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Voluntary fortification is ineffective to maintain the vitamin B_{12} and folate status of older Irish adults: evidence from the Irish Longitudinal Study on Ageing (TILDA)

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Abstract

Mandatory fortification of staple grains with folic acid and/or vitamin B_{12} (B_{12}) is under debate in many countries including Ireland, which has a liberal, but voluntary, fortification policy. Older adults can be at risk of both deficiency and high folate status, although little is known on the actual prevalence and the major predictors. Population prevalence estimates from older adults (*n* 5290 ≥50 years) from the Irish Longitudinal Study on Ageing (TILDA) (Wave 1) are presented here. Measures included plasma total vitamin B_{12} and folate, whereas predictors included detailed demographic, socio-economic, geographic, seasonal and health/lifestyle data. The prevalence of deficient or low B_{12} status (<185 pmol/l) was 12 %, whereas the prevalence of deficient/low folate status was 15 %. High folate status (>45 nmol/l) was observed in 8·9 %, whereas high B_{12} status was observed in 3·1 % (>601 pmol/l). The largest positive predictor of B_{12} concentration was self-reported B_{12} injection and/or supplement use (coefficient 51·5 pmol/; 95 % CI 9·4, 93·6; $P=0·016$) followed by sex and geographic location. The largest negative predictor was metformin use (−33·6; 95 % CI −51·9, −15·4; $P<0·0001$). The largest positive predictor of folate concentration was folic acid supplement use (6·0; 95 % CI 3·0, 9·0 nmol/l; $P<0·001$) followed by being female and statin medications. The largest negative predictor was geographic location (−5·7; 95 % CI −6·7, −4·6; $P<0·0001$) followed by seasonality and smoking. B-vitamin status in older adults is affected by health and lifestyle, medication, sampling period and geographic location. We observed a high prevalence of low B_{12} and folate status, indicating that the current policy of voluntary fortification is ineffective for older adults.

Key words: B-vitamins: Biomarkers: Fortification: Supplements: Folate: Vitamin B_{12}

National surveys have shown that the proportion of older adults within most populations (defined as those >60 years) is increasing, and by 2050 global projections indicate that the number of older adults worldwide will be more than 2 billion (21 % of the population)[1]. With such profound shifts in demographics also comes the corresponding increase in the prevalence of the chronic diseases of ageing including CVD, cognitive impairment and associated co-morbidities[2]. Maintenance of optimal nutritional status has been cited as an achievable goal in preventing or delaying the development of chronic disease[3], and recent emphasis has been placed on the protective roles of micronutrients, including the B-vitamins.

Both vitamin B_{12} (B_{12}) and folate are essential co-factors in intracellular one-carbon metabolism, which is involved in DNA synthesis, cellular regulation processes and amino acid metabolism[4]. Both vitamins are thought to be associated with cardiovascular health and cognitive function. A recent meta-analysis (*n* 82 334) indicated a 10 % lower risk of stroke and a 4 % lower risk of overall CVD with folic acid (FA) supplementation[5], whereas in a longitudinal study of older adults (>60 years, *n* 501) higher concentrations of vitamin B_{12} were associated with a decreased rate of brain volume loss over 6 years[6]. Estimates of vitamin B_{12} deficiency in older populations range from 5 to 40 %, depending on the marker of measurement and the deficiency cut-off selected[7]. The consequences of a B_{12}-deficient state can include the development of megaloblastic anaemia, irreversible demyelinating neurological disease/paresthesias and the potential for impaired immune and cognitive function[8]. Hence, the early detection of deficiency and awareness of the factors that can affect B_{12} status is important. In terms of folate, since the introduction of mandatory FA fortification of grains in the USA to reduce the

Abbreviations: B_{12}, vitamin B_{12}; FA, folic acid; NANA, National Adult Nutrition Study; NHANES, National Health and Nutritional Examination Survey; TILDA, Irish Longitudinal Study on Ageing.

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incidence of neural tube defects, the prevalence of deficient and low folate status in the US population has substantially decreased[9]. Conversely, there is an accompanying increased incidence of high blood folate concentrations (>45 nmol/l), which has led to concerns of potential adverse effects, especially when combined with low vitamin B_{12} status, as frequently observed in older adults[10].

To date, mandatory FA fortification does not occur in the European Union (EU). Uniquely within the EU, however, both Ireland and the UK have a voluntary but liberal food fortification policy that includes FA. The foods most commonly fortified with FA are breakfast cereals, but not all are fortified[11,12]. In addition, there are reports of varying degrees of FA enrichment of foods, and enrichment also appears to be inconsistent across time and across products[13]. Moreover, foods that are fortified or rich in B_{12} are not necessarily consumed by the older population. For instance, dairy foods, which are a particularly good source of B_{12}, are only consumed in the recommended amounts by only 4 % of older Irish adults[14].

A recent Irish National Survey that previously examined vitamin B_{12} and folate status was limited to a small number of older adults and had a narrow range of demographic and clinical data[15]. Therefore, the aims of this study were (i) to report the B_{12} and folate status of a large, nationally representative sample of the older Irish population (n >5000) and (ii) to investigate the demographic, socio-economic, geographic, seasonal, health and lifestyle determinants of these vitamin concentrations in this population, which is subject to a voluntary, but liberal, food fortification policy.

Methods

Study population

Participants were recruited as part of The Irish Longitudinal Study on Ageing (TILDA), a nationally representative cohort of community-dwelling adults aged ≥50 years, designed to investigate how the health, social and economic circumstances of the older Irish population interact in the determination of 'healthy' ageing. Full details of the study design, sampling and methodology have been published previously[16,17]. The first wave of data collection (Wave 1) was conducted between October 2009 and July 2011, using a stratified clustered procedure to randomly sample postal addresses from the Irish Geo-Directory (a listing of all residential addresses in the Republic of Ireland). All postal addresses in Ireland were assigned to one of 3155 geographic clusters using RANSAM (a random sampling design for Ireland) where all household residents aged ≥50 years were eligible to participate; a sample of 640 of these clusters was selected, stratified by socio-economic group and geography to maintain a population representative sample. Clusters were selected with a probability proportional to the number of individuals aged ≥50 years in each cluster. At Wave 1, 8175 adults completed a computer-aided personal interview (CAPI) (response rate of 62 %). Approximately 72·1 % (n 5895) consented to, and participated in, a health assessment that included donating a blood sample. In total, folate measurements were available for 90·7 % (n 5350) and B_{12} measurements were available for 88·5 % (n 5219) of these participants. Missing data were randomly dispersed across clusters and were generally because of sample assay failures or insufficient sample volume. The study was conducted according to the guidelines set out in the Declaration of Helsinki, and ethical approval was granted by the Faculty of Health Sciences Research Ethics Committee of Trinity College Dublin.

Lifestyle and supplement use

During the CAPI, data were collected regarding sex, age, province of residence (Leinster – East/South East; Munster – South/South West; Ulster and Connacht – North and West), habitation status (living alone yes/no), current smoker status (yes/no), current alcohol status (yes/no) and household housing wealth (measure of economic resource 'asset wealth' coded above or below the average for the sample). A list of up to twenty medications including both prescription and non-prescription medications and dietary/vitamin supplements were recorded by interviewers. Once entered into the data set, they were assigned WHO Anatomical Therapeutic Chemical Classification codes and binarised to yes/no for FA supplement use (single tablet or multi-vitamin) and for use of vitamin B_{12} (injection or single tablet or B_{12} in multi-vitamin). Classification codes were also binarised to yes/no for the commonly prescribed medications of older adults, which have been associated with B-vitamin concentrations including metformin, proton pump inhibitors (PPI), statins and thyroxin medications[18,19]. Self-reported physical activity was assessed by the International Physical Activity Questionnaire (IPAQ). Physical activity levels were also dichotomised and defined according to the IPAQ categories: physically active = minimally or health enhancing physically active; not physically active = inactive or insufficiently active. During the health assessment, anthropometric measurements including height (to the nearest 0·01 m) and weight (to the nearest 0·01 kg) were measured with Seca™ (Seca Ltd) using a standardised protocol. BMI was then calculated and obesity was classified as a BMI >30 kg/m^2. Seasonality was examined and defined as winter (December through February), spring (March through May), summer (June through August) and autumn (September through November).

Blood samples

A non-fasting blood sample was collected by venepuncture into one 10-ml K_2EDTA tube (BD, Becton, Dickinson Limited) by a trained phlebotomist. Samples were kept chilled and centrifuged (3000 rpm for 15 min) and aliquots were labelled and stored at −80°C until required for analysis. Plasma total B_{12} and folate concentrations were determined by microbiological assays, as previously described[20,21]. The inter-assay CV for plasma B_{12} and folate were <10·9 %. B_{12} status profiles (pmol/l) were defined as follows: <148, deficient; 148–<185, low; 185–<258, low normal; >258–601, normal; and >601, high[22,23]. Folate status profiles (nmol/l) were defined as follows: <6·8, deficient; 6·8–10·0, low; >10–23·0, low normal; >23·0–45·0, normal; >45·0, high[24]. These cut-offs were selected to allow for comparison with previous studies and to give an indication of the population range.

Statistical analyses

Base population weights were calculated by comparing TILDA with the 2011 Irish Census with respect to age, sex, educational attainment, marital status and urban *v.* rural residence (weights reflected the reciprocal of the selection probability). The staged stratified clustering sample design of TILDA was accounted for when computing CI and standard errors. Prevalence estimates and descriptive statistics were adjusted using modified base weights that accounted for survey non-response, non-attendance at the health assessment component of the study and whether or not respondents provided a blood sample. TILDA participants with plasma total B_{12} and folate concentrations more than 3 SD above the mean or who were missing data on other covariates were excluded from the analysis, resulting in 5158 results for total B_{12} and 5290 results for plasma folate (the +3 SD cut-off was applied to remove outlying high values, likely attributable to measurement error). Weighted prevalence estimates are reported as percentages with 95% CI. Weighted geometric means are presented along with B_{12} and folate concentrations categorised by the stated biomarker cut-offs, stratified by the population characteristics. Pairwise comparisons of proportions by B_{12} and folate status cut-offs were computed across variables of interest, and false-discovery rate correction for multiple comparisons was applied ($P<0{\cdot}041$, achieving $q<0{\cdot}05$). Multiple linear regression estimated the effects of health, lifestyle, geographic and socio-economic factors on B_{12} and folate concentrations. Multiple imputation (twelve cycles) was used to impute missing information on asset wealth in the final statistical models (information was missing for <5% of participants). All analyses were carried out using STATA 14 (StataCorp).

Results

The population characteristics of the TILDA cohort have been published comprehensively elsewhere[16,17]. In short, of the participants included in this combined analysis, the mean age was 63·6 years (age range 50–98) and 53% were female. The proportion who reported currently consuming alcohol was 76·8%, whereas 15·6% were current smokers. The prevalence of reported combined B_{12} supplementation (injection and/or supplements) was 2·6%, with women reporting significantly higher usage than men (3·5 *v.* 1·7%; $P<0{\cdot}001$, respectively). The prevalence of FA supplementation was 2·8%, with significantly more women than men reporting supplementation use (3·6 *v.* 2·0%; $P<0{\cdot}001$, respectively).

Prevalence of biomarker status

The estimated distribution of both B_{12} and folate status by age group is displayed in Fig. 1. The prevalence of deficient or low B_{12} status was 12%, whereas 15% had deficient or low folate status. High B_{12} status (>601 pmol/l) was observed in 3·1% of the population, whereas 8·9% had high folate status (>45 nmol/l). The prevalence estimates by demographic and lifestyle factors are displayed in Table 1 for total B_{12} and Table 2 for plasma folate. Participants reporting supplement use had significantly higher concentrations of both total B_{12} and

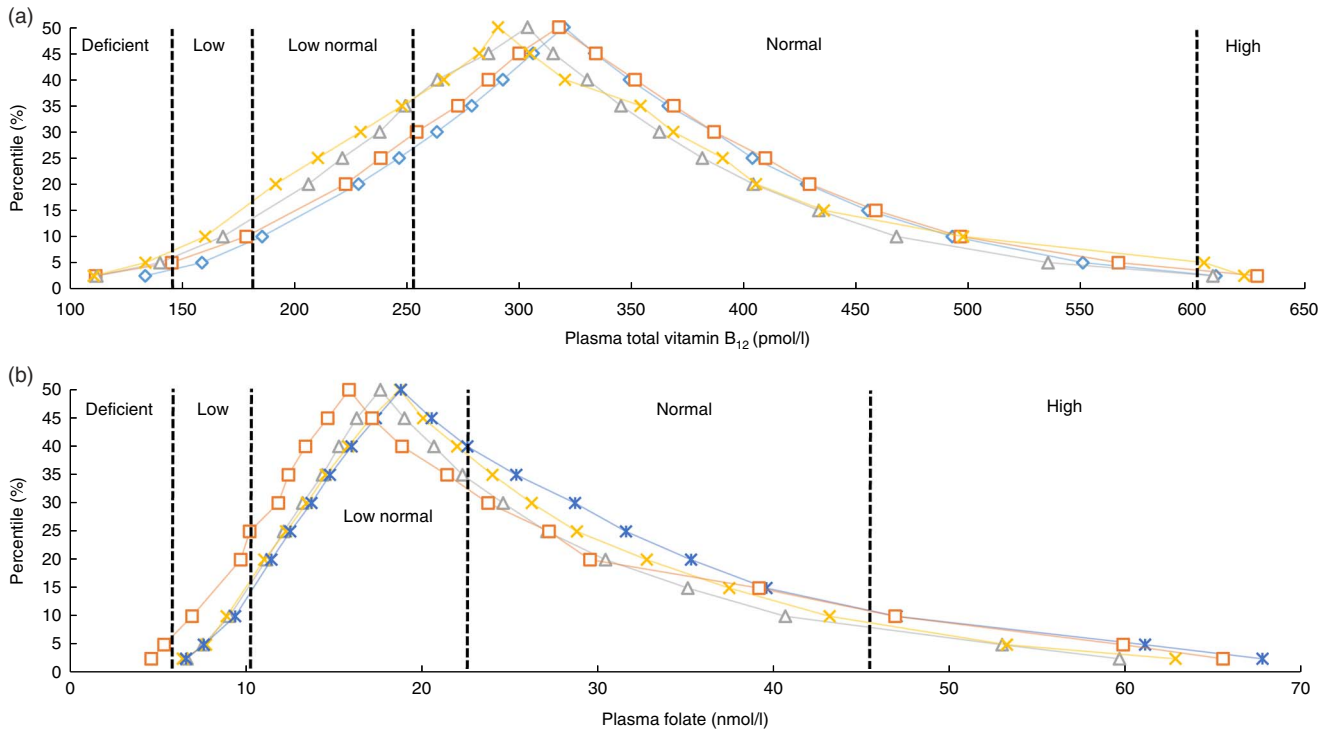

Fig. 1. Frequency distribution of plasma total vitamin B_{12} (a) and folate (b) of older Irish adults from the Irish Longitudinal Study on Ageing study by age group. a: ◇, 50–59; □, 60–69; △, 70–79; ×, >80; b: △, 50–59; ×, 60–69; ✳, 70–79; □, >80.

Table 1. Mean plasma total vitamin B_{12} with weighted prevalence of status by demographic and lifestyle characteristics†
(Weighted means and prevalence estimates and 95 % confidence intervals)

		Vitamin B_{12}									
				<148 pmol/l (*n* 237)		148– <185 pmol/l (*n* 320)		185– <258 pmol/l (*n* 994)		>258 pmol/l (*n* 3627)	
Characteristics	Subjects (*n*)	Average (pmol/l)	95 % CI	%	95 % CI	%	95 % CI	%	95 % CI	%	95 % CI
Supplements and/or B_{12} injection	147	352·3	326·8, 378·3	2·9	1·0, 7·8	3·9	1·7, 8·5	14·7	9·7, 21·5	78·5	70·7, 84·5
No supplements or B_{12} injection	5011	312·5	308·3, 316·4	5·1	4·4, 5·9	7·0	6·1, 7·8	19·6	18·4, 20·7	68·3	66·8, 69·7
P			0·0022*		<0·0001*		<0·0001*		<0·0001*		<0·0001*
Sex											
Male	2428	303·4	298·5, 308·7	5·1	4·1, 6·2	7·5	6·3, 8·8	20·2	18·5, 22·0	67·2	65·0, 69·1
Female	2730	323·2	317·5, 329·0	5·0	4·1, 6·1	6·3	5·2, 7·5	18·7	17·1, 20·3	70·0	67·8, 71·8
P			<0·0001*		0·8516		0·3239		0·5619		0·0147*
Age (years)											
50–59	2196	320·7	314·7, 326·5	4·0	3·0, 5·1	5·7	4·6, 6·9	17·9	16·2, 19·6	72·4	70·3, 74·4
60–69	1757	317·1	310·4, 324·0	5·4	4·2, 6·7	6·1	4·8, 7·6	19·7	17·8, 21·8	68·8	66·3, 71·1
70–79	929	299·2	290·0, 308·3	6·4	4·8, 8·5	8·9	6·9, 11·3	22·6	19·4, 25·9	62·1	58·2, 65·7
>80	276	297·2	281·2, 313·2	6·6	4·2, 10·1	11·1	7·7, 15·6	19·8	15·2, 25·2	62·5	56·3, 68·2
P			<0·0001*		0·0431		0·0013*		0·1011		<0·0001*
Obesity											
BMI >30 kg/m^2	1734	298·9	292·7, 305·5	5·9	4·6, 7·3	8·2	6·6, 9·9	21·6	19·6, 23·7	64·3	61·7, 66·7
BMI <30 kg/m^2	3410	321·1	315·7, 326·1	4·7	3·8, 5·6	6·2	5·3, 7·2	18·3	16·8, 19·7	70·8	68·9, 72·5
P			<0·0001*		0·0053*		0·0032*		<0·0001*		<0·0001*
Smoking status											
Smoker	803	296·8	287·3, 306·2	6·9	5·0, 9·4	7·3	5·5, 9·6	21·3	18·4, 24·4	64·5	60·7, 68·0
Non-smoker	4355	317·5	313·2, 322·2	4·7	3·9, 5·4	6·8	5·9, 7·7	19·0	17·7, 20·3	69·5	67·9, 71·0
P			0·0002*		<0·0001*		<0·0001*		<0·0001*		<0·0001*
Alcohol consumption											
Alcohol consumer	3638	314·3	309·7, 319·3	4·8	4·0, 5·8	6·3	5·4, 7·2	19·0	17·5, 20·3	69·9	68·1, 71·6
Non-consumer	1103	315·7	306·6, 324·7	5·4	4·0, 7·1	8·1	6·3, 10·2	19·5	17·0, 22·2	67·0	63·7, 70·1
P			0·8346		<0·0001*		<0·0001*		<0·0001*		<0·0001*
Physical activity											
Physically active	3637	312·5	305·2, 320·0	5·5	4·6, 6·5	6·1	5·2, 7·0	18·9	17·5, 20·2	69·5	67·8, 71·1
Not physically active	1479	313·9	309·0, 318·6	4·2	3·1, 5·5	8·6	7·0, 10·5	20·9	18·5, 23·3	66·3	63·4, 69·0
P			0·8000		<0·0001*		0·0008*		<0·0001*		<0·0001*
Habitation											
Lives alone	988	304·8	295·4, 314·3	6·5	4·8, 8·7	7·8	6·1, 10·0	20·2	17·5, 23·0	65·5	62·0, 68·7
Does not live alone	4170	316·4	311·8, 320·7	4·6	3·9, 5·3	6·6	5·7, 7·5	19·2	17·9, 20·5	69·6	68·0, 71·1
P			0·0319*		<0·0001*		<0·0001*		<0·0001*		<0·0001*
Asset wealth											
<Mean asset wealth	2622	309·0	303·4, 314·3	5·9	4·8, 6·9	7·2	6·1, 8·4	19·1	17·6, 20·5	67·8	65·7, 69·7
> Mean asset wealth	2270	320·0	314·7, 326·1	4·0	3·0, 4·9	6·4	5·1, 7·5	19·9	18·1, 21·7	69·7	67·6, 71·8
P			0·0080*		<0·0001*		0·0004*		0·0004*		<0·0001*
Season											
Summer	1290	304·5	296·8, 312·2	5·2	4·0, 6·6	7·8	6·2, 9·7	22·4	19·8, 25·1	64·6	61·5, 67·6
Autumn	1057	311·5	302·4, 320·7	6·3	4·7, 8·2	8·4	6·6, 10·5	17·7	15·1, 20·5	67·6	64·4, 70·6
Winter	1113	317·9	309·4, 326·5	4·6	3·3, 6·1	5·7	4·3, 7·5	20·6	18·0, 23·3	69·1	65·8, 72·2
Spring	1695	318·9	312·5, 325·8	4·6	3·4, 6·0	6·0	4·7, 7·4	17·6	15·8, 19·6	71·8	69·4, 74·0
P			<0·0001*		0·3126		0·0520		0·0173*		0·0031*
Location											
East/South East	2553	310·4	305·2, 316·1	5·5	4·4, 6·6	7·1	5·9, 8·4	19·5	17·9, 21·1	67·9	65·9, 69·9
South/South West	1562	311·5	304·5, 318·9	4·5	3·3, 5·8	7·0	5·6, 8·7	20·5	18·3, 22·9	68·0	65·2, 70·6
North and West	1043	324·0	314·7, 333·7	5·1	3·8, 6·8	6·1	4·7, 7·8	17·7	15·5, 20·1	71·1	67·8, 74·0
P			<0·0001*		0·4841		0·6132		0·2476		0·2387

* Statistically significant after adjustment for multiple comparisons ($P < 0·041$).
† *P* values indicate statistically significant pairwise comparisons of the difference in proportion of column criteria across row variables. For variables with more than two levels linear regression was used.

folate and had lower rates of deficiency compared with non-supplement users. For both biomarkers, men had significantly lower concentrations as did the oldest old (>80 years) ($P < 0·001$). For both total B_{12} and folate, those who were obese, smoked or reported lower mean asset wealth had significantly lower biomarker concentrations and higher rates of deficiency/low status. Seasonality was also significantly associated with both biomarker concentrations ($P < 0·001$). Concentrations of total B_{12} were the highest in spring and the lowest in summer (318·9 *v.* 304·5 pmol/l), whereas plasma folate concentrations were also the highest in spring but lowest in autumn (19·5 *v.* 16·4 nmol/l) (but we note that in the regression analyses, the effects of seasonality were only robust for folate concentrations in autumn *v.* winter). When examined by geographic area, participants residing in the North and West of the Island (Connaught and Ulster) had lower plasma

Table 2. Mean plasma folate with weighted prevalence of status by demographic and lifestyle characteristics†
(Weighted means and prevalence estimates and 95 % confidence intervals)

		Plasma folate									
				<10 nmol/l (*n* 731)		10–23 nmol/l (*n* 2610)		> 23–45 nmol/l		> 45 nmol/l (*n* 479)	
Characteristics	Subjects (*n*)	Average (nmol/l)	95 % CI	%	95 % CI	%	95 % CI	%	95 % CI	%	95 % CI
Supplements	162	24·5	20·7, 29·1	8·0	3·9, 15·3	35·5	28·0, 43·7	29·3	21·7, 38·1	27·2	19·9, 35·9
No supplements	5128	18·5	18·1, 19·0	15·1	13·8, 16·4	49·8	48·2, 51·4	26·7	25·2, 28·1	8·4	7·4, 9·4
P		0·0013*		<0·00001*		<0·00001*		<0·00001*		<0·00001*	
Sex											
Male	2476	17·9	17·4, 18·4	15·9	14·2, 17·6	51·9	49·7, 54·0	25·0	23·1, 26·9	7·2	6·0, 8·5
Female	2814	19·5	18·9, 20·1	14·0	12·4, 15·6	47·0	44·9, 49·1	28·4	26·5, 30·2	10·6	9·8, 12·1
P		<0·00001*		0·3284		0·2314		0·0009*		<0·00001*	
Age (years)											
50–59	2256	18·3	17·8, 18·9	14·3	12·6, 16·1	52·2	49·9, 54·4	26·0	23·9, 28·0	7·5	6·3, 8·9
60–69	1784	19·1	18·4, 19·7	14·5	12·7, 16·5	47·8	45·2, 50·3	28·8	26·5, 31·0	8·9	7·3, 10·7
70–79	968	19·8	18·9, 20·9	12·7	10·5, 15·3	48·0	44·2, 51·6	28·0	24·8, 31·4	11·3	9·0, 14·0
>80	282	16·7	15·2, 18·4	23·1	17·7, 29·6	45·1	38·4, 51·8	21·0	16·1, 26·6	10·8	7·6, 15·2
P		<0·00001*		0·0004*		0·041		0·0309*		0·0235*	
Obesity											
BMI >30 kg/m^2	1782	17·6	17·0, 18·2	16·8	14·7, 19·0	51·8	49·0, 54·4	24·4	22·1, 26·7	7·1	5·8, 8·6
BMI <30 kg/m^2	3493	19·2	18·7, 19·8	14·0	12·5, 15·4	48·3	46·4, 50·2	27·8	26·1, 29·5	9·9	8·7, 11·3
P		<0·00001*		<0·00001*		<0·00001*		<0·00001*		<0·00001*	
Smoking status											
Smoker	828	16·7	15·9, 17·6	19·2	16·5, 22·2	51·5	47·7, 55·2	22·6	19·4, 26·1	6·7	4·9, 8·8
Non-smoker	4462	19·2	18·7, 19·7	13·9	12·5, 15·2	48·9	47·2, 50·6	27·7	26·2, 29·2	9·5	8·3, 10·7
P		<0·00001		<0·00001		<0·00001		<0·00001		<0·00001*	
Alcohol consumption											
Alcohol consumer	3738	19·0	18·5, 19·5	14·0	12·6, 15·4	49·1	47·3, 50·9	27·7	26·0, 29·4	9·2	8·0, 10·4
Non-consumer	1123	18·1	17·4, 19·0	16·8	14·2, 19·6	48·9	45·7, 52·1	25·3	22·6, 28·0	9·0	7·2, 11·1
P		0·056		<0·00001*		<0·00001*		<0·00001*		<0·00001*	
Physical activity											
Physically active	3728	18·7	18·2, 19·3	14·5	13·1, 16·0	49·8	47·9, 51·5	26·6	25·0, 28·3	9·1	7·9, 10·3
Not physically active	1516	18·5	17·8, 19·2	15·7	13·5, 18·1	48·8	45·9, 51·6	27·0	24·5, 29·6	8·5	6·8, 10·4
P		0·571		<0·00001*		<0·00001*		<0·00001*		<0·00001*	
Habitation											
Lives alone	1011	18·2	17·3, 19·1	17·9	15·2, 20·9	46·8	43·2, 50·3	25·2	22·2, 28·3	10·1	8·1, 12·4
Does not live alone	4279	18·8	18·4, 19·3	13·9	12·6, 15·2	50·3	48·5, 51·9	27·2	25·7, 28·7	8·6	7·5, 9·7
P		0·2069		<0·00001*		<0·00001*		<0·00001*		<0·00001*	
Asset wealth											
<Asset wealth	2683	17·6	17·1, 18·2	16·3	14·6, 18·2	51·9	49·7, 54·00	24·7	22·8, 26·6	7·1	5·9, 8·3
> Asset wealth	2335	20·3	19·6, 21·1	12·3	10·6, 14·2	46·3	43·9, 48·6	30·0	27·8, 32·2	11·4	9·6, 11·3
P		<0·00001*		<0·00001*		<0·00001*		0·0633		0·2547	
Season											
Summer	1335	19·1	18·3, 19·8	13·1	11·0, 15·4	49·6	46·6, 52·5	29·7	26·9, 32·6	7·6	6·0, 9·5
Autumn	1069	16·4	15·6, 17·2	19·8	16·7, 23·3	51·5	47·7, 55·1	25·7	22·6, 29·0	3·0	1·9, 4·3
Winter	1145	19·3	18·4, 20·3	13·6	11·4, 16·0	49·4	45·9, 52·8	24·7	21·9, 27·6	12·3	10·2, 14·7
Spring	1738	19·5	18·8, 20·3	14·0	12·0, 16·0	48·1	45·3, 50·8	26·5	24·2, 28·9	11·4	9·5, 13·6
P		<0·00001*		0·0004*		0·5235		0·0852		<0·00001*	
Location											
East/South East	2604	21·1	20·4, 21·9	10·5	8·9, 12·0	46·4	44·2, 48·5	30·1	28·2, 32·1	13·0	11·3, 14·8
South/South West	1398	16·2	15·7, 16·8	20·3	17·7, 22·9	52·2	49·4, 54·9	23·2	20·9, 25·7	4·3	3·3, 5·4
North and West	1088	17·0	16·3, 17·7	18·0	15·4, 20·6	52·7	49·1, 56·2	23·6	20·7, 26·8	5·7	4·3, 7·5
P		<0·0001*		<0·0001*		0·0008*		<0·0001*		<0·0001*	

* Statistically significant after adjustment for multiple comparisons ($P < 0{\cdot}041$).
† *P* values indicate statistically significant pairwise comparisons of the difference in proportion of column criteria across row variables. For variables with more than two levels linear regression was used.

folate compared with those living in the East and South East (Leinster region) (16·2 *v.* 21·1 nmol/l). For medications, only metformin use was associated with lower total B_{12} concentrations (272·9 *v.* 315·7 pmol/l; $P < 0{\cdot}0001$) and a higher prevalence of B_{12} deficiency (11·0 *v.* 4·8 %; $P < 0{\cdot}0001$). There were no statistically significant differences in the concentration of total B_{12} or folate by statin, PPI or thyroid medication use (data not shown).

Regression analyses

In a multiple linear regression analysis (Table 3), the largest positive predictor of total B_{12} concentration was self-reported B_{12} injection and/or supplement use (coefficient 51·5 pmol; 95 % CI 9·4, 93·6; $P = 0{\cdot}016$). This was closely followed by being female and geographic location (North and West *v.* East and South East). The largest negative predictor was metformin

Table 3. Determinants of plasma total vitamin B_{12} concentrations (pmol/l) in older Irish adults (Regression coefficients and 95 % confidence intervals)

		Linearised		
Characteristics	Coefficient	SE	*P*	95 % CI
Age (years)	−0·94	0·25	<0·0001	−1·44, −0·45
Sex				
Female	17·91	4·13	<0·0001	9·80, 26·03
BMI				
Obese*	−21·70	4·33	<0·0001	−30·21, −13·20
Smoking status				
Current	−22·39	5·62	<0·0001	−33·43, −11·34
Physical activity				
Inactive†	0·69	5·72	0·584	−8·11, 14·39
B_{12} injection/supplement use				
User‡	51·53	21·42	0·016	9·45, 93·61
Folate supplement use				
User	−20·59	16·92	0·224	−53·82, 12·63
Season sampled				
Spring	3·13	5·72	0·584	−8·11, 14·39
Summer	−9·10	6·31	0·150	−21·49, 3·29
Autumn	−1·46	6·51	0·822	−14·25, 11·31
Alcohol use				
Consumer	−7·04	5·18	0·175	−17·23, 3·13
Living arrangement				
Alone	7·17	5·65	0·205	−3·93, 18·28
Province				
Ulster and Connacht	15·60	5·85	0·008	4·10, 27·11
Munster	3·15	4·86	0·517	−6·93, 12·69
Asset wealth				
> Mean asset wealth	8·89	4·35	0·042	0·33, 17·45
Metformin use				
User	−33·69	9·29	<0·0001	−51·94, −15·43
Statin use				
User	3·46	4·52	0·444	−5·41, 12·34
PPI use				
User	6·48	6·05	0·285	−5·41, 18·37
Thyroxine use				
User	6·05	8·64	0·484	−10·93, 23·03

PPI, proton pump inhibitors.
* Obesity = BMI >30 kg/m^2.
† Physical activity levels were defined by IPAQ categories.
‡ Vitamin B_{12} injections or vitamin B_{12}-containing supplements (including multi-vitamins) were defined by the use of the World Health Organization's Anatomical Therapeutic Chemical codes.

medication use (−33·6; 95 % CI −51·9, −15·4; $P<0·001$). Other smaller negative predictors included smoking and obesity. FA supplement use was not a significant predictor of total B_{12} concentrations. The largest positive predictor of plasma folate concentration was FA supplement use (6·0 nmol/l; 95 % CI 3·0, 9·0); $P<0·001$) followed by being female and statin medication use (Table 4). The largest negative predictor was geographic location (North, West and South *v.* East and South East; $P<0·001$) followed by data being sampled in the autumn, smoking and obesity.

Discussion

To our knowledge, we are the first to present prevalence data for B_{12} and folate status and their determinants in a large representative sample of the older Irish population. In a country with a voluntary fortification policy, the estimated prevalence of folate deficiency/low folate status is 15 % or one in seven people aged >50 years. These data are in stark contrast to countries with mandatory fortification (of enriched cereal grains or flour) such as the US where rates of low folate status (<10 nmol/l) have been reported in only 1·2 % of those aged ≥60 years (post-fortification)[25]. Such a high rate of low folate status is important given the implications of sub-optimal concentrations, particularly for older adults. Deficiencies in folate can result in impaired biosynthesis of DNA with clinical symptoms including the development of megaloblastic anaemia, alopecia, achromotrichia and neuropathy[26,27]. Longer-term, low folate status has been associated with higher rates of cognitive impairment, CVD and cancer mortality[28,29]. For instance, in the US National Health and Nutritional Examination Survey (NHANES) (1999–2010), women with folate concentrations of approximately 20 nmol/l had a 1·5-fold increased all-cause mortality risk in comparison with those >20 nmol/l folate concentrations[29]. In terms of B_{12}, the estimated prevalence of B_{12} deficiency and low B_{12} status (<185 pmol/l) in older Irish adults is 12 % or one in eight. Our data are similar to observations across other older adult surveys (aged >60 years). In NHANES (1999–2002), the prevalence of B_{12} deficiency (<148 pmol/l) was about 3 %, whereas 7 % had a low B_{12} status

Table 4. Determinants of plasma folate concentrations (nmol/l) in older Irish adults (Regression coefficients and 95 % confidence intervals)

		Linearised		
Characteristics	Coefficient	SE	*P*	95 % CI
Age (years)	0·35	0·29	0·237	−0·23, 0·93
Sex				
Female	2·04	0·43	<0·0001	1·19, 2·90
BMI				
Obese*	−2·16	0·47	<0·0001	−3·10, −1·23
Smoking status				
Current	−2·27	0·61	<0·0001	−3·49, −1·05
Physical activity				
Inactive†	−0·28	0·54	0·607	−1·35, 0·79
Folate supplement use				
User‡	6·02	1·53	<0·0001	3·01, 9·03
Season sampled				
Spring	−0·09	0·74	0·900	−1·54, 1·36
Summer	−1·37	0·72	0·059	−2·79, 0·05
Autumn	−4·81	0·69	<0·0001	−6·18, −3·44
Alcohol use				
Consumer	0·72	0·55	0·192	−0·36, 1·81
Living arrangement				
Alone	−0·33	0·66	0·616	−1·64, 0·97
Province				
Ulster and Connacht	−4·86	0·67	<0·0001	−6·17, −3·54
Munster	−5·70	0·55	<0·0001	−6·79, −4·61
Asset wealth				
> Mean asset wealth	1·59	0·52	0·003	0·56, 2·63
Metformin use				
User	0·61	1·17	0·598	−1·68, 2·91
Statin use				
User	1·36	0·57	0·019	0·22, 2·49
PPI use				
User	−1·11	0·70	0·114	−2·50, 0·26
Thyroxine use				
User	0·90	0·92	0·332	−0·92, 2·72

PPI, proton pump inhibitors.
* Obesity = BMI >30 kg/m^2.
† Physical activity levels were defined by IPAQ categories.
‡ Folate-containing supplements (including multi-vitamins) were defined by the use of the World Health Organization's Anatomical Therapeutic Chemical codes.

(<185 pmol/l)[30]. In three combined UK surveys (*n* 3511), one in twenty people (aged 65–74 years) and one in ten people (aged >75 years) had B_{12} deficiency (defined as <150 pmol/l)[31,32]. These observed high rates of B_{12} deficiency in older adults are of concern given that early diagnosis is important to avoid irreversible neurologic damage.

Both vitamin B_{12} and folate status have been examined previously in a much smaller, representative study of older Irish adults (*n* 358)[15]. In the National Adult Nutrition Study (NANS), the average B_{12} concentration for adults >50 years was 281 pmol/l, which is close to the value of 308 pmol/l observed in the current study. Our data for folate are somewhat lower than in NANS, which may be explained by using the matrix of plasma instead of serum[33]. Interestingly, our plasma folate data were closely aligned with a study from Northern Ireland (*n* 662) where the folate concentrations of low-medium consumers of FA fortified foods were similar (16·2–22·6 nmol/l)[34]. However, unlike the NANS or Hoey *et al.* study, which were both limited to a small sample number and a narrow range of potential determinants, using the TILDA data allowed for the examination of a wide range of possible health and lifestyle determinants. We observed that the oldest old (>80 years) had the highest rates of both B_{12} deficiency and low folate status (6·6 and 23·1 %, respectively). Older adults can be at a particular risk of deficiency owing to increased malabsorption from atrophic gastritis, infection or medication interactions[8]. Interestingly, we also observed that men and those with a lower asset wealth also had lower folate and B_{12} concentrations. It has been reported that men often display a lower B-vitamin status than women[15,35], which in some studies has been attributed to a higher intake of supplements (which also appears to be a factor in the current study). In addition, it has been observed that older men tend to have a poorer awareness of healthy diet recommendations compared with women who were also more likely to have healthier dietary patterns[36,37]. Few studies have examined measures of economic wealth and B-vitamins. Pfeiffer *et al.*[35] in NHANES (2003–2006) reported that those with a lower poverty income ratio had a significantly lower serum folate concentration. Similar dietary mechanisms may explain the decrease in B-vitamin status with age and lower asset wealth including compromised food quantity, quality and variety and lower fruit and vegetables intakes in those with lower incomes[36–38].

Obesity and smoking were also observed as risk factors for lower B-vitamin status in the current study. Obesity has been

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associated previously with higher erythrocyte folate and lower serum folate in 3767 adults from NHANES. The suggested mechanisms included volumetric dilution, lower folate intakes, lower folate intestinal absorption and higher folate uptake by red blood cells[39]. This is of concern for Ireland, as over half of the over 50s population are classified as centrally obese, with only 16 % of men and 26 % of women having a normal BMI[40]. Smoking has also been associated with lower B-vitamin concentrations in both the British National Diet and Nutrition Survey and in the US NHANES (2003–2006)[35,41]. It has been hypothesised that nitric oxide or hydrogen sulphide could oxidise or compete for the B_{12} cobalt atom, whereas tetrahydrofolates could react with cyanates to form biologically inactive derivatives[42].

Uniquely, we were also able to examine the associations with geographic location and with seasonality. We observed that concentrations of folate were lowest in autumn and concentrations of B_{12} were lowest in summer compared with winter. Some[43–46] but not all studies[47,48] examining folate concentrations after exposure to UV light from sun-bed use or outdoor exposure have observed a lower status. For example, in a small study of forty-five Australian women (aged 18–47 years), UV exposure appeared to reduce the effectiveness of FA supplementation[45]. Other studies have also observed that UV exposure may affect B_{12} status. In a cohort of 1013 elderly Chileans, solar radiation was associated with B_{12} deficiency[49]. Similar mechanisms for both B_{12} and folate have been proposed including direct and indirect photo-degradation by UV radiation and by the formation of free radicals[50,51]. These observations may not only be important for B-vitamin status but also for other micronutrients as UV exposure is the main determinant of vitamin D status.

It is important to note that FA supplement use was not a contributor to B_{12} concentrations or status in our population, as there have been some concerns that high folate status could exacerbate the clinical effects of B_{12} deficiency[52]. Within TILDA, 8·9 % had a folate concentration >45 nmol/l. This is lower than the frequency observed either in NANS (19 %) or in post-fortification NHANES in the USA (38 %)[15,53]. The same methodology was used in all three studies and the folate measurements for this study and for NANS were carried out in the same laboratory. It is unclear what the clinical implications are of high folate status. Previously, high folate concentrations have been correlated with unmetabolised FA in plasma[54], which has been suggested to be a driver of some adverse health effects[54]. In a cross-sectional study of 219 older adults (>65 years), higher folate concentrations were associated with lower brain volumes[55], whereas a higher cancer incidence was reported in the folate and B_{12} intervention group of the B-PROOF study of older adults, with the effect on cancer incidence more pronounced in the oldest old (>80 years)[56]. FA has been suggested to be a preventive agent for cancer initiation but a promoter of established neoplastic cells[57]. However, this effect is still not clear as higher levels of folate have been associated with a lower risk of lung cancer diagnosis in a case–control study of 366 adults[58], whereas lower folate status in comparison with high status has been associated with a higher risk of all-cause mortality[29].

The major strength of this study is that it is the largest nationally representative older population sample reported with detailed data on a wide range of potential determinants. A limitation is that no dietary intake data were available including the consumption of fortified food products or the dosage of B-vitamin supplements taken. However, previous studies have shown a weak relationship between intake and blood biomarker data for these micronutrients[15,34]. One recent study has indicated that dietary intake data and biomarkers reflect the same underlying folate status with a systematic under-estimation bias in intake data[59]. Furthermore, our study was limited to the use of plasma total B_{12} and folate as indicators of status with the corresponding benefits and drawbacks associated with each of these biomarkers (i.e. total B_{12} measurement in blood is known to have relatively low sensitivity for estimating B_{12} deficiency[60], whereas plasma folate is subject to variations from recent dietary intakes). It is also important to note that because of the recruitment sampling criteria these findings may not be applicable to non-Caucasians, older adults in institutional care or those with severe cognitive impairment.

In summary, we have observed high rates of low B_{12} and folate status coupled with a much smaller rate of high folate status in the older Irish population. Ideally, encouraging older persons to improve their normal eating practices and to follow the food pyramid has the potential to enhance the B_{12} and folate status of this population group. However, compliance to healthy eating recommendations, as well as practical issues such as cooking practices, bioavailability, medication intakes and age-related changes to the gastrointestinal tract, means that the holistic unfortified approach can be ineffectual. Our data (and others previously) indicate that the current policy of voluntary fortification is ineffective at preventing the occurrence of deficiency and low blood status. Serious consideration must now be given to the implementation of mandatory FA and B_{12} fortification. Such a policy would ensure that the requirements of the 'at-risk' older adult population are met while also helping to reduce the prevalence of folate-responsive neural tube defects in the unborn child. The continued delay in consideration of this implementation could put >180 000 and 145 000 older Irish adults at risk of the health consequences of low folate and B_{12} status, respectively.

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