B-vitamins in Relation to Depression in Older Adults over 60 Years of Age: The TUDA Cohort Study

Katie Moore, PhD[a], Catherine F. Hughes, PhD[a], Leane Hoey, PhD[a], Mary Ward, PhD[a], Conal Cunningham, MD[b], Anne M. Molloy, PhD[c], J.J. Strain, PhD[a], Kevin McCarroll, MD[b], Miriam C. Casey, MD[b], Fergal Tracey, MD[d], Eamon Laird, PhD[c], Maurice O'Kane, MD[e], Helene McNulty, PhD[a]

[a]Nutrition Innovation Centre for Food and Health (NICHE), School of Biomedical Sciences, Ulster University, Coleraine, Northern Ireland, United Kingdom

[b]Mercers Institute for Research on Aging, St James's Hospital, Dublin 8, Ireland

[c]School of Medicine, Trinity College Dublin, Dublin b, Ireland

[d]Causeway Hospital, Northern Health and Social Care Trust, Coleraine, Northern Ireland, United Kingdom

[e]Clinical Chemistry Laboratory, Altnagelvin Hospital, Western Health and Social Care Trust, Londonderry, Northern Ireland, United Kingdom

Corresponding author: Helene McNulty, Nutrition Innovation Centre for Food and Health (NICHE), School of Biomedical Sciences, Ulster University, Cromore Road, Coleraine, Northern Ireland BT521SA. Email: h.mcnulty@ulster.ac.uk

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Brief Summary:

This study draws on data from over 5000 European adults of 60+ years and shows that better folate and related B-vitamin status may have a positive impact on mental health in older adults.

Abstract

Objectives: Mental health disorders are major contributors to disease burden in older people. Deficient status of folate and the metabolically related B-vitamins may be implicated in these conditions. This study aimed to investigate folate, vitamin B12, vitamin B6 and riboflavin in relation to depression and anxiety in aging and also considered the role of fortified foods as a means of optimizing B-vitamin status and potentially reducing the risk of these mental health disorders.

Design: The TUDA aging study was a cross-sectional cohort study.

Setting and Participants: Community-dwelling adults (n = 5186; ≥ 60 years) recruited from two jurisdictions within the island of Ireland from 2008 to 2012.

Measures: Depression and anxiety were assessed using the Centre for Epidemiological Studies Depression (CES-D) and the Hospital Anxiety and Depression (HAD) scales, respectively. The following B-vitamin biomarkers were measured: red blood cell folate, serum total vitamin B12, plasma pyridoxal-5-phosphate (PLP; vitamin B6) and erythrocyte glutathione reductase activation coefficient (EGRac; riboflavin).

Results: Biomarker values in the lowest 20% of status for folate (Odds Ratio (OR) 1.79; 95% CI 1.23-2.61), vitamin B6 (OR 1.45; 1.01-2.06) or riboflavin (OR 1.56; 1.10-

2.00), but not vitamin B12, were each associated with an increased risk of depression (CES-D score ≥16). Correspondingly, B-vitamin fortified foods if consumed daily were associated with a reduced risk depression (OR 0.54; 0.41-0.70). A deficient status of vitamin B6 (OR 1.73; 1.07-2.81), but not other vitamins, was associated with increased anxiety.

Conclusions/Implications: Better B-vitamin status may have a role in impacting positively on mental health in older adults. Regular intake of fortified foods can provide a means of optimizing B-vitamin status and thus could contribute to reducing depression. If confirmed by a randomized trial, these results may have implications for nutrition and mental health policy, and thus quality of life, in older people.

Introduction

Globally the population is aging and by 2050 the number of people aged ≥60 years is predicted to reach 2.1 billion.[1] Mental health disorders are a leading cause of disability and ill health in older age,[2] affecting an estimated 20% of adults ≥60 years worldwide.[3] Given the considerable human and economic cost of mental health conditions and the generally poor response rates to costly pharmacological treatments,[4,5] there is much interest in the potential roles of certain dietary components as modifiable risk factors for depression. Folate and vitamin B12 have received particular attention in this regard.[6] These B-vitamins have interrelated roles within one-carbon metabolism, where folate in the form of 5 methyltetrahydrofolate, and vitamin B12 in the form of methylcobalamin, are required for the remethylation of homocysteine to methionine which subsequently forms S-adenosylmethionine (SAM).[7] SAM, in turn, is the essential methyl donor required for the production of monoamine neurotransmitters, phospholipids and nucleotides.[8]

Historically, clinical deficiencies of folate and vitamin B12 were associated with a range of neuropsychiatric symptoms, including depression,[9-11] raising the possibility that optimizing relevant B-vitamin intake and status could be protective. Research to date in this area has however focused predominantly on folate, and to a lesser extent vitamin B12[12] whereas related B-vitamins - vitamin B6 and riboflavin - also required for one-carbon metabolism have received much less attention. The aim of this study therefore was to investigate biomarker status of all relevant B-vitamins - folate, vitamin B12, vitamin B6 and riboflavin - in relation to mental health in a well characterized cohort of 5186 older adults born in Ireland. Furthermore, this study considered the role of fortified foods as a means of optimizing B-vitamin status, and potentially reducing the risk of depression and anxiety, in older adults.

Methods

Study design and participants

The study involved new analysis of data from the TUDA aging cohort study (ClinicalTrials.gov Identifier: NCT02664584). As described in detail elsewhere,[13] 5,186 community-dwelling adults aged ≥60 years were recruited between 2008 and 2012 from two jurisdictions within the island of Ireland - Northern Ireland (United Kingdom, UK) and the Republic of Ireland. The TUDA study initially aimed to investigate the role of nutrition and lifestyle factors in the etiology of common age-related diseases, namely, dementia, osteoporosis and cardiovascular disease. Participants were recruited in both jurisdictions using standardized protocols by centrally trained staff, either from general practice or hospital outpatient clinics, and deemed suitable if they were born on the island of Ireland and were without a diagnosis of dementia. For the current study, participants receiving vitamin B12 injections were excluded from the analysis (**Fig. 1**).

Ethical approval was granted by the Office for Research Ethics Committees Northern Ireland (ORECNI; reference 08/NI/RO3113), with corresponding approvals from The Northern and Western Health and Social Care Trusts in Northern Ireland, and the Research Ethics Committee of St James Hospital and The Adelaide and Meath Hospital in Dublin. All participants provided written informed consent.

Neuropsychiatric assessment

During the participant appointment, depression was assessed using the Centre for Epidemiological Studies Depression (CES-D) scale, which is a 20 item self-reported questionnaire, with a minimum score of 0 (no symptoms of depression) and maximum score of 60 (significant symptoms of depression). A score of ≥16 was used

as a cut-off value suggestive of clinical depression.[14] Anxiety was assessed using the 7 item Hospital Anxiety and Depression (HAD) scale, with a minimum score of 0 (suggestive of no symptoms of anxiety) and a maximum score of 21 (significant anxiety). A score ≥11 was used as a cut-off value for probable anxiety.[15]

For the purpose of the current analysis, cognitive function was assessed using the Folstein Mini-Mental State Examination (MMSE),[16] a short, structured cognitive test. The maximum score achievable is 30, with a score <25 indicating a possibility of cognitive impairment and a score <20 indicating dementia.

Blood sampling and laboratory analysis

A non-fasting blood sample was obtained and analyzed on the day of sampling for routine biomarkers of health in participating hospital laboratories. For research biomarkers, all sample preparation and fractionation was carried out within 4 hours of collection and fractions were stored at -70 °C (for up to five years) for batch analysis at the end of the study. B-vitamins were analyzed centrally in laboratories in Dublin (vitamin B12, folate, homocysteine) or Coleraine (vitamin B6, riboflavin) using established methods. Red blood cell (RBC) folate and serum total vitamin B12 were measured by microbiological assay using *Lactobacillus casei* and *Lactobacillus leichmanni*, respectively.[17,18] Plasma homocysteine was measured by fluorescence polarization immunoassay.[19] Vitamin B6 status (plasma pyridoxal-5-phosphate, PLP) was analyzed by HPLC with fluorescence detection.[20] Riboflavin status was measured by erythrocyte glutathione reductase activation coefficient (EGRac), a functional assay that measures the activity of glutathione reductase before and after in-vitro reactivation with its prosthetic group flavin adenine dinucleotide (FAD), the active cofactor form of riboflavin; results are reported as a ratio, a higher EGRac ratio indicates lower

riboflavin status.[21] For each assay, quality controls were provided by the repeated analysis of pooled samples covering a wide range of values.

Dietary assessment

Dietary information on habitual intake of specified foods (for the purpose of this paper, B-vitamin fortified foods) was collected using a researcher-assisted food frequency questionnaire (FFQ), previously validated for B-vitamin intake against B-vitamin biomarkers.[22] Using a 7-item section for fortified foods (from a larger FFQ used in the TUDA study), brand names of fortified food products were collected so that up-to-date details on relevant nutrient profiles could be obtained. Using this approach, participants were categorized according the number of portions of fortified food consumed per week. A small number of participants (n = 110; 2.2%) could not be classified as regards fortified food intake and/or supplement use and are not included in this analysis.

General health, lifestyle and biophysical measures

Health and lifestyle information was gathered using a researcher-assisted, questionnaire which included information on smoking, alcohol, medical history and use of prescription drugs, including antidepressant medications. To facilitate the accuracy of recorded drugs and vitamin supplements, participants were asked to bring these items to their appointment for inspection by the researcher. Anthropometric measurements were recorded (including weight, height, waist and hip) and blood pressure measurements were taken in accordance with standard operating procedures by trained researchers. The Timed Up-and-Go (TUG) test,[23] the Physical Self-Maintenance Scale (PSMS) and the Instrumental Activities of Daily Living (IADL)

scale were used to assess functional mobility and general ability of participants. Socio-economic status was measured as area-based deprivation by adopting a novel cross-jurisdictional appraoch, whereby geo-referenced address-based information was used to map and link participants to official socioeconomic indicators of deprivation within Northern Ireland (UK) and the Republic of Ireland, as previously described in detail.[13]

Statistical Analysis

All statistical analysis was performed using SPSS software (Statistical Package for Social Sciences, Version 23.0, SPSS UK Ltd., Chersey, United Kingdom). Data were checked for normality and log-transformed as appropriate. Analysis of covariance with Bonferroni post hoc test was used for analysis of continuous data and chi-squared tests were used for categorical variables. Relationships of demographic, clinical and lifestyle factors with depression (CES-D score) and anxiety (HAD score) were investigated using multiple linear regression analysis. The risk of depression (CES-D score ≥16) and anxiety (HAD score ≥11) in relation B-vitamin biomarker status was determined using logistic regression. For this purpose, B-vitamin biomarkers were examined in quintiles ranging from the highest 20% (reference category) to lowest 20% of values, and the model was adjusted for relevant co-variates. The associations of B-vitamin fortified food intake with risk of depression (CES-D score ≥16) and anxiety (HADS score ≥11) were also determined using logistic regression, with adjustment for relevant co-variates; the reference category was non-consumers, against which the remaining categories (low, medium and high fortified food frequencies) were compared.

Results

General characteristics

The general characteristics of the study population are described in **Table 1**. Participants were predominantly female (67%), the majority were fortified food consumers (72%) and 11% were B-vitamin supplement users. Overall, higher rates of depression (CES-D score ≥16.0) and anxiety (HAD score ≥11.0) were recorded in females compared to males; likewise, self-reported depression and anxiety were also higher in females. B-vitamin biomarker status was generally lower, and homocysteine concentrations higher, in men compared to women. Although mean B-vitamin biomarker concentrations fell within normal reference ranges, some evidence of deficiency (using accepted laboratory cut-offs) was identified for specific B-vitamin biomarkers (data not shown): folate (RBC folate 2.3%); vitamin B12 (serum B12 11.6%); vitamin B6 (PLP 12.2%); riboflavin (EGRac 48.6%).

Relationships of demographic, clinical and lifestyle factors with depression and anxiety

The relationship of clinical and lifestyle factors with depression (CES-D score) and anxiety (HAD score) was examined by linear regression (**Supplemental Table 1**). The following factors were significantly associated with depression: female sex (β = 0.04, *P* = .008), socioeconomic status (β = 0.09, *P* <.001), physical frailty (β = 0.19, *P* < .001), living alone (β = 0.08, *P* <.001), antidepressant usage (β = 0.21, *P* < .001), previous ischemic attack (β = 0.04, *P* = .02) and smoking (β = 0.05, *P* =.001), whereas age (β = - 0.10 *P* <.001) and education (β = - 0.06, *P* <.001) were negatively related to depression. The following factors were identified as being positively associated with anxiety: female sex (β = 0.08, *P* <.001), socioeconomic status (β = 0.08, *P* <.001), hypertension (β = 0.04, *P* = .027) and anti-depressant usage (β = 0.18, *P* <.001),

whereas age (β = -0.138, *P* <.001), education (β = -0.10, *P* <.001) and BMI (β = -0.05, *P* <.001) were inversely related to anxiety.

B-vitamin biomarker status in relation to depression and anxiety

The associations of B-vitamin biomarker status with risk of depression (CES-D score ≥16) was examined after adjustment for the above co-variates and vitamin supplement use (**Fig. 2**). Each B-vitamin was examined in quintiles of biomarker status; the reference category was set at the highest 20% of values. Compared with the reference category, the lowest quintile of folate (Odds Ratio (OR) 1.79; 95% CI 1.23-2.61, *P* = .002), vitamin B6 (OR 1.45; 1.01-2.06, *P* = .043) or riboflavin (OR 1.56; 1.10-2.00, *P* = .012) status was associated with increased risk of depression. No significant relationship of serum total B12 was observed with depression (*P* = 0.577). Similarly, the relationship of B-vitamins with anxiety was examined in quintiles of biomarker status (data not shown). After adjustment for relevant co-variates (i.e. age, gender, anti-depressant drug usage, education, BMI, socioeconomic status and hypertension) and vitamin supplement use, only low/deficient status of B6 - but not other B-vitamins - was associated with an increased risk of anxiety (OR 1.73; 1.07-2.81, *P* = .024).

B-vitamin intakes, biomarker status and risk of depression or anxiety

The influence of B-vitamin fortified food and supplement intake on B-vitamin biomarker status was examined (**Table 2**). Participants were categorized by fortified food intake (0, low, medium, high) and supplement usage; ‘non-consumers’ did not consume fortified foods or supplements and hence depended on natural food sources of B-vitamins only. As dietary intake of B-vitamin fortified foods increased, biomarker

status of each vitamin increased in a stepwise manner, with the highest B-vitamin biomarker status being observed in those participants who consumed the highest intakes of fortified foods (i.e. at least once daily) and in those taking B-vitamin supplements. Supplement users were identified on the basis of their reported current use of supplemental B vitamins in tablet form (irrespective of fortified food) and accounted for 10.8% of overall TUDA sample. A small number of participants (n = 110; 2.2%) could not be classified as regards fortified food intake and supplement use and thus were excluded from this part of the analysis. Fortified breakfast cereals (65%), spreads (55 %) and drinks (20 %) were the most commonly consumed fortified foods within this cohort (data not shown).

The risk of depression was examined in relation to B-vitamin fortified food intake (**Fig. 3**); for this purpose, the reference category was 'non-consumers' i.e. no fortified food or supplement usage. High fortified food intake (> 1 portion per day) was associated with significantly lower depression (OR 0.54; 95% CI 0.41-0.70, *P* <.001). After adjustment for relevant co-variates (i.e. age, gender, anti-depressant medication, education, vitamin supplement usage, smoking status, physical frailty, living alone, socioeconomic status and transient ischemic attack) and fortified food intake, B-vitamin supplement usage was not associated with risk of depression (OR 0.941; 0.68-1.30, *P* = .712). No significant relationship was identified between B-vitamin fortified food intake (OR 0.97; 0.69-1.36, *P* = .861) or supplement usage (OR 0.99; 0.64-1.54, *P* = .974) and anxiety.

Discussion

This study is the first large cross sectional study to investigate biomarker status of all four B-vitamins involved in one-carbon metabolism in relation to depression and

anxiety in older adults. The findings suggest that low biomarker status of folate, vitamin B6 or riboflavin, but not vitamin B12, were each independently associated with increased depression. Correspondingly, consuming at least one portion per day of B-vitamin fortified food was associated with lower depression (by 50% relative to non-consumers). Only deficient status of vitamin B6 (but not the other B-vitamins) was associated with higher risk of anxiety, and no significant relationship of fortified food with anxiety was shown.

The current results estimated that having RBC folate concentrations in the lowest 20% was associated with an increased risk of depression (by almost 80%), adding to the considerable body of evidence linking low folate with depression. Likewise, published meta-analyses of observational studies in adults reported that low biomarker status of folate was associated with between 23%[12] and 42%[24] increased risk of depression. The stronger relationship of folate with depression identified in the current study compared with the aforementioned studies,[12,24] may be explained to some extent by the use of RBC folate. RBC folate is widely considered to be a better index of long-term folate status, compared to plasma or serum folate as it parallels liver concentrations (accounting for about 50% of total body folate) and is thus considered to represent tissue folate stores, whereas serum folate is the earliest indicator of folate exposure and reflects recent dietary intake.[7,25] The evidence linking folate with depression is however not entirely consistent. The Chicago Health and Aging Study (CHAP) (n = 3503) and the Quebec longitudinal study on nutrition and Aging (NuAge) (n = 1368) found no association of folate with depression; however these observations were based on dietary intakes only with no corresponding folate biomarker data.[26,27] Furthermore, the studies were conducted in regions with mandatory folic acid fortification policies, where more optimal folate status throughout

the population would make a relationship with depression less likely. The current study found no association of vitamin B12 with depression, which is in line with the findings from one large cohort study (n = 2,524) conducted in the USA,[28] but at odds with other research which reported inverse associations of vitamin B12 intake[26,27] or biomarkers[29] with depression. The explanation for such discrepancy in the evidence linking vitamin B12 with depression is unclear, but may possibly relate to differences in B12 status among populations under investigation or methodological variation among studies, including the use of different B12 biomarkers to measure status, especially considering that no consensus exists as to the best biomarker for assessing B12 status in the laboratory.[30]

Low status of vitamin B6 or riboflavin were each significantly associated with depression. Likewise, previous studies have reported inverse associations of vitamin B6 biomarkers with depression.[31] In contrast to the other relevant B-vitamins, riboflavin has received very little attention as regards its potential role in depression, with previous evidence limited to one early study which reported that 27% of patients admitted to a psychiatric inpatient unit had riboflavin deficiency,[32] whilst a recent study showed no significant relationship of dietary riboflavin intake with depression.[33] The finding that both vitamins show similar relationships with depression is perhaps unsurprising. There is a well established metabolic dependency of vitamin B6 on riboflavin, in that the generation in tissues (via pyridoxine 5'phosphate oxidase) of the active B6 form, PLP, requires riboflavin in its co-factor form flavin mononucleotide (FMN). This interrelationship in humans was previously confirmed by showing that riboflavin supplementation of older adults not only improved riboflavin biomarker status, but also enhanced vitamin B6 concentrations, suggesting that riboflavin may be the more limiting nutrient.[34]

In the current study, low/deficient vitamin B6 status was associated with an increased risk of anxiety, while no significant associations with anxiety were found for any other B-vitamin biomarkers or fortified foods. The findings are generally in line with those of the Hordaland Homocysteine Study (n = 5948) which also reported no significant relationships of folate or vitamin B12 with anxiety in Norwegian adults.[35] Few previous studies have investigated vitamin B6 in relation to anxiety and the evidence is unclear, although one randomized trial in 60 patients observed short term benefits in symptoms of anxiety in response to a supplement containing vitamin B6 (combined with vitamin B12 and folate) in patients suffering from depression,[36] perhaps suggesting potential benefits of optimizing B6 status in this patient group. In line with the conclusions of a recent meta-analysis, we observed a positive association of anxiety with hypertension in the current study, the mechanism for which has been previously reviewed but remains unclear.[37] Further work would be required to investigate whether vitamin B6 plays a role in this complex relationship.

The current results not only showed that low biomarker status of specific B-vitamins was associated with a higher risk of depression, but importantly suggested (for the first time) the potential for fortified foods to contribute to reducing depression in older age. Fortified foods are known to provide a highly bioavailable source of B-vitamins, particularly folate,[7] and their contribution to optimal B-vitamin biomarker status among adults (not taking B-vitamin supplements) has previously been reported.[22] The current results suggest that regular consumption of fortified foods, by improving B-vitamin biomarkers, may provide a practical means of reducing the risk of depression in older adults. Indeed the findings, showing a potential benefit of fortified foods in relation to mental health, may contribute to the current risk-benefit debate surrounding mandatory fortification with folic acid, and specifically the issue of whether

there are any benefits to older people from a folic acid fortification policy directed primarily at preventing neural tube defects in women of reproductive age.[38]

The biological mechanism explaining these and previous results linking folate and related B-vitamins with depression is not known, but invariably must relate to their roles in one-carbon metabolism. In particular, these B-vitamins are required for methylation reactions; lower status may thus reduce the methylation of neurotransmitters.[6] Furthermore, folate is required for monoamine synthesis and lower concentrations of monoamine metabolites in cerebral spinal fluid have been found in folate deficient patients suffering from depresssion.[8] Additionally, the active form of vitamin B6 (PLP) is the cofactor for aromatic L-amino acid decarboxylase in the tryptophan serotonin pathway, thus deficient B6 status (and/or riboflavin required to generate PLP in tissues)[34] may lead to reduced concentrations of serotonin.[39]

This study had both strengths and limitations. Although the TUDA study is one of the largest and most comprehensively characterized cohorts of its kind, its cross-sectional design means that the possibility of residual confounding and reverse causality cannot be excluded. Also, the data have been derived from only two jurisdictions within Europe, Ireland and the UK, therefore the results may not necessarily be generalizable to other populations. Furthermore the CES-D scale used in this study to assess depression, while widely considered to have an acceptable screening accuracy in primary care settings, is not as robust as certain other diagnostic instruments and this may have limited the interpretation of the findings to some extent.[40] However, this is the first human study to investigate the associations of all relevant B-vitamin biomarkers (including riboflavin, rarely assessed in cohort studies or nutritional surveys) with depression and anxiety in older adults, and thus allowed an in-depth examination of the role of one-carbon metabolism in mental

health. Finally, this is the first study to have considered the potential role of fortified foods as a practical means of reducing depression in older age.

Conclusions/Relevance

This study shows that lower biomarker status of folate or vitamin B6 or riboflavin was associated with depression in older adults, while deficient status of vitamin B6 was associated with anxiety. Higher intakes of B-vitamin fortified foods (e.g. fortified breakfast cereals) or B-vitamin supplement use resulted in the achievement of optimal B-vitamin biomarker status, whereas fortified foods consumed daily were associated with lower depression. Further work in the form of well-designed randomized controlled trials, investigating relevant B-vitamins and in populations with sub-optimal B-vitamin status, are needed to confirm these observational findings. If confirmed, these results may have implications for dietary recommendations and health policy involving low cost non-drug options to improve mental health and thus quality of life in older adults.

Conflicts of Interest

The authors have no financial or personal conflicts of interest.

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List of Figure Captions

Fig.1. Flow Diagram and Study Design of the TUDA Aging Cohort

Fig. 2. Risk of Depression in Relation to B-vitamin Biomarker Status

RBC, red blood cell; PLP, pyridoxal 5-phosphate; EGRac, erythrocyte glutathione reductase co-efficient. Values are odds ratios for risk of CES-D score ≥16 with 95% CI relative to reference category, with adjustment for age, gender, anti-depressant drug usage, age finished education, vitamin supplement usage, smoking status, physical frailty (Timed up and go), living alone, area-based socioeconomic deprivation and transient ischemic attack. *P < .05 †P <.01.

Fig. 3. Risk of Depression in Relation to B-vitamin Fortified Food Intake

Values are odds ratios for risk of CES-D score ≥ 16 with 95% CI relative to reference category, with adjustment for age, gender, anti-depressant drug usage, age finished education, smoking status, physical frailty (Timed up and go), living alone, area-based socioeconomic deprivation and transient ischemic attack. *P < 0.001.

Table 1

General Characteristics of TUDA Study Participants

	Males (n = 1665)	Females (n = 3406)	*P**
Age, mean (SD) (year)	73.4 (8.0)	74.3 (8.4)	< .001
Education, mean (SD) (years)	16.0 (3.2)	16.0 (2.9)	.543
Health and Lifestyle			
Instrumental Activities of Daily Living	24.1 (0.1)	24.1 (0.1)	.895
Physical Self Maintenance Score	23.1 (0.05)	22.9 (0.3)	< .001
Timed Up and Go (seconds)	14.1 (0.2)	14.0 (0.1)	.461
Living alone % (n)	22.4 (373)	39.2 (1335)	< .001
Current Smoker % (n)	11.6 (193)	12.1 (411)	.651
Alcohol (units/week)	8.8 (0.2)	2.5 (0.2)	< .001
Fortified Food Consumer % (n)	71.2 (1186)	71.7 (2443)	.888
B-vitamin Supplement User % (n)	9.8 (163)	11.4 (3820	.098
Vitamin D Supplement User % (n)	32.1 (533)	55.3 (1867)	< .001
Socio-economic Status (most deprivation) % (n)	26.4 (429)	26.2 (867)	.856
Medical			
BMI (kg/m^2)	28.4 (0.1)	27.7 (0.01)	< .001
Waist to Hip ratio	0.97 (0.02)	0.88 (0.01)	< .001
Diabetes % (n)	18.7 (311)	9.6 (327)	< .001
Hyperlipidemia % (n)	55.3 (919)	52.1 (1774)	.037
Hypertension % (n)	79.2 (1318)	68.1 (2318)	< .001
Previous Myocardial infarction % (n)	16.0 (266)	7.2 (244)	< .001
Previous Transient Ischemic Attack % (n)	8.1 (135)	8.4 (286)	.774
Previous Stroke % (n)	11.4 (189)	5.8 (199)	< .001
Brain Health			
Depression (CES-D Score)	5.5 (0.2)	6.3 (0.1)	.267
Identified Depressed (CES-D Score ≥16)% (n)	8.3 (137)	12.0 (407)	< .001
Self-reported depression % (n)	19.5 (325)	26.2 (893)	< .001
Anti-depressant drugs % (n)	10.2 (169)	15.9 (542)	< .001
Anxiety (HAD score)	2.8 (0.1)	3.4 (0.1)	.513
Identified Anxious (HAD score ≥11) % (n)	3.7 (61)	5.6 (190)	.004
Self-reported anxiety % (n)	15.9 (264)	24.4 (832)	< .001
Cognition (MMSE score)	27.0 (0.1)	27.1 (0.0)	< .001
Cognitive impairment (MMSE <25) % (n)	11.9 (187)	13.5 (444)	.134
Biomarker			
Red blood cell folate (nmol/L)	1043 (13.5)	1094 (9.2)	.001
Serum vitamin B12 (pmol/L)	263 (3.1)	288 (2.1)	< .001
Plasma vitamin B6 (PLP; nmol/L)	65.4 (1.0)	72.0 (0.7)	< .001
Riboflavin (EGRac)	1.34 (0.00)	1.33 (0.00)	.146
Plasma total Homocysteine (µmol/L)	15.2 (0.1)	14.3 (0.1)	< .001
MTHFR 677TT genotype % (n)	11.9 (192)	12.2 (405)	.689

TUDA, Trinity Ulster Department of Agriculture; MMSE, Mini Mental State Examination; CES-D, Centre for Epidemiologic Studies Depression; HAD, Hospital Anxiety and Depression Scale; PLP, pyridoxal 5-phosphate; EGRac, erythrocyte glutathione reductase activation co-efficient; MTHFR methylenetetrahydrofolate reductase. Continuous variables presented as adjusted means (SEM) unless otherwise stated.

*ANCOVA with Bonferroni post hoc tests on log-transformed data when applicable, with adjustment for age, BMI, smoking status, alcohol, anti-depressant medication usage, vitamin supplement usage and fortified food and categorical variables were assessed using χ^2 analysis

Table 2
B-vitamin Intakes from Fortified Food and Supplements in Relation to Biomarker Status

	Non Consumer	**Fortified Food Consumer**			**Supplement User** [†]
		Low consumer	Medium consumer	High consumer	
Servings of Fortified Foods/week	0	1-4	5-7	8+	0-8+
TUDA Total n *(%)* [*]	*1164 (23.0)*	*479 (9.5)*	*1049 (20.7)*	*1724 (34.0)*	*545 (10.8)*
Vitamin Biomarker					
RBC folate (nmol/L)	691 (525, 910)[‡]	802 (612, 1089)[§]	909 (664, 1238)[‖]	1138 (809, 1577)[**]	1554 (1034, 2023)[††]
Serum folate (nmol/L)	16.5 (11.1, 24.4)[‡]	19.5 (14.2,28.9)[§]	24.6 (163, 37.7)[‖]	34.0 (21.5, 57.0)[**]	51.1 (32.6, 77.5)[††]
Serum total vitamin B12 (pmol/L)	238 (174, 318)[‡]	243 (180, 323)[‡§]	260 (188, 336)[*§]	271 (208, 361)[‖]	293 (213, 392)[‖]
Plasma vitamin B6 PLP (nmol/L)	47.0 (31.9, 70.0)[‡]	54.1 (37.5, 80.0)[§]	60.8 (41.5, 87.6)[‖]	70.3 (47.5, 97.6)[**]	70.6 (39.0, 115.0)[**]
EGRac (riboflavin status; ratio)	1.35 (1.25, 1.47)[‡]	1.32 (1.22, 80.0)[§]	1.28 (1.20, 1.38)[‖]	1.28 (1.20, 1.39)[‖]	1.24 (1.15, 1.34)[**]
Homocysteine (µmol/L)	15.2 (12.2, 19.1)[‡]	13.7 (11.4, 16.7)[§]	13.7 (11.3, 17.1)[§]	12.6 (10.7, 15.7)[‖]	12.2 (10.3, 15.0)[‖]

RBC, red blood cell; PLP, pyridoxal 5-phosphate; EGRac, erythrocyte glutathione reductase activation co-efficient
Data presented as median (25th, 75th percentiles). Differences were assessed using ANCOVA with Bonferroni post hoc tests on log-transformed data when applicable, controlling for age, gender, BMI and smoking. Values within a row without a common superscript symbol ([‡, §, ‖, **,††]) are significantly different ($P < 0.001$). Normal reference ranges for the laboratory assay from lab where analysis was conducted: RBC folate >340 nmol/L; Serum vitamin B12 >148pmol/L; Vitamin B6 ≥30 nmol/L; Riboflavin ≤1.3; Homocysteine <15µmol/L.
[*]A small number of participants (n = 110; 2.2%) could not be classified as regards fortified food intake and supplement use and are not included in this analysis
[†]Supplement User' was identified as current user of supplemental B-vitamins in tablet form (irrespective of fortified food).