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

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Abbreviations: DFE, dietary folate equivalent, EFSA, European Food Safety Authority, INFID, Irish National Food and Ingredient Database, MMA, methylmalonic acid, MTHFR, methylene tetrahydrofolate reductase, NANS, National Adult Nutrition Survey, NTD, neural tube defect, RBC, red blood cell, tHcy, total plasma homocysteine

Running head: Dietary intakes and status of folate and vitamin B-12 in Irish adults

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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: 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 using brand information for participants from the cross sectional National Adult Nutrition Survey 2008-10. Dietary and biomarker values were compared across six mutually exclusive consumption groups formed on the basis of folic acid intake.

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

Conclusion: 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 sub optimally protected against NTDs.

INTRODUCTION

Folate has a well-established role in the prevention of NTDs (1, 2) and more recently, the metabolically related B vitamin, vitamin B-12 has also been shown to have a protective role independent of folate (3). Folate is available in the diet either in natural forms occurring in a variety of foodstuffs or in the synthetic form as folic acid which is present only in dietary supplements and 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 anaemia (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 National Health and Nutrition Examination Survey has extended its monitoring programme 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 last decade, mandatory folic acid fortification has been considered by European countries including Ireland (12) and the United Kingdom (13) but to date remains non-existent 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 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 paper 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.

SUBJECTS AND 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 years (men: n = 760; women: n = 740). A detailed description of the methodology used in the NANS has been 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, are 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.

As 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 twenty 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 over 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 sub-groups. There were few exclusion criteria, other than pregnancy/lactation and inability to complete the survey due to disability. The sample was representative of the Irish adult population with respect to age, gender, social class and urban/rural location when compared to 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 socio-demographics (including education and social class) and health and lifestyle factors (including smoking status and medication usage) and anthropometric measures including height, weight, waist and hip circumference and body composition were measured in the participant's homes (17). Participation in the survey did not require provision of a blood sample. The overall response rate of the survey which was calculated as the number of participants who completed the 4 day food diary divided by the total number selected was 59.6%. For the purpose of this paper, only participants who provided dietary intake data and 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 (≥5mg) (n=6) were excluded resulting in a final sample size of 1126.

Dietary Assessment

Food and beverage intake data were collected using a 4 consecutive day semi-weighed food diary which included at least one 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 brand level where possible and were asked to retain packaging of foods they consumed which was later used to develop the Irish Food and Ingredients Database version 3.0 (INFID) (20). INFID is a multifaceted database recording 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 analysed using the food composition database WISP© version 3.0 (Tinuviel Software, Anglesey, UK) which uses data from McCance and Widdowson's 'The Composition of Foods' sixth and fifth editions plus all nine 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 one of 21 food groups.

Folate and vitamin B-12 intakes from natural food sources and fortified foods were estimated 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 using INFID, manufacturer's 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 paper to include the contribution of supplements. The vitamin content of supplements was obtained from INFID or directly from product labels.

Overall, five descriptors for folate and vitamin B-12 intakes were created and will be referred to throughout the paper as **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 (DFE's) were also calculated based on the following equation: Dietary folate equivalent (µg) = natural folate (µg) + 1.7 x added folic acid in foods (µg) (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 fast from food, beverages and supplements overnight for 12hrs prior to 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 in University College Dublin or University College Cork within 5 h of collection (time delays between 30 min and5 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 florescence polarization immunoassay (24). Full blood counts were performed on the Beckman coulter counter from which packed cell volume was obtained for the calculation of RBC folate concentrations. The 5, 10 methylenetetrahydrofolate reductase 677C→T genotype (MTHFR) (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 analysed 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 inter-assay coefficients of variation were ≤10.9% for serum folate; ≤13.8% for RBC folate; ≤11.0% for serum vitamin B-12 and ≤7.3% for tHcy.

Statistical Analysis

All statistical analyses were performed using PASW version 18 (SPSS Inc. Chicago, IL, USA). The distributions of all dietary variables and biomarkers were positively skewed; therefore the data were presented as medians and interquartile ranges. 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 two-way ANOVA with scheffe post hoc tests was used to assess the impact of sex and age on biomarkers of folate and vitamin B-12 status. The relationships between dietary and biomarker variables were examined using Pearson's correlation and Pearson's partial correlation coefficients. To examine the relative impact of voluntary fortification and supplement use, participants were categorised into six mutually exclusive consumption groups formed according to their source of folic acid intake from the 4-day food diary. Non consumers consumed no folic acid during the food diary recording period. Fortified food consumers consumed a folic acid fortified food at least once during the recording period and were further stratified into low, medium and high consumers based on 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. As 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 across consumption groups using chi square analysis for categorical variables and one-way ANOVA for continuous variables with scheffe post hoc tests. B vitamin intakes and biomarker concentrations were compared using ANCOVA with Bonferroni post hoc tests controlling for

199 sex, smoking, body mass index and energy intakes. In a similar sub analysis, the proportion of
200 women of reproductive age with optimal RBC folate (>907nmol/L) and serum vitamin B-12
201 status (>221pmol/L) for protection against NTDs (26, 3) were compared across five folic acid
202 consumption groups using binary logistic regression adjusting for the MTHFR genotype and
203 smoking status. For all statistical analyses, continuous variables were log transformed to
204 normalise their distribution and $P<0.05$ was considered statistically significant.

205

206

207 **RESULTS**

208 The final sample comprised of 50% males and 50% females and included 67.7%, 19.7% and
209 12.5% in the age groups 18-50, 51-64 and ≥65 years respectively. The majority of the sample
210 were from an urban location (70%) and almost half (45%) were classified as professionals or
211 in technical or managerial occupations. The final sample did not differ from the total recruited
212 sample in terms of age, sex, education level and location and remained representative of the
213 Irish population with respect to these demographics. (18). Furthermore, the use of folic acid
214 or vitamin B-12 supplements was similar between those included in the present analysis
215 (14%) and those participants who did not provide a blood sample (13%).There were no
216 significant differences in biomarker concentrations between fasting (n=895) and non-fasting
217 (n=231) participants, except for serum folate concentrations which were significantly higher
218 in non-fasting participants [median (IQR) 31.6 (17.4, 40.4) nmol/L compared to 28.9 (15.6,
219 36.1) nmol/L]. Removal of participants who provided non-fasting blood samples (21% of the
220 overall sample) did not change the main findings and these participants were therefore
221 included in the final analysis (Data not shown).

222

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 323μg/d, 312μg/d, 223μg/d and 64μg/d respectively with the lowest intakes of each form of folate reported among women aged 18-50yrs (**Table 1**). The majority of the population were consumers of folic acid fortified foods (79%) while the use of folic acid supplements ranged from 8% in older men (≥65y) to 20% in women aged 51-64y. The median intake of total vitamin B-12 was 4.2μg/d for the total population with a very small reported intake from fortified foods and supplements (0.3μg/d). When adjusted for energy intakes, intakes of both total and 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 (≥65yrs) consumers (**Supplemental Table 1**). Among supplement users, females had significantly higher energy adjusted intakes of folic acid and vitamin B-12 from supplements than males (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 while 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 872nmol/L, 25.5nmol/L, 298pmol/L and 11.8μmol/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 to men while men had significantly higher concentrations of tHcy (P<0.001). Men and women aged ≥65yrs had significantly higher concentrations of RBC folate (P=0.001) and tHcy (P<0.001) compared to the youngest age group (18-50yrs). The proportion of the total population with low serum folate (<6.8nmol/L), low-marginal RBC folate (<453nmol/L) and vitamin B-12 (<148pmol/L) concentrations was < 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 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 DFE (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 non-consumers of vitamin B-12 supplements, serum vitamin B-12 was weakly though significantly correlated with vitamin B-12 intake, both total (including fortified food) and natural vitamin B-12 intake 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) while 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) while 11% also consumed folic acid from fortified foods (group 6). No significant differences across 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 across 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 as only supplement users had a significantly higher concentration of serum vitamin B-12 compared to non-consumers (P<0.001). Concentrations of tHcy were 1-2μmol lower among medium and high fortified food consumers and supplement users compared to non-consumers and low fortified food consumers (P<0.001). Only 3 participants had an intake of folic acid exceeding the tolerable upper level (1,000μg/d); of which, one participant was a high fortified food consumer and two were supplement users only. The prevalence of high serum folate concentrations (>45nmol/L) was 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 out of 371) achieved an optimal folate status for NTD protection (>907nmol/L), of which, a significantly higher proportion were in the high fortified food consumer group (47%) and supplement user group (64%) compared to the other three groups (16-33%) (P=0.008) (**Figure 3**). An optimal serum vitamin B-12 concentration (>221pmol/L) was observed for 70% of women and there was no significant difference in the proportion achieving this concentration across the consumption groups (Figure 3).

DISCUSSION

The results showed that consumption of voluntarily fortified foods and supplements were each associated with 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. 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 a recent Irish study (27), we observed an adequate folate status and a low prevalence of clinical deficiency in the general population. This could be partially attributed to an increase in voluntary fortification practices in Ireland as our results show an increase both in the number of consumers (from 67% to 79%) and total folate intakes (by ≈ 50µg/d) over the past decade (28). As per similar studies in Northern Ireland (29) and the US (11), with increased consumption of folic acid from fortified foods there was a significant increase in RBC folate status, a long term measure of folate status. Furthermore, the magnitude of increase in RBC folate status (26%) between the 'medium' (825nmol/L) and 'high' (1040nmol/L) folic acid consumer groups was similar to that estimated for a doubling of supplemental doses in the range of 50-400µg/d (30). Moreover, high fortified food consumers (median intake 180µg/d) had similar RBC folate and tHcy concentrations to that of supplement users, supporting previous research showing the effectiveness of chronic low dose folic acid intake in improving RBC folate status (31, 32), and 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 US (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 non-consumers of fortified foods (compared with mean population folate values) may have been overlooked previously. The current results showed that non-consumers of folic acid from fortified food or supplements (18% of the Irish population) were at much greater risk of suboptimal folate status compared with 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) and this was most evident among non-consumers of folic acid (84%), thus highlighting the disproportionate efficacy of voluntary fortification as a public health measure to prevent NTD. Furthermore, only 16% of young women reported 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 has 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 level (1,000µg/d) were only evident in 0.2% of the population (n=3) and the 95th percentile of intake was 437µg/d in high fortified food consumers ,733 µg/d in consumers of supplements only and 1320µg/d in consumers of both. This indicates that supplement use was more likely to contribute to intakes above the tolerable upper level rather than fortification, which is in agreement with data from the US and Canada (11, 36). The significance of high serum folate concentrations (>45nmol/l) detected in 19% of the population is unclear but it has been correlated with unmetabolised folic acid in plasma (37) which in turn has been hypothesised to mediate some of the adverse effects associated with

high folic acid (9). In the interim, continued monitoring of folic acid intakes from voluntary fortification is warranted while further analysis of this cohort could establish baseline levels of unmetabolised 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 has been recommended that consideration be given to differences in bioavailability in folate and folic acid when establishing dietary recommendations, chiefly through the use of DFE (38). Indeed, this is the approach used currently in the US (21) and recently proposed by the European Food Safety Authority (EFSA) (39). Our intake-status data, showing that natural food folate compared to added folic acid was poorly correlated with folate status biomarkers (whether RBC or serum folate) , confirms 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 to 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 level of 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 as almost one third had a vitamin B-12 concentration below 221pmol/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 B-12 mal-absorption. The higher vitamin B-12 status among older women compared to 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 policymakers of vitamin B-12 fortification as well as folic acid.

One of the main strengths of this study is the nationally representative nature of the sample and the collection of detailed dietary data (to brand level) which facilitated the estimation of synthetic vitamin intakes from fortification and supplement use. Additionally, the measurement of RBC folate using the microbiological assay is considered as the gold standard method for measuring long term folate status (42). Under- or over-estimation of intakes was possible given the cross-sectional nature of the study and the reliance on nutrient information from food labels with no account for overage. Of note, the number of adults who consumed supplements only and not fortified foods (n=36) was relatively small compared to the other consumption groups, therefore conclusions related to supplement use only should be interpreted with caution. Furthermore, the creation of the consumption groups were based on the consumption of folic acid fortified foods and supplements rather than vitamin B-12. The main results, however, remained unchanged when grouped by vitamin B-12 (data not shown). The lack on data on methylmalonic acid (MMA) which is considered a more specific functional marker of vitamin B-12 status may have been a further limitation; however, MMA 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 now considering fortification.

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Table 1. Median intakes of folate and vitamin B-12 according to dietary source in the total population and percentage consumers of fortified foods and supplements by sex and age group

	All Ages	Males			Females		
	18-91y	**18-50y**	**51-64y**	**≥ 65y**	**18-50y**	**51-64y**	**≥ 65y**
	(n=1126)	*(n=392)*	*(n=112)*	*(n=63)*	*(n=371)*	*(n=110)*	*(n=78)*
Folate intakes (µg/d)							
DFE	323 (234, 474)[1]	389 (294, 554)	409 (292, 580)	356 (233, 581)	267 (197, 363)	303(230, 464)	269 (210, 451)
Total[2]	312 (228, 448)	372 (280, 506)	351 (265, 497)	314 (214, 470)	260 (192, 349)	297 (225, 423)	277 (198, 391)
Natural	223 (173, 286)	274 (204, 344)	271 (213, 325)	223 (184, 274)	189 (151, 231)	207 (171, 268)	210 (158, 248)
Total folic acid[3]	64 (14, 167)	74 (20, 175)	68 (9, 205)	71 (12, 183)	52 (13, 135)	66 (24, 196)	63 (12, 177)
Fortified foods[4]	50 (9, 118)	58 (12, 125)	60 (8, 169)	64 (11, 180)	35 (6, 87)	40 (9, 109)	47 (0, 138)
Consumers of folic acid (%)							
Fortified foods	79	80	80	81	78	80	74
Supplements	14	14	9	8	16	20	14
Vitamin B-12 intakes (µg/d)							
Total[2]	4.2 (2.9, 6.1)	5.0 (3.4, 7.0)	4.8 (3.3, 6.9)	4.8 (2.9, 6.6)	3.5 (2.4, 4.8)	4.2 (3.2, 6.0)	4.1 (2.8, 5.6)
Natural	3.7 (2.5, 5.1)	4.3 (3.0, 5.9)	4.4 (2.8, 6.1)	4.2 (2.8, 5.6)	2.9 (2.0, 4.0)	3.8 (2.7, 4.8)	3.7 (2.7, 4.8)
Total synthetic[3]	0.3 (0, 0.8)	0.3 (0.0, 0.8)	0.2 (0.0, 0.9)	0.2 (0.0, 1.2)	0.2 (0.0, 0.7)	0.3 (0.0, 0.9)	0.2 (0.0, 0.8)
Fortified foods[4]	0.1 (0.0, 0.5)	0.2 (0.0, 0.5)	0.2 (0.0, 0.8)	0.2 (0.0, 1.0)	0.1 (0.0, 0.4)	0.1 (0.0, 0.5)	0.1 (0.0, 0.4)
Consumers of synthetic B-12 (%)							
Fortified foods	65	67	63	65	64	63	60
Supplements	14	14	7	8	15	19	14

[1]Interquartile range in parentheses (all such values)

[2]Refers to total folate or vitamin B-12 intakes from natural food sources, fortified foods and supplements.

[3]Refers to total folic acid and total synthetic vitamin B-12 intakes from both fortified foods and supplements.

[4] Refers to folic acid and synthetic vitamin B-12 intakes from fortified foods only. Intakes from supplements are not given for the total population due to the low percentage of consumers.

DFE, dietary folate equivalents calculated as follows: natural folate (µg) + (folic acid from fortified foods (µg) x 1.7) (21)

Table 2. Effects of sex and age on median concentrations of red blood cell folate, serum folate, serum vitamin B-12 and total plasma homocysteine in Irish adults

	All ages		Age group						2-way ANOVA[1] (P-value)		
	n	18-91y	n	18-50y	n	51-64y	n	≥65y	Sex	Age	S X A
RBC folate (nmol/L)											
All	1118	872 (672, 1196)[2]	756	839 (660, 1137)[a]	221	924 (700, 1269)[ab]	141	960 (747, 1356)[b]	0.509	0.001	0.126
Males	563	923 (707, 1171)	388	905 (700, 1154)	112	924 (713, 1228)	63	964 (740, 1414)			
Females	555	836 (649, 1228)	368	799 (626, 1110)	109	936 (696, 1334)	78	926 (747, 1296)			
Serum folate (nmol/L)											
All	1115	25.5 (16.7, 38.8)	756	24.9 (16.6, 36.0)[a]	220	28.2 (17.9, 42.9)[b]	139	28.0 (16.0, 46.3)[ab]	0.016	0.032	0.071
Males	560	24.9 (17.1, 36.7)	387	24.4 (17.0, 35.5)	110	24.8 (16.9, 38.5)	63	30.2 (19.2, 38.6)			
Females	555	26.1 (16.4, 41.2)	369	25.2 (16.1, 37.2)	110	32.0 (18.9, 47.8)	76	27.0 (14.1, 54.9)			
Serum vitamin B_{12} (pmol/L)											
All	1114	298 (224, 378)	756	305 (226, 379)	220	285 (217, 383)	138	277 (216, 369)	0.259	0.475	<0.001
Males	559	314 (238, 388)	387	328 (257, 403)	110	295 (209, 383)	62	247 (205, 322)			
Females	555	289 (215, 369)	369	285 (205, 360)	110	282 (228, 400)	76	306 (250, 387)			
tHcy (µmol/L)											
All	1116	11.8 (10.1, 13.8)	756	11.4 (9.8, 13.1)[a]	219	12.4 (10.8, 14.8)[b]	141	13.2 (11.1, 15.8)[b]	<0.001	<0.001	0.866
Males	562	12.4 (10.8, 14.4)	388	12.0 (10.4, 13.7)	111	13.2 (11.5,15.4)	63	13.9 (11.7, 17.3)			
Females	554	11.1 (9.5, 12.9)	368	10.7 (9.3, 12.3)	108	11.7 (10.2, 14.3)	78	12.2 (10.4, 15.5)			

[1]Main effects and interaction effects were assessed by two way ANOVA.

[2]Interquartile range in parentheses (all such values)

[abc]Values across a row with unlike superscript letters are significantly different (scheffe post hoc test) $P < 0.05$

A, age; RBC, red blood cell; S, sex; tHcy, total plasma homocysteine

Table 3. Dietary intakes and biomarker status of folate and vitamin B-12 grouped by participants' intake of folic acid fortified foods and supplements[1]

	Non Consumers (1)	Low Consumers (2)	Medium Consumers (3)	High Consumers (4)	Supplement users (5)	Supplement users & FF consumers (6)	P-value[2]
Folic acid intake	**0µg/d**	**1-45µg/d**	**46-108µg/d**	**109-1044µg/d**	**10-2,000µg/d**	**33-958µg/d**	
n	*200*	*254*	*252*	*261*	*36*	*123*	
General Characteristics							
Male:female (%)	48:52	43:57	52:48	62:38	42:58	43:57	<0.001
Age (y)	45 (31, 56)[3]	43 (28, 55)	39 (27, 52)	45 (28.5, 58)	38 (27,52)	38 (26,57)	0.056
BMI (kg/m^2)	27.1 (23.7, 30.1)	26.6 (23.8, 29.9)	26.6 (23.8, 30.1)	26.3 (23.7, 29.1)	25.6 (22.9,28.3)	25.3 (22.8,28.2)	0.037
Energy (MJ/d)	7.9 (6.5, 9.7)^{a}	7.9 (6.2, 9.7)^{a}	8.2 (6.4, 10.6)^{ab}	9.0 (7.2, 11.3)^{b}	8.7 (6.8, 10.5)^{ab}	8.6 (7.2, 11.2)^{ab}	<0.001
Smoker (%)	30	23	20	12	19	13	<0.001
Dietary Intakes							
Folate (µg)							
Total	206 (160, 293)^{a}	233 (186, 291)^{b}	288 (242, 349)^{c}	445 (363, 535)^{d}	558 (267, 636)^{de}	582 (431, 746)^{e}	<0.001
Natural	206 (160, 293)	211 (161, 272)	214 (170, 278)	248 (195, 309)	237 (179, 306)	246 (185, 309)	0.366
Folic acid	0.0	22 (13, 32)^{a}	69 (56, 84)^{b}	180 (137, 248)^{c}	203 (150, 400)^{cd}	287 (220, 438)^{d}	<0.001
DFE	206 (160, 293)^{a}	249 (199, 310)^{b}	338 (291, 406)^{c}	572 (472, 709)^{d}	237 (179,306)^{ab}	373 (252, 546)^{e}	<0.001
Vitamin B-12 (µg)							
Total	3.4 (2.4, 4.9)^{a}	3.6 (2.3, 4.8)^{a}	3.9 (2.8, 5.4)^{a}	4.8 (3.6, 6.3)^{b}	8.0 (5.4, 24.7)^{c}	7.2 (4.9, 12.2)^{d}	<0.001
Natural	3.4 (2.4, 4.8)	3.4 (2.1, 4.6)	3.6 (2.4, 5.1)	4.0 (2.9, 5.5)	4.2 (3.2, 5.4)	4.3 (2.8, 5.4)	0.012
Synthetic	0.0	0.05 (0, 0.1)^{a}	0.3 (0.2, 0.4)^{b}	0.7 (0.4, 1.1)^{c}	2.9 (1.0, 17.6)^{d}	2.0 (1.1, 6.5)^{e}	<0.001
Biomarkers							
Serum folate (nmol/L)	17.0 (12.3, 24.8)^{a}	21.7(14.2, 30.3)^{b}	23.2 (16.8, 33.1)^{b}	36.9 (26.3,53.6)^{c}	32.9 (22.6, 48.1)^{c}	44.9 (29.2, 68.6)^{c}	<0.001
RBC folate (nmol/L)	699 (538, 934)^{a}	784 (623, 1018)^{b}	825 (695, 1083)^{b}	1040 (83, 1390)^{c}	1013 (812, 1487)^{c}	1156 (831, 1501)^{c}	<0.001
Serum vitamin B-12 (pmol/L)	288 (202, 357)^{ab}	261 (197, 343)^{a}	296 (239, 368)^{ab}	315 (252, 410)^{bc}	380 (295, 497)^{cd}	373 (287, 485)^{d}	<0.001
Plasma tHcy (µmol/L)	12.7 (11.0, 15.5)^{a}	12.2 (10.7, 14.5)^{a}	11.9 (10.2, 13.9)^{b}	11.5 (9.7, 12.9)^{bc}	10.6(9.6, 13.0)^{abc}	10.3 (9.2, 12.2)^{c}	<0.001

[1]Non consumers (1) consumed no folic acid from fortified foods or supplements during the food diary. Those who consumed folic acid from fortified foods at least once during the food diary were categorised as low (2) medium (3) or high (4) consumers based on tertiles of folic acid intake from fortified foods. Supplement users (5) consumed folic acid from supplements at least once during the food diary and no fortified foods. Supplement users and fortified consumers (6) consumed folic acid from both supplements and fortified foods.

[2]General characteristics were compared across the groups using Chi square analysis and one-way ANOVA (scheffe post hoc tests). B vitamin dietary intakes and biomarkers were compared using one-way ANCOVA controlling for gender, BMI, energy intakes and smoking status. [abc]Values across a row with unlike superscript letters were significantly different (bonferroni post hoc tests). P <0.05.

[3]Median and interquartile range in parentheses (all such values).

DFE, dietary folate equivalents calculated as follows: natural folate (µg) + (folic acid from fortified foods (µg) x 1.7) (21); MJ, megajoule; RBC, red blood cell; tHcy, total plasma homocysteine.

Figure 1

Relation between RBC folate concentration and dietary folate intake expressed as dietary folate equivalents (panel 1 a), added folic acid (panel 1 b) and natural folate only (panel 1 c) in fortified food consumers who were non-users of folic acid supplements. Correlations were calculated on log transformed data using Pearson's correlation coefficients (r). $P<0.05$ was considered significant. Dietary folate equivalents were calculated as µg natural folate + [1.7 x added folic acid (µg) in foods] (20). Corresponding intake-status correlations for serum folate were: with DFE ($r=0.417$, $P<0.001$); added folic acid ($r=0.396$, $P<0.001$); natural food folates ($r=0.163$, $P<0.001$); n=757. For all correlations, one participant with an outlying intake value was removed from the analysis on the basis that the estimated intake was considered highly unlikely to be representative of true intake from food sources (i.e. DFE=2400 µg/d)

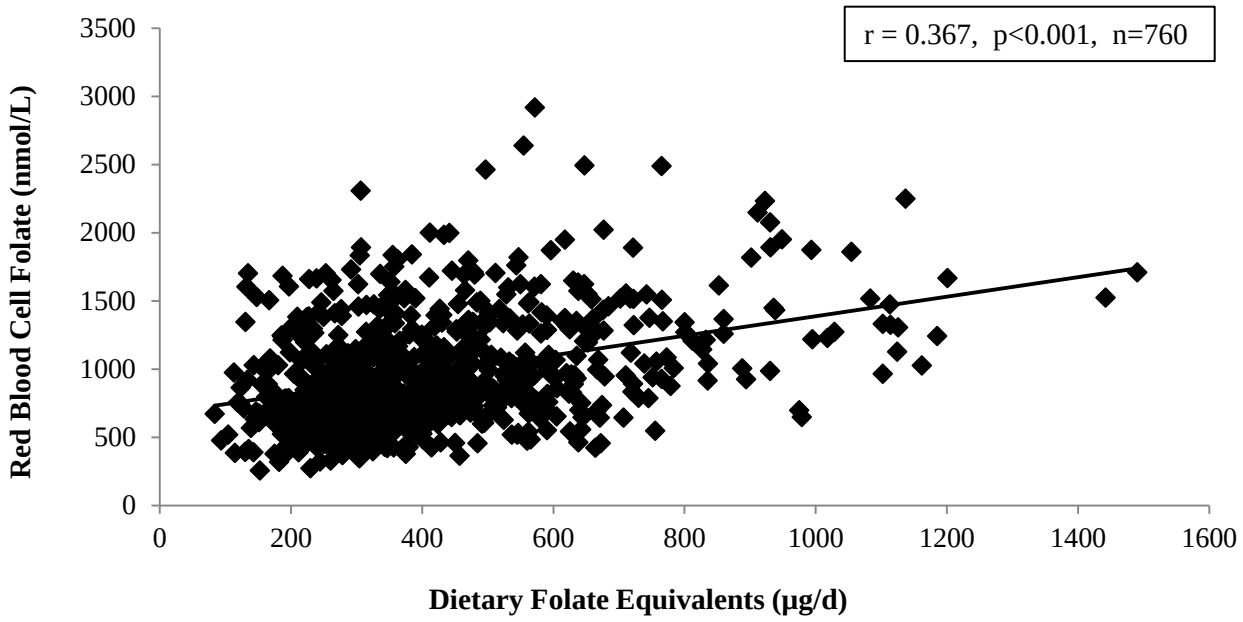
Figure 2

Relation between serum vitamin B-12 concentration and total dietary vitamin B-12 intake (panel 2 a) and natural vitamin B-12 intake (panel 1 b) in non-users of vitamin B-12 supplements (representing 86% of the population). Correlations were calculated on log transformed data using Partial Pearson's correlation coefficients (r) controlling for age. $P<0.05$ was considered significant. Two participants with outlying vitamin B-12 intake values (36.9µg/d and 33.4 µg/d) and one participant with an outlying serum vitamin B-12 concentration (2216pmol/L) were removed from the analysis.

Figure 3

Proportion of women of reproductive age (18-50ys) with optimal folate (≥907nmol/L) (26) and vitamin B-12 status (>221pmol/L) (3) for protection against NTDs according to intake of folic acid fortified foods and supplements. Non-consumers consumed no folic acid during food diary. Those who consumed folic acid from fortified foods during the food diary but not supplements were stratified into tertiles of folic acid intake; low consumers (1-33μg/d), medium consumers (34-86μg/d) and high consumers (≥87μg/d). Supplement users consumed folic acid supplements during the food diary. As the majority of supplement users also consumed fortified foods (48 out of 58), they were merged into one group. Median RBC folate concentrations were 638, 705, 775, 859 and 1233nmol/L across non consumers, low, medium and high consumers and supplements users respectively. The proportion of women with an optimal folate and vitamin B-12 status was compared across consumption groups using binary logistic regression controlling for smoking status and MTHFR genotype. ‡Denotes significantly different from non-consumers, low and medium consumers; †Denotes significantly different from non-consumers (Bonferonni post hoc test) $P<0.05$. MTHFR, methylene tetrahydrofolate reductase, NTD, neural tube defect, RBC, red blood cell.

A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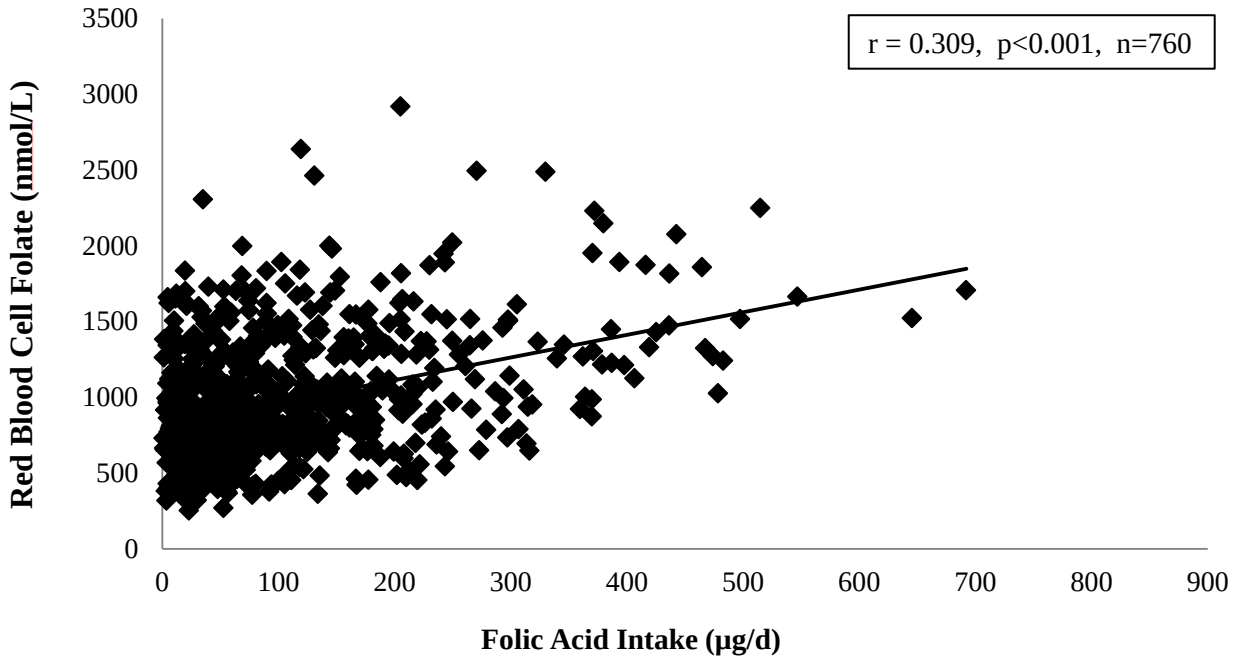
Red Blood Cell Folate (nmol/L)
3500
3000
2500
2000
1500
1000
500
0
0
200
400
600
800
1000
1200
1400
1600
Dietary Folate Equivalents (µg/d)

B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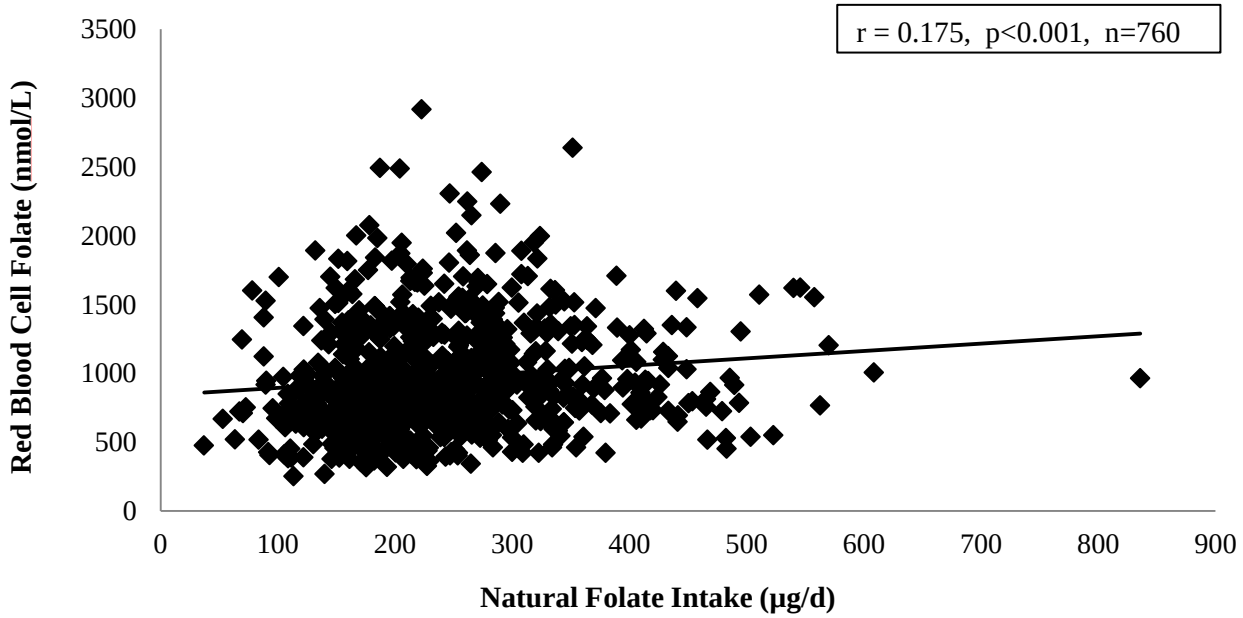
Red Blood Cell Folate (nmol/L)
3500
3000
2500
2000
1500
1000
500
0
0
100
200
300
400
500
600
700
800
900
Folic Acid Intake (µg/d)

C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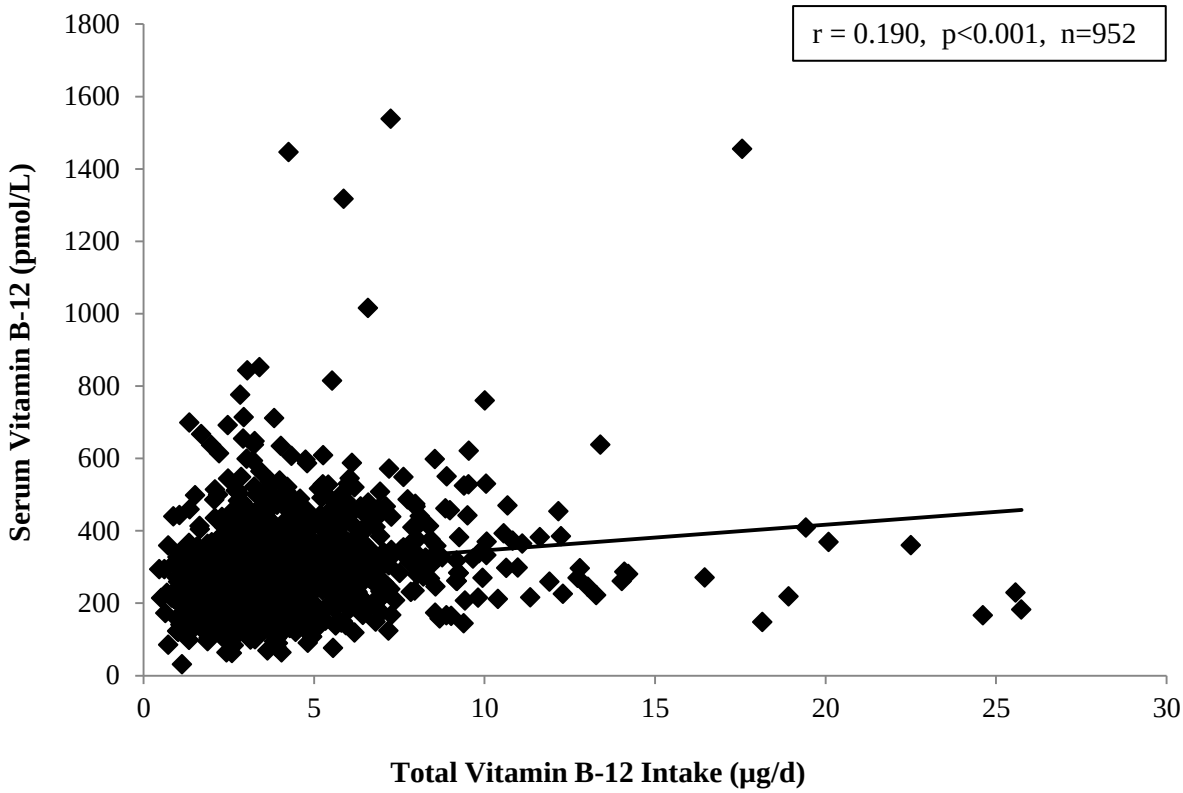
Red Blood Cell Folate (nmol/L)
3500
3000
2500
2000
1500
1000
500
0
0
100
200
300
400
500
600
700
800
900
Natural Folate Intake (µg/d)

A


r = 0.190, p<0.001, n=952
Serum Vitamin B-12 (pmol/L)
1800
1600
1400
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
Total Vitamin B-12 Intake (µg/d)

B

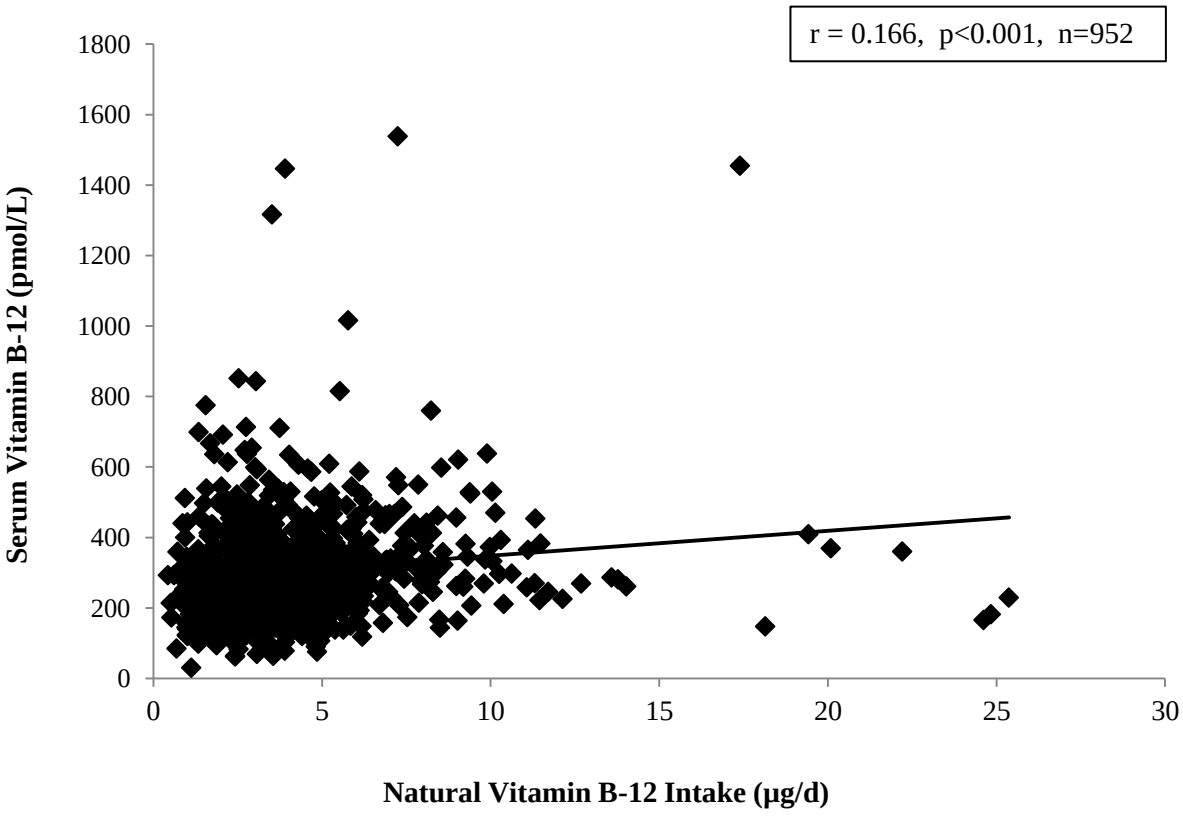
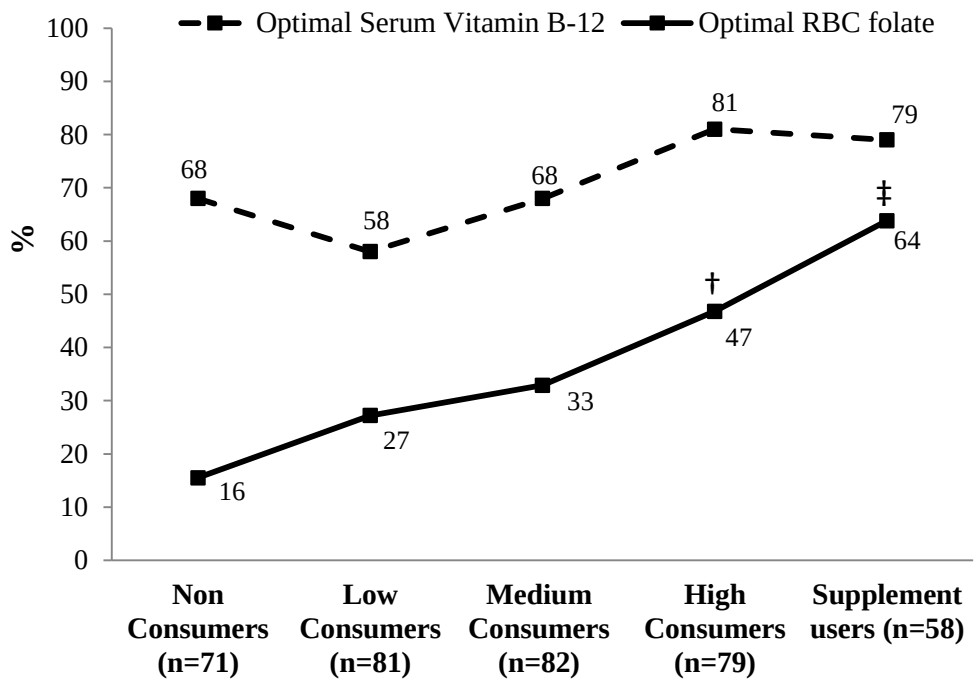

r = 0.166, p<0.001, n=952
Serum Vitamin B-12 (pmol/L)
1800
1600
1400
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
Natural Vitamin B-12 Intake (µg/d)

Figure 3


Optimal Serum Vitamin B-12
Optimal RBC folate
%
100
90
80
70
60
50
40
30
20
10
0
68
58
68
81
79
16
27
33
47
64
†
‡
Non Consumers (n=71)
Low Consumers (n=81)
Medium Consumers (n=82)
High Consumers (n=79)
Supplement users (n=58)