Impact of voluntary fortification and supplement use on dietary intakes and biomarker status of folate and vitamin B-12 in Irish adults

Sinéad M Hopkins, Michael J Gibney, Anne P Nugent, Helene McNulty, Anne M Molloy, John M Scott, Albert Flynn, JJ Strain, Mary Ward, Janette Walton, and Breige A McNulty

ABSTRACT

Background: Ireland has traditionally operated a liberal policy of voluntary fortification, but little is known about how this practice, along with supplement use, affects population intakes and status of folate and vitamin B-12.

Objective: The aim was to examine the relative impact of voluntary fortification and supplement use on dietary intakes and biomarker status of folate and vitamin B-12 in Irish adults.

Design: Folic acid and vitamin B-12 from fortified foods and supplements were estimated by using brand information for participants from the cross-sectional National Adult Nutrition Survey 2008–2010. Dietary and biomarker values were compared between 6 mutually exclusive consumption groups formed on the basis of folate acid intake.

Results: The consumption of folic acid through fortified foods at low, medium, and high levels of exposure [median (IQR) intakes of 22 (13, 32), 69 (56, 84), and 180 (137, 248) μg/d, respectively]; from supplements [203 (150, 400) μg/d]; or from both sources [287 (220, 438) μg/d] was associated with significantly higher folate intakes and status compared with nonconsumption of folic acid (18% of the population). Median (IQR) red blood cell (RBC) folate increased significantly from 699 (538, 934) nmol/L in nonconsumers to 1040 (83, 1390) nmol/L in consumers with a high intake of fortified foods (P < 0.001), with further nonsignificant increases in supplement users. Supplement use but not fortification was associated with significantly higher serum vitamin B-12 concentrations relative to nonconsumers (P < 0.001). Two-thirds of young women had suboptimal RBC folate for protection against neural tube defects (NTDs); among nonconsumers of folic acid, only 16% attained optimal RBC folate.

Conclusions: The consumption of voluntarily fortified foods and/or supplement use was associated with significantly higher dietary intakes and biomarker status of folate in Irish adults. Of concern, the majority of young women remain suboptimally protected against neural tube defect; RBC, red blood cell; tHcy, total plasma homocysteine; WISP, Weighed Intake Software Package.

Keywords: folate intakes, vitamin B-12 intakes, B vitamin biomarkers, voluntary fortification, supplements

INTRODUCTION

Folate has a well-established role in the prevention of neural tube defects (NTDs)5 (1, 2) and the metabolically related B vitamin, vitamin B-12, was also shown to have a protective role independent of folate (3). Folate is available in the diet either in natural forms that occur in a variety of foodstuffs or in the synthetic form as folic acid, which is present only in dietary supplements and in fortified foods. Government bodies worldwide advise women of reproductive age to consume a daily folic acid supplement for NTD prevention. However, public health campaigns have been largely unsuccessful (4), and as a result, some countries have opted for a policy of mandatory folic acid fortification of flour or bread alongside recommendations on supplement use. Mandatory fortification has been highly effective in reducing the number of NTD-affected pregnancies in these countries (5, 6). Nonetheless, certain concerns have been raised that the subsequent increase in folic acid intakes across all population subgroups may have unintended harmful effects on health, such as masking of pernicious anemia (7), colorectal cancer promotion in people with pre-existing lesions (8), or even incident cancer in elderly populations (9) or adverse cognitive effects in older adults with low vitamin B-12 status (10). Consequently, the US NHANES extended its monitoring program to examine folic acid intakes and corresponding folate biomarker status from all sources of folic acid in the US diet, including mandatory fortification, voluntary fortification, and supplements (11).

In the past decade, mandatory folic acid fortification has been considered by European countries, including Ireland (12) and the
United Kingdom (13), but to date remains nonexistent in Europe. In contrast, voluntary fortification with micronutrients including folic acid and vitamin B-12 is permitted in some countries, with Ireland considered to have one of the most liberal policies (14, 15). Currently, there is no routine monitoring in place to measure the impact of such voluntary fortification on micronutrient intakes and status, in part due to the ad hoc nature of voluntary fortification and also due to the aggregated presentation of all vitamin forms in European food-composition tables (16). Moreover, supplement use is often not accounted for in national dietary surveys, nor are blood samples routinely collected for the measurement of biomarker status of folate, vitamin B-12, or other micronutrients.

The Irish National Adult Nutrition Survey (NANS) 2008–2010 is one of the few national dietary surveys in Europe to have collected comprehensive, brand-level dietary intake data on both fortified foods and dietary supplements in addition to biomarker data on B-vitamin status. Thus, it provides an excellent opportunity to assess the impact of voluntary fortification and supplement use in a nationally representative population exposed to a high level of voluntary fortification. The aims of this article were therefore to evaluate dietary intakes and status of folate and vitamin B-12 in the Irish adult population and to examine the relative contribution of voluntary fortification and supplement use to these intakes and corresponding biomarkers.

METHODS

Sampling procedure

Data for this analysis were derived from the NANS, a cross-sectional food-consumption survey carried out between May 2008 and April 2010 in the Republic of Ireland in a national sample of 1500 adults aged 18–90 y (men, n = 760; women, n = 740). A detailed description of the methodology used in the NANS was reported elsewhere (17). However, a concise overview of subject sampling and recruitment procedures, as well as methods of data collection and laboratory analysis pertinent to the objectives of the present work, is outlined below. Ethical approval was obtained from University College Cork Clinical Research Ethics Committee of the Cork Teaching Hospitals and the Human Ethics Research Committee of University College Dublin. Written consent was obtained from all participants in accordance with the Declaration of Helsinki. Because the Republic of Ireland does not have a national identification system for adults, a database of names and addresses held by Data Ireland (National Postal Service) was used to randomly select persons in 20 geographical clusters across the country, selected to provide proportional representation across the urban-rural continuum. A sample of 1500 free-living adults to represent a population of >4 million people participated in the dietary survey. The sample size was chosen to deliver at least 100 individuals in the least-populated age and sex subgroups. There were few exclusion criteria, other than pregnancy/lactation and inability to complete the survey because of disability. The sample was representative of the Irish adult population with respect to age, sex, social class, and urban/rural location when compared with the 2006 Irish census (18).

In addition to the collection of food and beverage intake data and blood samples for nutritional biochemistry, questionnaires were administered to collect data on sociodemographic characteristics (including education and social class) and health and lifestyle factors (including smoking status and medication usage); anthropometric measurements, including height, weight, waist and hip circumference and body composition, were measured in the participants’ homes (17). Participation in the survey did not require the provision of a blood sample. The overall response rate of the survey, which was calculated as the number of participants who completed the 4-d food diary divided by the total number selected, was 59.6%. For the purpose of this article, only participants who provided dietary intake data and who had biochemical data on folate and vitamin B-12 status were included (n = 1136). A further 10 participants who were receiving vitamin B-12 injections (n = 4) or taking high-dose folic acid (≥5 mg; n = 6) were excluded, which resulted in a final sample size of 1126 participants.

Dietary assessment

Food and beverage intake data were collected by using a 4-consecutive-day semiweighed food diary, which included at least 1 weekend day. Participants were asked to record the type and amount of all food, beverages, and supplements consumed and, where applicable, record recipes, cooking method, and details of leftovers. A quantification protocol developed by the Irish Universities Nutrition Alliance for the North/South Ireland Food Consumption Survey was updated for NANS and is described elsewhere (19). Participants recorded their food intake at the brand level wherever possible and were asked to retain packaging of foods they consumed, which was later used to develop the Irish Food and Ingredients Database (INFID), version 3.0 (20). The INFID is a multifaceted database that records detailed information (including nutritional content and ingredients list) from the packaging of branded foods and beverages consumed during NANS and previous food consumption surveys in Ireland. Food intake data were analyzed by using the food-composition database Weighed Intake Software Package (WISP), version 3.0 (Tinuviel Software), which uses data from the sixth and fifth editions of McCance and Widdowson’s The Composition of Foods plus all 9 supplemental volumes to generate nutrient intake, as described elsewhere (17). Adjustments were made to the food-composition database to take account of recipes, nutritional supplements, commonly consumed generic Irish foods, and new foods on the market. All food and beverages consumed in NANS were grouped into 1 of 21 food groups. Folate and vitamin B-12 intakes from natural food sources and fortified foods were estimated by using WISP, customized for NANS as described above and further modified for the purposes of the current analysis in relation to folate and vitamin B-12 values. WISP provides compositional data on the total folate and vitamin B-12 content of foods but does not distinguish between the natural form of the vitamin and any synthetic form that may be added through fortification. Therefore, fortified foods containing folic acid and vitamin B-12 were initially identified from the presence of the vitamin on the ingredients list by using INFID, manufacturers’ websites, or by supermarket audits. To distinguish between the natural folate and vitamin B-12 content and that which is added during fortification, manufacturers were contacted to determine how B vitamins are declared on their nutrition labels. The majority reported that the vitamin value on
the label was a combination of the natural and synthetic forms of the vitamin. Therefore, the natural B-vitamin content of each food was estimated from published food-composition data (17) and subtracted from the total to determine the synthetic content. Existing fortified foods in the database were updated to reflect current levels of fortification, and newly identified fortified foods were allocated a new food code. Apart from these modifications, WISP was also customized for the purpose of this study to include the contribution of supplements. The vitamin content of supplements was obtained from INFID or directly from product labels. Overall, 5 descriptors for folate and vitamin B-12 intakes were created and will be referred to throughout the article as follows: 1) natural (folate or vitamin B-12 naturally occurring in foods), 2) synthetic–fortified foods (folic acid or crystalline vitamin B-12 added during fortification), 3) synthetic–supplements (folic acid or crystalline vitamin B-12 used in supplement formulations), 4) total synthetic (a combination of folic acid and vitamin B-12 from fortified foods and supplements), and 5) total (a combination of natural and total synthetic intakes). Henceforth, both synthetic forms of folate will be referred to as folic acid. Dietary folate equivalents (DFEs) were also calculated on the basis of the following equation: DFE (\(\mu g\)) = natural folate (\(\mu g\)) + 1.7 \times added folic acid in foods (\(\mu g\)) (21).

**Blood sampling and biomarker analysis**

Participants who consented to give a blood sample were asked to attend a designated phlebotomy clinic within their area, or for older adults who were unable to travel the samples were collected in the participant’s home by a qualified phlebotomist. All participants were asked to refrain from consuming food, beverages, and supplements overnight for 12 h before their appointment the following morning. A total of 1136 respondents (75.7% of the total sample) successfully provided a blood sample, of which 79% were fasting samples. The blood samples reached laboratories at University College Dublin or University College Cork within 5 h of collection (time delays between 30 min and 5 h) and were processed and stored at –80°C until required for further analysis. Red blood cell (RBC) folate, serum folate (22), and serum vitamin B-12 (23) were measured by microbiological assay, and total plasma homocysteine (tHcy) was measured by fluorescence polarization immunoassay (24). Full blood counts were conducted by using the Beckman Coulter counter from which packed cell volume was obtained for the calculation of RBC folate concentrations. The 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C>T genotype (25) was determined by polymerase chain reaction amplification followed by \(Hin F1\) restriction digestion, which was carried out by LGC (www.lgcgroup.com). Samples were analyzed blind for all assays, and quality control was carried out by repeated analysis of stored batches of pooled samples covering a wide range of values. Intra- and interassay CVs were \(\leq10.9%\) for serum folate, \(\leq13.8%\) for RBC folate, \(\leq11.0%\) for serum vitamin B-12, and \(\leq7.3%\) for tHcy.

**Statistical analysis**

All statistical analyses were performed by using PASW, version 18 (SPSS). The distributions of all dietary variables and biomarkers were positively skewed; therefore, the data are presented as medians and IQRs. The \(n\) value was reduced slightly for analyses on biomarker variables to take account of missing data for some participants: serum folate (\(n = 11\)), RBC folate (\(n = 8\)), serum vitamin B-12 (\(n = 12\)), and tHcy (\(n = 10\)). A 2-factor ANOVA with Scheffé post hoc tests was used to assess the impact of sex and age on biomarkers of folate and vitamin B-12 status. The relations between dietary and biomarker variables were examined by using Pearson correlation and partial correlation coefficients. To examine the relative impact of voluntary fortification and supplement use, participants were categorized into 6 mutually exclusive consumption groups formed according to their source of folic acid intake from the 4-d food diary. Nonconsumers did not consume any folic acid during the food-diary recording period. Fortified-food consumers consumed a folic acid–fortified food at least once during the recording period but were further stratified into low, medium, and high consumers on the basis of tertiles of folic acid intake. Supplement users were defined as participants who consumed folic acid from a supplement at least once during the recording period but no folic acid from fortified food. Supplement users and fortified-food consumers consumed folic acid from both sources. Because most vitamin B-12–fortified foods also contained folic acid and almost all consumers of vitamin B-12 supplements also consumed folic acid supplements, a separate analysis according to mutually exclusive vitamin B-12 consumption groups was not conducted; instead, the fortified-food and supplement consumption groups based on folic acid intakes were also applied to vitamin B-12. Population characteristics were compared between consumption groups by using \(\chi^2\) analysis for categorical variables and 1-factor ANOVA for continuous variables with Scheffé post hoc tests. B-vitamin intakes and biomarker concentrations were compared by using ANCOVA with Bonferroni post hoc tests controlling for sex, smoking, BMI, and energy intakes. In a similar subanalysis, the proportions of women of reproductive age with optimal RBC folate (>907 nmol/L) and serum vitamin B-12 status (>221 pmol/L) for protection against NTDs (3, 26) were compared across 5 folic acid–consumption groups by using binary logistic regression with adjustment for the MTHFR genotype and smoking status. For all statistical analyses, continuous variables were log-transformed to normalize their distribution and \(P < 0.05\) was considered significant.

**RESULTS**

The final sample comprised 50% men and 50% women and included 67.7%, 19.7%, and 12.5% in the age groups 18–50, 51–64, and \(\geq65\) y, respectively. Most of the sample were from an urban location (70%), and almost half (45%) were classified as professionals or in technical or managerial occupations. The final sample did not differ from the total recruited sample in terms of age, sex, educational level, and location and remained representative of the Irish population with respect to these demographic characteristics (18). Furthermore, the use of folic acid or vitamin B-12 supplements was similar between those included in the present analysis (14%) and those participants who did not provide a blood sample (13%). There were no significant differences in biomarker concentrations between fasting (\(n = 895\)) and nonfasting (\(n = 231\)) participants, except...
for serum folate concentrations, which were significantly higher in nonfasting participants [median (IQR) of 31.6 (17.4, 40.4) nmol/L compared with 28.9 (15.6, 36.1) nmol/L]. The removal of participants who provided nonfasting blood samples (21% of the overall sample) did not change the main findings, and these participants were therefore included in the final analysis (data not shown).

### Folate and vitamin B-12 intakes in the total population

Median intakes of DFEs, total folate, natural folate, and total folic acid for the total population were 323, 312, 223, and 64 μg/d, respectively, with the lowest intakes of each form of folate reported among women aged 18–50 y (Table 1). The majority of the population were consumers of folic acid–fortified foods (79%), whereas the use of folic acid supplements ranged from 8% in older men (≥65 y) to 20% in women aged 51–64 y. The median intake of total vitamin B-12 was 4.2 μg/d for the total population, with a very small reported intake from fortified foods and supplements (0.3 μg/d). When adjusted for energy intakes, intakes of both total natural folate and total and natural vitamin B-12 increased significantly with age, which may be partially driven by the higher folic acid and synthetic vitamin B-12 intakes from fortified foods among older adult (≥65 y) consumers (Supplemental Table 1). Among supplement users, women had significantly higher energy-adjusted intakes of folic acid and vitamin B-12 from supplements compared with men (Supplemental Table 1). Overall, natural food sources made the greatest contribution to mean intakes of total folate (74%) and vitamin B-12 (87%) (data not shown). Folic acid and vitamin B-12 from fortified foods (mainly from breakfast cereals and fat spreads) contributed 20% and 8% to total folate and vitamin B-12 intakes, respectively, whereas supplements contributed only 5–6% to total intakes of both vitamins (data not shown).

### Folate and vitamin B-12 status and correlation with dietary intakes

Median concentrations of RBC folate, serum folate, serum vitamin B-12, and tHcy were 872 nmol/L, 25.5 nmol/L, 298 pmol/L, and 11.8 μmol/L, respectively, for the total population (Table 2). A significant sex-by-age interaction was observed for serum vitamin B-12 (P < 0.001) whereby concentrations tended to decrease with age for men but increased with age for women. Overall, women had significantly higher concentrations of serum folate (P = 0.016) compared with men, whereas men had significantly higher concentrations of tHcy (P < 0.001). Men and women aged ≥65 y had significantly higher concentrations of RBC folate (P = 0.001) and tHcy (P < 0.001) compared with the youngest age group (18–50 y). The proportions of the total population with low serum folate (<6.8 nmol/L) and low-marginal RBC folate (<453 nmol/L) and vitamin B-12 (<148 pmol/L) concentrations were <2%, 6%, and 7%, respectively (data not shown). Among consumers of fortified foods who did not consume folic acid supplements, RBC folate was significantly correlated with dietary folate intake expressed as DFEs (r = 0.367, P < 0.001) and was found to be more strongly correlated

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### TABLE 1

Intakes of folate and vitamin B-12 according to dietary source in the total population and percent consumers of fortified foods and supplements by sex and age group

<table>
<thead>
<tr>
<th>Source</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All ages</td>
<td>(18–50 y)</td>
</tr>
<tr>
<td>Folate intakes, μg/d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dietary folate equivalents</td>
<td>323 (234, 474)</td>
<td>389 (294, 554)</td>
</tr>
<tr>
<td>Total</td>
<td>312 (228, 448)</td>
<td>372 (280, 506)</td>
</tr>
<tr>
<td>Natural</td>
<td>223 (173, 286)</td>
<td>274 (204, 344)</td>
</tr>
<tr>
<td>Total folate</td>
<td>64 (14, 167)</td>
<td>74 (20, 175)</td>
</tr>
<tr>
<td>Fortified foods</td>
<td>50 (9, 118)</td>
<td>58 (12, 125)</td>
</tr>
<tr>
<td>Consumers of folic acid, %</td>
<td>79</td>
<td>80</td>
</tr>
<tr>
<td>Fortified foods</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Supplements</td>
<td>4.2 (2.9, 6.1)</td>
<td>5.0 (3.4, 7.0)</td>
</tr>
<tr>
<td>Natural</td>
<td>3.7 (2.5, 5.1)</td>
<td>4.3 (3.0, 5.9)</td>
</tr>
<tr>
<td>Total synthetic</td>
<td>0.3 (0.0, 0.8)</td>
<td>0.3 (0.0, 0.8)</td>
</tr>
<tr>
<td>Fortified foods</td>
<td>0.1 (0.0, 0.5)</td>
<td>0.2 (0.0, 0.5)</td>
</tr>
</tbody>
</table>

1Dietary folate equivalents were calculated as follows: natural folate (μg) + [folic acid from fortified foods (μg) × 1.7] (21).
2Median; IQR in parentheses (all such values).
3Refers to total folic acid–fortified foods only. Intakes from supplements are not given for the total population because of the low percentage of consumers.
4Refers to folic acid and synthetic vitamin B-12 intakes from fortified foods only. Intakes from supplements are not given for the total population because of the low percentage of consumers.
5Refers to total folic acid and total synthetic vitamin B-12 intakes from both fortified foods and supplements.
with added folic acid ($r = 0.309$, $P < 0.001$) than with natural food folate ($r = 0.175$, $P < 0.001$) (Figure 1). Corresponding intake-status correlations for serum folate showed a similar pattern when intakes were expressed as DFEs ($r = 0.417$, $P < 0.001$), added folic acid ($r = 0.396$, $P < 0.001$), or natural food folates ($r = 0.163$, $P < 0.001$). In nonconsumers of vitamin B-12 supplements, serum vitamin B-12 was weakly, although significantly, correlated with vitamin B-12 intake, including both total (including fortified foods) and natural vitamin B-12 intakes only ($r = 0.190$ and 0.166, respectively; $P < 0.001$) (Figure 2).

### B-vitamin dietary intakes and biomarker status according to intakes of folic acid–fortified foods and supplements

Almost one-fifth (18%) of the population reported consuming no folic acid from either fortified foods or supplements (group 1), whereas the majority (68%) consumed folic acid from fortified foods only (groups 2–4) (Table 3). Among supplement users, 3% did not consume fortified foods (group 5), whereas 11% also consumed folic acid from fortified foods (group 6). No significant differences between consumption groups in terms of social class, education, location, MTHFR 677C>T genotype, and fasting status were observed, but there were significant differences in sex, energy intake, smoking status ($P < 0.001$), and BMI ($P < 0.05$). Intakes of natural folate did not differ significantly between the consumption groups ($P = 0.366$); however, there was a significant stepwise increase in total folate and folic acid intakes with increasing intake of fortified foods and with supplement use ($P < 0.001$). The dietary intake pattern was typically reflected in serum folate ($P < 0.001$) and RBC folate ($P < 0.001$), but concentrations of both reached a plateau among high-fortified-food consumers with no further significant increases in supplement users. The pattern was less marked for vitamin B-12 because only supplement users had a significantly higher concentration of serum vitamin B-12 compared with nonconsumers ($P < 0.001$). Concentrations of tHcy were 1–2 μmol lower among medium- and high-fortified-food consumers and supplement users compared with nonconsumers and low-fortified-food consumers ($P < 0.001$). Only 3 participants had an intake of folic acid exceeding the Tolerable Upper Intake Level (1000 μg/d), of whom 1 participant was a high-fortified-food consumer and 2 were supplement users only. Prevalences of high serum folate concentrations (>45 nmol/L) were 4%, 8%, 10%, 34%, 28%, and 50% across consumption groups 1–6, respectively (data not shown). Overall, only 36% of women of reproductive age (134 of 371) achieved an optimal folate status for NTD protection (≥907 nmol/L), of whom a significantly higher proportion were in the high fortified-food consumer group (47%) and supplement user group (64%) compared with the other 3 groups (16–33%; $P = 0.008$) (Figure 3). An optimal serum vitamin B-12 concentration (>221 pmol/L) was observed for 70% of women, and there was no significant difference in the proportion achieving this concentration between consumption groups (Figure 3).

### DISCUSSION

The results showed that the consumption of voluntarily fortified foods and supplements was each associated with a significantly higher biomarker status of folate in a nationally representative sample of Irish adults. Nevertheless, their population impact was unevenly distributed and current biomarker concentrations of folate were deemed insufficient to adequately protect the majority of women of reproductive age against NTDs.

### TABLE 2

**Effects of sex and age on median concentrations of RBC folate, serum folate, serum vitamin B-12, and tHcy in Irish adults**

<table>
<thead>
<tr>
<th>Age group</th>
<th>Sex</th>
<th>Age</th>
<th>n</th>
<th>Value</th>
<th>Sex</th>
<th>Age</th>
<th>n</th>
<th>Value</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC folate, nmol/L</td>
<td>All ages (18–91 y)</td>
<td>18–50 y</td>
<td>51–64 y</td>
<td>≥65 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>1118</td>
<td>872 (672, 1196)</td>
<td>756</td>
<td>839 (660, 1137)</td>
<td>221</td>
<td>924 (700, 1260)</td>
<td>141</td>
<td>960 (747, 1356)</td>
<td>0.509</td>
</tr>
<tr>
<td>Men</td>
<td>563</td>
<td>923 (707, 1171)</td>
<td>388</td>
<td>905 (700, 1154)</td>
<td>112</td>
<td>924 (713, 1228)</td>
<td>63</td>
<td>964 (740, 1414)</td>
<td>0.126</td>
</tr>
<tr>
<td>Women</td>
<td>555</td>
<td>836 (649, 1228)</td>
<td>368</td>
<td>799 (626, 1110)</td>
<td>109</td>
<td>936 (696, 1334)</td>
<td>78</td>
<td>926 (747, 1296)</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum folate, nmol/L</td>
<td>All</td>
<td>1115</td>
<td>25.5 (16.7, 38.8)</td>
<td>756</td>
<td>24.9 (16.6, 36.0)</td>
<td>220</td>
<td>28.2 (17.9, 42.9)</td>
<td>139</td>
<td>28.0 (16.0, 46.3)</td>
</tr>
<tr>
<td>Men</td>
<td>560</td>
<td>24.9 (17.1, 36.7)</td>
<td>387</td>
<td>24.4 (17.0, 35.5)</td>
<td>110</td>
<td>24.8 (16.9, 38.9)</td>
<td>63</td>
<td>30.2 (19.2, 38.6)</td>
<td>0.071</td>
</tr>
<tr>
<td>Women</td>
<td>555</td>
<td>26.1 (16.4, 41.2)</td>
<td>369</td>
<td>25.2 (16.1, 37.2)</td>
<td>110</td>
<td>32.0 (18.9, 47.8)</td>
<td>76</td>
<td>27.0 (14.1, 54.9)</td>
<td>0.016</td>
</tr>
<tr>
<td>Serum vitamin B-12, pmol/L</td>
<td>All</td>
<td>1114</td>
<td>298 (224, 378)</td>
<td>756</td>
<td>305 (226, 379)</td>
<td>220</td>
<td>285 (217, 383)</td>
<td>138</td>
<td>277 (216, 369)</td>
</tr>
<tr>
<td>Men</td>
<td>559</td>
<td>314 (238, 388)</td>
<td>387</td>
<td>328 (257, 403)</td>
<td>110</td>
<td>295 (209, 383)</td>
<td>62</td>
<td>247 (205, 322)</td>
<td>0.475</td>
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<tr>
<td>Women</td>
<td>555</td>
<td>289 (215, 369)</td>
<td>369</td>
<td>285 (205, 360)</td>
<td>110</td>
<td>282 (228, 400)</td>
<td>76</td>
<td>306 (250, 387)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>tHcy, μmol/L</td>
<td>All</td>
<td>1116</td>
<td>11.8 (10.1, 13.8)</td>
<td>756</td>
<td>11.4 (9.8, 13.1)</td>
<td>219</td>
<td>12.4 (10.8, 14.8)</td>
<td>141</td>
<td>13.2 (11.1, 15.8)</td>
</tr>
<tr>
<td>Men</td>
<td>562</td>
<td>12.4 (10.8, 14.4)</td>
<td>388</td>
<td>12.0 (10.4, 13.7)</td>
<td>111</td>
<td>13.2 (11.5, 15.4)</td>
<td>63</td>
<td>13.9 (11.7, 17.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Women</td>
<td>554</td>
<td>11.1 (9.5, 12.9)</td>
<td>368</td>
<td>10.7 (9.3, 12.3)</td>
<td>108</td>
<td>11.7 (10.2, 14.3)</td>
<td>78</td>
<td>12.2 (10.4, 15.5)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

1Values across a row without a common superscript letter are significantly different (Scheffé post hoc test, $P < 0.05$). RBC, red blood cell; tHcy, total homocysteine.
2Main effects and interaction effects were assessed by 2-factor ANOVA.
3Median; IQR in parentheses (all such values).
In the case of vitamin B-12, only supplement use was associated with improved biomarker status. These outcomes could help inform the international debate on mandatory folic acid fortification and assist in the establishment of evidence-based dietary reference values for folate. Similar to an Irish study (27), we observed an adequate folate status and a low prevalence of
This could be partially attributed to an increase in voluntary fortification practices in Ireland because our results show an increase both in the percentage of consumers (from 67% to 79%) and total folate intakes (by ~50 μg/d) over the past decade (28). As per similar studies in Northern Ireland (29) and the United States (11), with increased consumption of folic acid from fortified foods there was a significant increase in RBC folate concentration, a long-term measure of folate status. Furthermore, the magnitude of increase in RBC folate status (26%) between the “medium” (825 nmol/L) and “high” (1040 nmol/L) folic acid consumer groups was similar to that estimated for a doubling of supplemental doses in the range of 50–400 μg/d (30). Moreover, high-fortified-food consumers (median intake: 180 μg/d) had RBC folate and tHcy concentrations similar to those in supplement users, supporting previous research showing the effectiveness of chronic low-dose folic acid intake in improving RBC folate status (31, 32), and in lowering tHcy concentrations (33). Also,
of note, high-fortified-food consumers in the current study had comparable serum folate and RBC folate concentrations to non–supplement users in the United States (34). Collectively, these observations support the view that chronic low-dose folic acid consumption from voluntary fortification has the potential to be as effective as both supplement use and mandatory fortification, at least when mean folate values are considered. The notable difference between mandatory and voluntary fortification, however, is that the impact of the latter will be entirely dependent on individual food choices, and the much lower folate status of nonconsumers of fortified foods (compared with mean population folate values) may have been overlooked previously. The current results showed that nonconsumers of folic acid from fortified food or supplements (18% of the Irish population) were at much greater risk of suboptimal folate status than the population as a whole. Of greatest concern, the majority of young women (66%) in the present study had suboptimal RBC folate for maximum protection against NTDs (26); this was most evident among nonconsumers of folic acid (84%), thus highlighting the disproportionate efficacy of voluntary fortification as a public health measure to prevent NTDs. Furthermore, only 16% of young women reported the use of folic acid supplements. These findings would therefore support the view that mandatory fortification is the only way to ensure some protection to all women against NTDs. Recently, a competent authority in Ireland advised against further increases in voluntary folic acid fortification (35), which may be driven by concerns related to the potential adverse health effects of high folic acid intakes. In the present study, folic acid intakes exceeding the Tolerable Upper Intake Level (1000 μg/d) were evident only in high-fortified-food consumers, 733 μg/d in consumers of fortified foods, 733 μg/d in consumers of supplements and fortified foods. Values across a row without a common superscript letter are significantly different (Bonferroni post hoc test, P < 0.05). DFE, dietary folate equivalent [calculated as natural folate (μg) + (folic acid from fortified foods, μg) × 1.7] (21); RBC, red blood cell; tHcy, total plasma homocysteine. 4General characteristics were compared between groups by using χ² analysis and 1-factor ANOVA (Scheffe post hoc tests). B-vitamin dietary intakes and biomarkers were compared by using 1-factor ANCOVA controlling for sex, BMI, energy intakes, and smoking status. 5Median; IQR in parentheses (all such values).
folic acid intakes from voluntary fortification is warranted and further analysis of this cohort could establish baseline levels of unmetabolized folic acid in Irish adults. The intake-status data presented in the current study will be of high relevance for international bodies tasked with setting dietary reference values for folate. It was recommended that consideration be given to differences in bioavailability of folate and folic acid when establishing dietary recommendations, chiefly through the use of DFEs (38). Indeed, this is the approach used currently in the United States (21) and recently proposed by the European Food Safety Authority (39). Our intake-status data, which showed that natural food folate compared with added folic acid was poorly correlated with folate status biomarkers (whether RBC or serum folate), confirm the relatively greater bioavailability of the latter (40). Therefore, the use of the DFE to express folate intake [which inherently adjusts for the higher bioavailability of added folic acid compared with natural food folate (21)] is more appropriate than the alternative approach of disregarding differences in folate bioavailability and treating natural food folates and added folic acid equally when considering dietary intakes or setting dietary reference values. Although vitamin B-12–fortified foods were consumed by 65% of the population, their impact on vitamin B-12 status was minimal, with only supplement use having a significant effect as reported elsewhere (11, 29, 41). The low vitamin B-12 supplement use and the low concentration of vitamin B-12 used in fortified products in Ireland may have implications for women of reproductive age because almost one-third had a vitamin B-12 concentration <221 pmol/L, exposing them to greater NTD risk (3). Older adults are also an “at risk” group due to the well-recognized problem of food-bound vitamin B-12 malabsorption. The higher vitamin B-12 status among older women compared with men may be explained by their higher intake of crystalline vitamin B-12 from supplements. Thus, more targeted dietary recommendations and health promotion campaigns may be required for these subgroups and consideration by policy makers of vitamin B-12 as well as folic acid fortification. One of the main strengths of this study is the nationally representative nature of the sample and the collection of detailed dietary data (to the brand level), which facilitated the estimation of synthetic vitamin intakes from fortification and supplement use. In addition, the measurement of RBC folate with the use of the microbiological assay is considered the gold standard method for measuring long-term folate status (42). Under- or overestimation of intakes was possible given the cross-sectional nature of the study and the reliance on nutrient information from food labels with no accounting for overage. Of note, the number of adults who consumed supplements only and not fortified foods (n = 36) was relatively small compared with the other consumption groups; therefore, conclusions related to supplement use only should be interpreted with caution. Furthermore, the creation of the consumption groups was based on the consumption of folic acid–fortified foods and supplements rather than on vitamin B-12.

The main results, however, remained unchanged when grouped by vitamin B-12 (data not shown). The lack of data on methylmalonic acid, which is considered a more-specific functional marker of vitamin B-12 status, may have been a further limitation; however, methylmalonic acid is specific only at concentrations indicative of deficiency (43). In conclusion, voluntary fortification and supplement use in Ireland were each associated with significantly improved dietary intakes and biomarker status of folate in the general population, but their impact was inadequate in providing the majority of women of reproductive age an optimal RBC folate concentration to protect against NTDs. These folate and vitamin B-12 intake-status data will serve as an important population-based baseline against which future changes in fortification practices and supplement use in Ireland can be monitored. Furthermore, it should help inform future policy decisions in countries that are now considering fortification.

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The authors’ responsibilities were as follows—SMH: conduct of experiment, data analyses, data interpretation, and manuscript writing; MG, APN, AF, JW, and BAM: survey design and implementation; MG, APN, HM, JMS, JJS, MW, AMM, and BAM: data interpretation and writing of the manuscript; APN: contribution to data analyses. All of the authors reviewed and approved the manuscript. None of the authors had a conflict of interest.

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HOPKINS ET AL.


