognitive decline over a 10-year period associated with B_{12} status according to folate status. In the multivariate analyses included in the previous report, a doubling in holoTC concentrations (e.g. from 50 to 100 pmol/l) was associated with 30 % slower rate of cognitive decline as assessed using change in Mini-Mental State Examination score per year (−0·137 to −0·083), whereas a doubling in tHcy (e.g. from 10 to 20 μmol/l) or MMA (e.g. from 0·25 to 0·50 μmol/l) concentrations was associated with > 50 % more rapid cognitive decline (−0·090 to −0·169) and (−0·104 to −0·169), respectively. The latter analyses were repeated with three-factor interaction terms for folate and holoTC by age to assess any interaction in the rates of cognitive decline associated with B_{12} status and high folate status. The interaction terms were not statistically significant, thereby providing no evidence that a high folate status was associated

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Table 3. High serum folate levels and the association of low vitamin B_{12} status with mean levels of homocysteine (tHcy) and methylmalonic acid (MMA) and with risk of anaemia and cognitive impairment*

(Values presented for anaemia and cognitive impairment are the OR and 95 % CI after adjustment for age, sex, smoking and study (*n* 2257))

HoloTC status (pmol/l)	Folate status (nmol/l)	No. of individuals	HoloTC (pmol/l)		Folate (nmol/l)		THcy (μmol/l)		MMA (μmol/l)		Anaemia†			Cognitive impairment‡			
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	*n*	%	OR	95 % CI§	*n*	%	OR	95 % CI§
45 +	< 30	1301	83	40	14	7	14	5	0·30	0·20	80	6·1	1·0	0·79, 1·26	315	24	1·0	0·87, 1·15
45 +	30 +	386	92	43	53	28	12	3	0·28	0·12	21	5·4	0·77	0·49, 1·22	67	17	0·64	0·49, 0·85
< 45	< 30	494	32	9	13	7	18	8	0·49	0·40	45	9·1	1·32	0·97, 1·81	170	34	1·45	1·19, 1·76
< 45	30 +	76	29	11	51	26	19	13	0·98	13·0	7	9·2	1·10	0·49, 2·43	26	34	1·50	0·91, 2·46
45 +	< 60	1593	84	40	19	13	14	5	0·30	0·19	96	5·0	1·0	0·81, 1·23	362	23	1·0	0·88, 1·13
45 +	60 +	94	98	53	90	35	12	3	0·27	0·11	5	5·3	0·72	0·29, 1·80	20	21	0·87	0·52, 1·46
< 45	< 60	553	32	9	16	11	18	8	0·52	0·42	51	9·2	1·36	1·01, 1·83	189	34	1·56	1·30, 1·88
< 45	60 +	17	24	12	89	32	26	23	2·07	4·72	1	5·9	0·79	0·10, 6·03	7	41	2·46	0·90, 6·71

HoloTC, holotranscobalamin.
*For details of subjects and procedures, see Methods.
† Anaemia was defined as Hb < 120 g/l in men and < 110 g/l in women.
‡ Cognitive impairment was defined as Mini-Mental State Examination < 25/30.
§ Adjusted for age, sex, smoking and study.

with a more rapid rate of cognitive decline associated with low B_{12} status[18]. In addition, a recent study carried out in North America[19] after the introduction of mandatory fortification reported higher mean levels of MMA among individuals with low B_{12} status with high compared with low folate status. However, in the present study, there was also no evidence of effect modification on the levels of tHcy or MMA associated with low B_{12} according to high folate status.

Irrespective of the apparent adverse effect of high folate status observed in North America but not in the UK, both the present UK study and the North American study[19] reported a high prevalence of low B_{12} status. The Scientific Advisory Committee on Nutrition in the UK has recommended the introduction of mandatory folic acid fortification and that this should be introduced together with a strategy for management of B_{12} deficiency in older people[1]. However, screening asymptomatic older people for biochemical evidence of B_{12} deficiency is likely to generate more false positive than true positive test results[5].

In conclusion, we undertook the present study to assess possible hazards for older people associated with voluntary folic acid fortification in the UK and found no evidence that high blood folate levels affected the associations of low B_{12} status with anaemia or with cognitive impairment. However, periodic surveys of vitamin status in relation to anaemia and cognitive impairment in older people are required to assess the effects of fortification. Moreover, further randomized trials of high-dose oral vitamin B_{12} supplements are required to assess the public health relevance of correction of biochemical evidence of low B_{12} status in the absence of relevant symptoms[20,21].

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