

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

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18 **Running title:** B-vitamin Status and Depression in Aging

19 **Key words:** B-vitamins, folate, depression, anxiety, aging, food fortification

20 **Funding sources:** This work was supported by the Irish Department of Agriculture,
21 Food and the Marine and Health Research Board (under the Food Institutional
22 Research Measure, FIRM) and from the Northern Ireland Department for Employment
23 and Learning (under its Strengthening the All-Island Research Base initiative).

24 **Word count:** Abstract: 300; Brief Summary: 185; Main text: 3536; Reference count:
25 40; Table/Figures: 5 and 1 Supplemental Table.

26 **Brief Summary:**

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

30

31 **Abstract**

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

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

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

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

47 *Results:* Biomarker values in the lowest 20% of status for folate (Odds Ratio (OR)
48 1.79; 95% CI 1.23-2.61), vitamin B6 (OR 1.45; 1.01-2.06) or riboflavin (OR 1.56; 1.10-

49 2.00), but not vitamin B12, were each associated with an increased risk of depression
50 (CES-D score ≥ 16). Correspondingly, B-vitamin fortified foods if consumed daily were
51 associated with a reduced risk depression (OR 0.54; 0.41-0.70). A deficient status of
52 vitamin B6 (OR 1.73; 1.07-2.81), but not other vitamins, was associated with increased
53 anxiety.

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

59

60 **Introduction**

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

74 Historically, clinical deficiencies of folate and vitamin B12 were associated with
75 a range of neuropsychiatric symptoms, including depression,⁹⁻¹¹ raising the possibility
76 that optimizing relevant B-vitamin intake and status could be protective. Research to
77 date in this area has however focused predominantly on folate, and to a lesser extent
78 vitamin B12¹² whereas related B-vitamins - vitamin B6 and riboflavin - also required
79 for one-carbon metabolism have received much less attention. The aim of this study
80 therefore was to investigate biomarker status of all relevant B-vitamins - folate, vitamin
81 B12, vitamin B6 and riboflavin - in relation to mental health in a well characterized
82 cohort of 5186 older adults born in Ireland. Furthermore, this study considered the role
83 of fortified foods as a means of optimizing B-vitamin status, and potentially reducing
84 the risk of depression and anxiety, in older adults.

85 **Methods**

86 *Study design and participants*

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

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

104

105 *Neuropsychiatric assessment*

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

110 as a cut-off value suggestive of clinical depression.¹⁴ Anxiety was assessed using the
111 7 item Hospital Anxiety and Depression (HAD) scale, with a minimum score of 0
112 (suggestive of no symptoms of anxiety) and a maximum score of 21 (significant
113 anxiety). A score ≥ 11 was used as a cut-off value for probable anxiety.¹⁵

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

118

119 *Blood sampling and laboratory analysis*

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

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

137

138 *Dietary assessment*

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

149

150 *General health, lifestyle and biophysical measures*

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

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

165

166 *Statistical Analysis*

167 All statistical analysis was performed using SPSS software (Statistical Package
168 for Social Sciences, Version 23.0, SPSS UK Ltd., Chersey, United Kingdom). Data
169 were checked for normality and log-transformed as appropriate. Analysis of
170 covariance with Bonferroni post hoc test was used for analysis of continuous data and
171 chi-squared tests were used for categorical variables. Relationships of demographic,
172 clinical and lifestyle factors with depression (CES-D score) and anxiety (HAD score)
173 were investigated using multiple linear regression analysis. The risk of depression
174 (CES-D score ≥ 16) and anxiety (HAD score ≥ 11) in relation B-vitamin biomarker status
175 was determined using logistic regression. For this purpose, B-vitamin biomarkers were
176 examined in quintiles ranging from the highest 20% (reference category) to lowest
177 20% of values, and the model was adjusted for relevant co-variates. The associations
178 of B-vitamin fortified food intake with risk of depression (CES-D score ≥ 16) and anxiety
179 (HADS score ≥ 11) were also determined using logistic regression, with adjustment for
180 relevant co-variates; the reference category was non-consumers, against which the
181 remaining categories (low, medium and high fortified food frequencies) were
182 compared.

183

184 **Results**

185 *General characteristics*

186 The general characteristics of the study population are described in **Table 1**.
187 Participants were predominantly female (67%), the majority were fortified food
188 consumers (72%) and 11% were B-vitamin supplement users. Overall, higher rates of
189 depression (CES-D score ≥ 16.0) and anxiety (HAD score ≥ 11.0) were recorded in
190 females compared to males; likewise, self-reported depression and anxiety were also
191 higher in females. B-vitamin biomarker status was generally lower, and homocysteine
192 concentrations higher, in men compared to women. Although mean B-vitamin
193 biomarker concentrations fell within normal reference ranges, some evidence of
194 deficiency (using accepted laboratory cut-offs) was identified for specific B-vitamin
195 biomarkers (data not shown): folate (RBC folate 2.3%); vitamin B12 (serum B12
196 11.6%); vitamin B6 (PLP 12.2%); riboflavin (EGRac 48.6%).

197

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

199 The relationship of clinical and lifestyle factors with depression (CES-D score)
200 and anxiety (HAD score) was examined by linear regression (**Supplemental Table 1**).
201 The following factors were significantly associated with depression: female sex ($\beta =$
202 0.04 , $P = .008$), socioeconomic status ($\beta = 0.09$, $P < .001$), physical frailty ($\beta = 0.19$, P
203 $< .001$), living alone ($\beta = 0.08$, $P < .001$), antidepressant usage ($\beta = 0.21$, $P < .001$),
204 previous ischemic attack ($\beta = 0.04$, $P = .02$) and smoking ($\beta = 0.05$, $P = .001$), whereas
205 age ($\beta = - 0.10$, $P < .001$) and education ($\beta = - 0.06$, $P < .001$) were negatively related
206 to depression. The following factors were identified as being positively associated with
207 anxiety: female sex ($\beta = 0.08$, $P < .001$), socioeconomic status ($\beta = 0.08$, $P < .001$),
208 hypertension ($\beta = 0.04$, $P = .027$) and anti-depressant usage ($\beta = 0.18$, $P < .001$),

209 whereas age ($\beta = -0.138$, $P < .001$), education ($\beta = -0.10$, $P < .001$) and BMI ($\beta = -0.05$,
210 $P < .001$) were inversely related to anxiety.

211

212 *B-vitamin biomarker status in relation to depression and anxiety*

213 The associations of B-vitamin biomarker status with risk of depression (CES-D
214 score ≥ 16) was examined after adjustment for the above co-variables and vitamin
215 supplement use (**Fig. 2**). Each B-vitamin was examined in quintiles of biomarker
216 status; the reference category was set at the highest 20% of values. Compared with
217 the reference category, the lowest quintile of folate (Odds Ratio (OR) 1.79; 95% CI
218 1.23-2.61, $P = .002$), vitamin B6 (OR 1.45; 1.01-2.06, $P = .043$) or riboflavin (OR 1.56;
219 1.10-2.00, $P = .012$) status was associated with increased risk of depression. No
220 significant relationship of serum total B12 was observed with depression ($P = 0.577$).
221 Similarly, the relationship of B-vitamins with anxiety was examined in quintiles of
222 biomarker status (data not shown). After adjustment for relevant co-variables (i.e. age,
223 gender, anti-depressant drug usage, education, BMI, socioeconomic status and
224 hypertension) and vitamin supplement use, only low/deficient status of B6 - but not
225 other B-vitamins - was associated with an increased risk of anxiety (OR 1.73; 1.07-
226 2.81, $P = .024$).

227

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

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

234 status of each vitamin increased in a stepwise manner, with the highest B-vitamin
235 biomarker status being observed in those participants who consumed the highest
236 intakes of fortified foods (i.e. at least once daily) and in those taking B-vitamin
237 supplements. Supplement users were identified on the basis of their reported current
238 use of supplemental B vitamins in tablet form (irrespective of fortified food) and
239 accounted for 10.8% of overall TUDA sample. A small number of participants (n = 110;
240 2.2%) could not be classified as regards fortified food intake and supplement use and
241 thus were excluded from this part of the analysis. Fortified breakfast cereals (65%),
242 spreads (55 %) and drinks (20 %) were the most commonly consumed fortified foods
243 within this cohort (data not shown).

244 The risk of depression was examined in relation to B-vitamin fortified food intake
245 (**Fig. 3**); for this purpose, the reference category was 'non-consumers' i.e. no fortified
246 food or supplement usage. High fortified food intake (> 1 portion per day) was
247 associated with significantly lower depression (OR 0.54; 95% CI 0.41-0.70, $P < .001$).
248 After adjustment for relevant co-variates (i.e. age, gender, anti-depressant medication,
249 education, vitamin supplement usage, smoking status, physical frailty, living alone,
250 socioeconomic status and transient ischemic attack) and fortified food intake, B-
251 vitamin supplement usage was not associated with risk of depression (OR 0.941; 0.68-
252 1.30, $P = .712$). No significant relationship was identified between B-vitamin fortified
253 food intake (OR 0.97; 0.69-1.36, $P = .861$) or supplement usage (OR 0.99; 0.64-1.54,
254 $P = .974$) and anxiety.

255

256 **Discussion**

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

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

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

284 the population would make a relationship with depression less likely. The current study
285 found no association of vitamin B12 with depression, which is in line with the findings
286 from one large cohort study (n = 2,524) conducted in the USA,²⁸ but at odds with other
287 research which reported inverse associations of vitamin B12 intake^{26,27} or
288 biomarkers²⁹ with depression. The explanation for such discrepancy in the evidence
289 linking vitamin B12 with depression is unclear, but may possibly relate to differences
290 in B12 status among populations under investigation or methodological variation
291 among studies, including the use of different B12 biomarkers to measure status,
292 especially considering that no consensus exists as to the best biomarker for assessing
293 B12 status in the laboratory.³⁰

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

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

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

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

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

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

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

361

362 **Conclusions/Relevance**

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

374

375 **Conflicts of Interest**

376 The authors have no financial or personal conflicts of interest.

377

378 **Acknowledgements**

379 The TUDA study was supported by the Irish Department of Agriculture, Food
380 and the Marine and Health Research Board (under the Food Institutional Research
381 Measure, FIRM) and from the Northern Ireland Department for Employment and
382 Learning (under its Strengthening the All-Island Research Base initiative).

383 Sponsor's role: The funders of this research had no role in the design, methods,
384 subject recruitment, data collections, analysis and preparation of paper.

385 The authors are grateful to all TUDA participants.

386

References

1. United Nations Department of Economic and Social Affairs/Population Division. World Population Prospects: The 2015 Revision, Key Findings and Advance Tables. United Nations, 2015.
2. Andreas S, Schulz H, Volkert J, Dehoust M et al. Prevalence of mental disorders in elderly people: the European MentDis_ICF65+ study. *Br J Psychiatry* 2016;210:125-131.
3. World Health Organisation. .Mental health and older adults. <http://www.who.int/mediacentre/factsheets/fs381/en/>. Accessed on April 2016.
4. Almeida OP, Flicker L, Hankey GJ, Yeap BB et al. Depression, Frailty, and All-Cause Mortality: A Cohort Study of Men Older than 75 Years. *J Am Med Dir Assoc* 2015;16:296-300.
5. Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA et al. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am J Psychiatry* 2006;163:1905-1917.
6. Moore K, Hughes CF, Ward M, Hoey L et al. Diet, nutrition and the ageing brain: current evidence and new directions. *Proc Nutr Soc* 2018;1-12.
7. Bailey LB, Stover PJ, McNulty H, Fenech MF et al. Biomarkers of Nutrition for Development-Folate Review. *J Nutr* 2015;145:1636-1680.
8. Bottiglieri T, Reynolds EH. Folate and Neurological Disease. In: LB Bailey, eds. *Folate in Health and Disease*. 2nd Ed. Florida: CRC Press, 2005.

9. Carney MWP. Serum Folate Values In 423 Psychiatric Patients. *Br Med J* 1967;4:512-516.
10. Reynolds EH, Preece JM, Bailey J, Coppen A. Folate Deficiency in Depressive Illness. *Br J Psychiatry* 1970;117:287.
11. Shorvon SD, Carney MWP, Chanarin I, Reynolds EH. The neuropsychiatry of megaloblastic anaemia. *Br Med J* 1980;281:1036.
12. Petridou ET, Kousoulis AA, Michelakos T, Papatoma P et al. Folate and B12 serum levels in association with depression in the aged: a systematic review and meta-analysis. *Aging Ment Health* 2016;20:965-973.
13. McCann A, McNulty H, Rigby J, Hughes CF et al. Impact of area-level socioeconomic deprivation on the risk of cognitive dysfunction in older adults. *J Am Geriatr Soc* 2018;66:1269-1275.
14. Radloff L, Locke B. The community mental health assessment survey and the CES-D Scale. . In: M Weissman, Myers J, Ross C, eds. *Community surveys of psychiatric disorders*. New Brunswick, NJ: Rutgers University Press, 1986.
15. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand* 1983;67:361-370.
16. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;12:189-198.

17. Molloy AM, Scott JM. Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. *Methods Enzymol* 1997;281:43-53.
18. Kelleher BP, Broin S. Microbiological Assay for Vitamin-B-12 Performed in 96-Well Microtitre Plates. *J Clin Pathol* 1991;44:592-595.
19. Leino A. Fully automated measurement of total homocysteine in plasma and serum on the Abbott IMx analyzer. *Clin Chem* 1999;45:569-571.
20. Bates CJ, Pentieva KD, Matthews N, Macdonald A. A simple, sensitive and reproducible assay for pyridoxal 5'-phosphate and 4-pyridoxic acid in human plasma. 1999;280:101-111.
21. Powers HJ, Bates CJ, Prentice AM, Lamb WH et al. The relative effectiveness of iron and iron with riboflavin in correcting a microcytic anaemia in men and children in rural Gambia. 1983;37:413-425.
22. Hoey L, McNulty H, Askin N, Dunne A et al. Effect of a voluntary food fortification policy on folate, related B vitamin status, and homocysteine in healthy adults. *Am J Clin Nutr* 2007;86:1405-1413.
23. Podsiadlo D, Richardson S. The timed 'Up and Go': A test of basic functional mobility for frail elderly persons. *J Am Geriatr Soc* 1991;39:142-148.
24. Gilbody S, Lightfoot T, Sheldon T. Is low folate a risk factor for depression? A meta-analysis and exploration of heterogeneity. *J Epidemiol Community Health* 2007;61:631-637.

25. Duffy ME, Hoey L, Hughes CF, Strain JJ et al. Biomarker responses to folic acid intervention in healthy adults: a meta-analysis of randomized controlled trials. *Am J Clin Nutr* 2014;99:96-106.
26. Skarupski KA, Tangney C, Li H, Ouyang B et al. Longitudinal association of vitamin B-6, folate, and vitamin B-12 with depressive symptoms among older adults over time. *Am J Clin Nutr* 2010;92:330-335.
27. Gougeon L, Payette H, Morais JA, Gaudreau P et al. Intakes of folate, vitamin B6 and B12 and risk of depression in community-dwelling older adults: the Quebec Longitudinal Study on Nutrition and Aging. *Eur J Clin Nutr* 2016;70:380.
28. Beydoun MA, Shroff MR, Beydoun HA, Zonderman AB. Serum folate, vitamin B-12, and homocysteine and their association with depressive symptoms among U.S. Adults. *Psychosom Med* 2010;72:862-873.
29. Kim JM, Stewart R, Kim SW, Yang SJ et al. Predictive value of folate, vitamin B-12 and homocysteine levels in late-life depression. *Br J Psychiatry* 2008;192:268-274.
30. McNulty H, Hughes C. Assessing biomarker status of vitamin B12 in the laboratory: no simple solution. *Ann Clin Biochem* 2017;55:188-189.
31. Merete C, Falcon LM, Tucker KL. Vitamin B6 is associated with depressive symptomatology in Massachusetts elders. *J Am Coll Nutr* 2008;27:421-427.
32. Carney MW, Ravindran A, Rinsler MG, Williams DG. Thiamine, riboflavin and pyridoxine deficiency in psychiatric in-patients. *Br J Psychiatry* 1982;141:271-272.

33. Murakami K, Mizoue T, Sasaki S, Ohta M et al. Dietary intake of folate, other B vitamins, and omega-3 polyunsaturated fatty acids in relation to depressive symptoms in Japanese adults. *Nutrition* 2008;24:140-147.
34. Madigan SM, Tracey F, McNulty H, Eaton-Evans J et al. Riboflavin and vitamin B-6 intakes and status and biochemical response to riboflavin supplementation in free-living elderly people. *Am J Clin Nutr* 1998;68:389-395.
35. Bjelland I, Tell GS, Vollset SE, Refsum H et al. Folate, vitamin B-12, homocysteine, and the MTHFR 677C -> T polymorphism in anxiety and depression - The Hordaland Homocysteine Study. *Arch Gen Psychiatry* 2003;60:618-626.
36. Lewis JE, Tiozzo E, Melillo AB, Leonard S et al. The Effect of Methylated Vitamin B Complex on Depressive and Anxiety Symptoms and Quality of Life in Adults with Depression. *ISRN Psychiatry* 2013;2013:621453.
37. Pan Y, Cai W, Cheng Q, Dong W et al. Association between anxiety and hypertension: a systematic review and meta-analysis of epidemiological studies. *Neuropsychiatr Dis Treat* 2015;11:1121-1130.
38. Mills J, Molloy AM, Reynolds E. Do the benefits of folic acid fortification outweigh the risk of masking vitamin B12 deficiency? *BMJ* 2018;360:k1334-k1334.
39. Hensler J. Serotonin. In: Siegel GJ, Albers RW, Brady ST, Price DL, eds. *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*. 7th Ed. Canada: Elsevier Academic Press, 2006.

40. Vilagut G, Forero CG, Barbaglia G, Alonso J. Screening for Depression in the General Population with the Center for Epidemiologic Studies Depression (CES-D): A Systematic Review with Meta-Analysis. PLoS One 2016;11:e0155431-e0155431.

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)	<i>P</i> *
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
<i>Health and Lifestyle</i>			
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
<i>Medical</i>			
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
<i>Brain Health</i>			
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
<i>Biomarker</i>			
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
<i>MTHFR 677TT genotype % (n)</i>	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)
<i>Vitamin Biomarker</i>					
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 (16.3, 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, 1.40)§	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).

