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This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1562059> since 2020-02-28T15:28:25Z

Published version:

DOI:10.3945/jn.115.225433

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Plasma riboflavin and vitamin B-6, but not homocysteine, folate or vitamin B-12 are inversely associated with breast cancer risk in the EPIC-Varese cohort^{1,2,3,4}

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PubMed indexing: Agnoli, Grioni, Krogh, Pala, Allione, Matullo, Di Gaetano, Tagliabue, Pedraglio, Garrone, Cancarini, Cavalleri, Sieri

Word count: 5154; **Number of tables:** 3.

Running title: B vitamins and homocysteine in breast cancer risk.

¹ **Abbreviations.** BMI: body mass index; CI: confidence interval; CV: coefficient of variation; EPIC: European Prospective Investigation into Cancer and Nutrition; ER: estrogen receptor; HER2: human epidermal growth factor receptor 2; ORDET: Hormones and Diet in the Etiology of Breast Cancer; PLP: pyridoxal-5'-phosphate; PR: progesterone receptor; RR: rate ratio.

² This study was supported by the Italian Ministry of Health.

³ All authors declare no conflict of interest.

⁴ Supplemental Table 1, 2, and 3 are available from the “Online Supporting Material” link in the online posting of the article.

1 ABSTRACT

2 **Background.** One-carbon metabolism – important for DNA stability and integrity – may play a role
3 in breast carcinogenesis. However, epidemiological studies addressing this issue have yielded
4 inconsistent results.

5 **Objective.** We prospectively investigated associations between breast cancer and plasma folate,
6 riboflavin, vitamin B-6, vitamin B-12, and homocysteine, in women recruited to the Varese (Italy)
7 cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC).

8 **Methods.** We performed a nested case-control study on women aged 35-65 years at recruitment,
9 median body mass index 25.3 kg/m², who gave blood samples in 1987-1992, and again in 1993-
10 1998. Breast cancer cases identified to 31 December 2009 were individually matched to controls.
11 Relative risks (RRs) of breast cancer (and subtypes defined by hormone receptor status) with 95%
12 confidence intervals (CIs) were estimated by unconditional logistic regression, controlling for
13 matching factors and breast cancer risk factors.

14 **Results.** After a median of 14.9 years, 276 breast cancer cases were identified and matched to 276
15 controls. Increasing plasma vitamin B-6 was associated with decreased risk of overall (RR: 0.78;
16 95%CI: 0.63, 0.96 for 1SD increase), premenopausal (RR: 0.66; 95%CI: 0.48, 0.92 for 1SD
17 increase), ER+ (RR: 0.79; 95%CI: 0.63, 1.00 for 1SD increase) and PR+ (RR: 0.72; 95%CI: 0.55,
18 0.95 for 1SD increase) breast cancers. Increasing plasma vitamin B-6 was also associated with
19 decreased breast cancer risk in alcohol consumers (≥ 7 g/d) compared to consumption of < 7 g/d plus
20 non-consumption (RR: 0.71; 95% CI: 0.51, 0.99).

21 High plasma riboflavin was associated with significantly lower risk in premenopausal women (RR:
22 0.45; 95%CI: 0.21, 0.94 highest vs. lowest quartile, *P* trend=0.021). Plasma homocysteine, folate,
23 and vitamin B-12 were not associated with breast cancer risk.

24 **Conclusions.** High plasma vitamin B-6 and riboflavin may lower breast cancer risk, especially in
25 premenopausal women. Additional research is necessary to further explore these associations.

26 297 WORDS

27

28 **Keywords:** Breast cancer, B vitamins, homocysteine, nested case-control study, EPIC.

29

30 INTRODUCTION

31 The micronutrients folate, vitamin B-12, vitamin B-6, riboflavin, and homocysteine are all involved
32 in one-carbon metabolism, and thus play important roles in maintaining DNA stability and integrity.
33 Folate, as 5-methyltetrahydrofolate, is required to remethylate homocysteine to methionine which is
34 converted to S-adenosylmethionine. The latter provides methyl groups for methylation reactions in
35 general, and DNA and RNA biosynthesis in particular (1-5). S-adenosylmethionine depletion
36 induces DNA hypomethylation which may lead to expression of proto-oncogenes and eventually
37 cancer (6). Folate deficiency also results in deficient methylation of uracil to thymine, so that uracil
38 is incorporated into DNA (2), leading to chromosome breaks and carcinogenesis (3,6). Vitamin B-
39 12 deficiency is expected to cause chromosome breaks by the same mechanism as folate since it is
40 an essential coenzyme in the methylation of homocysteine to methionine (2,4).

41 Vitamin B-6 is an essential coenzyme for several catabolic and anabolic reactions. In particular it is
42 required for the conversion of tetrahydrofolate to 5,10-methylenetetrahydrofolate by serine
43 hydroxymethyltransferase (2). 5,10-methylenetetrahydrofolate is required for the synthesis of
44 nucleotides, themselves necessary for DNA synthesis and repair. Vitamin B-6 deficiency decreases
45 the activity of serine hydroxymethyltransferase, thereby depleting the 5,10-
46 methylenetetrahydrofolate pool, so that uracil is incorporated into DNA and chromosome breaks
47 occur (2). Vitamin B-6 is also necessary for the synthesis of glutathione from homocysteine:
48 glutathione is a cofactor of glutathione-S-transferases and peroxidases, which detoxify many
49 carcinogenic compounds and protect against oxidative DNA damage (7-9). Riboflavin is the
50 precursor of flavin adenine dinucleotide, a necessary cofactor for 5,10-methylenetetrahydrofolate
51 reductase (10-13), which catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to
52 5-methyltetrahydrofolate: the latter being the methyl donor for DNA methylation (10,11,14,15).

53 Inadequate levels of folate, vitamin B-12, vitamin B-6, and riboflavin may all result in high levels
54 of blood homocysteine (5,16) by disrupting the pathways summarized above (17). In vitro studies
55 indicate that high homocysteine levels are associated with high proliferation rates of cancer cells
56 including breast cancer cells (18,19), and also with oxidative damage to cells (20). High

57 homocysteine levels in blood have been associated with increased breast cancer risk in women
58 with low folate status (21), and also in women with high body mass index (BMI), high plasma
59 triglycerides, and abnormal oxidation of low-density lipoproteins (20,22-26) – all of which are
60 associated with increased risk of certain cancers including breast cancer (27,28).

61 Studies on associations of plasma homocysteine (21,29-32), folate (29,30,32-34), vitamin B-12
62 (30,32,33), and vitamin B-6 (30,32,33) with breast cancer risk, have produced mixed results. To our
63 knowledge no previous study has assessed the effect of plasma riboflavin on breast cancer risk. We
64 carried out a case-control study, nested in the EPIC-Varese cohort, to prospectively evaluate
65 whether plasma levels of homocysteine, folate, vitamin B-12, vitamin B-6, and riboflavin, were
66 associated with risk of breast cancer, and risk of breast cancer subtypes defined by expression of
67 hormone receptors.

68

69 **MATERIALS AND METHODS**

70 **Study population and data collection**

71 This was a case-control study nested in the women participating in the EPIC-Varese cohort study –
72 part of the larger European Investigation into Cancer and Nutrition (EPIC). We considered 6071
73 women defined by the following eligibility criteria: recruitment to the prospective Hormones and
74 Diet in the Etiology of Breast Cancer (ORDET) study in 1987-1992; recruitment to EPIC-Varese in
75 1993-1998 (70% of women who participated in ORDET were subsequently recruited in EPIC-
76 Varese); and either premenopausal or postmenopausal at the ORDET and EPIC baselines
77 (perimenopausal women and those with uncertain menopausal status were excluded).

78 The date of entry to the present study was the EPIC recruitment date. At EPIC baseline, after
79 participants had given written informed consent, detailed information was collected on reproductive
80 and medical history, physical activity, alcohol consumption, smoking, education and other
81 socioeconomic variables using a standardized lifestyle questionnaire. Diet over the previous year
82 was investigated using a food frequency questionnaire specifically developed to capture local
83 dietary habits. Also at baseline, weight, height, and blood pressure were measured and a 30 mL

84 fasting blood sample was collected, using standardized procedures. The blood samples were
85 divided into 0.5 mL aliquots of plasma, serum, red blood cells, and buffy coat, on the day of
86 collection, and stored in liquid nitrogen at -196 °C (35).

87 All study participants had also been recruited to the earlier ORDET study and at ORDET baseline
88 had given a blood sample. The stored plasma samples were analyzed and the results of these
89 analyses were combined with those obtained from the samples collected at the EPIC baseline, so as
90 to obtain mean estimates that were more reliable than those provided by a single measurement. The
91 study protocol was approved by the ethics committee of the Fondazione IRCCS Istituto Nazionale
92 dei Tumori (Milan, Italy).

93 **Breast cancer cases and selection of control women**

94 The 6071 women were followed-up to December 31, 2009 (median 14.9 years), through the
95 Lombardy Cancer Registry, Varese Province, characterized by high data completeness and quality.
96 A total of 276 new breast cancer cases were identified among the women over the follow-up period
97 from the registry database. Information on estrogen receptor (ER), progesterone receptor (PR), and
98 human epidermal growth factor receptor (HER2) expression of the cancers was obtained from
99 electronic pathology reports.

100 For each case, one matched control was chosen, using an incidence density sampling protocol, from
101 appropriate risk sets consisting of cohort members alive and free of cancer at the time of diagnosis
102 of the index case. Matching criteria were age at recruitment (± 5 years), date of recruitment (± 180
103 days), distance between ORDET and EPIC recruitment (± 90 days), menopausal status
104 (postmenopausal at both ORDET and EPIC baseline, premenopausal at ORDET baseline,
105 postmenopausal at EPIC baseline, premenopausal at ORDET baseline and still premenopausal at
106 EPIC baseline), and micronutrient analysis in the same batch.

107 **Analysis of plasma samples**

108 Analyses were performed both on EPIC and ORDET plasma samples. Folate and vitamin B-12
109 were determined on a Cobas 8000 modular analyzer (Roche Diagnostic GmbH) by Roche Elecsys
110 electrochemoluminescence assays. Homocysteine was determined immuno-enzymatically as S-

111 adenosylhomocysteine produced from serum homocysteine on a Siemens Dimension Vista Lab
112 System analyser (Siemens Healthcare Diagnostics Products GmbH). All assays were performed
113 according to recommendations of the equipment manufacturers.

114 Levels of riboflavin and vitamin B-6 (the latter as pyridoxal-5'-phosphate – PLP – principal active
115 form of vitamin B-6) were measured by LC/MS using a Thermo Fisher LCQ Vantage mass
116 spectrometer coupled to a Thermo Fisher Scientific Transcend HPLC system. Perchloric acid was
117 used to precipitate out proteins from aliquots of plasma to which had been added appropriate
118 internal standards (^{15}N ^{13}C -labelled riboflavin; ^2H -labelled PLP). After incubation at 50°C for 10
119 minutes, the treated aliquots were filtered and injected into the HPLC system and eluted with a
120 water, methanol, ammonium acetate gradient. The mass spectrometer was operated in single
121 reaction monitoring mode to minimize interference from other compounds. Use of internal
122 standards made it possible to correct for losses during purification and variation in instrument
123 response. We excluded 17 cases and 18 controls because EPIC or ORDET plasma samples were not
124 available.

125 **Statistical analysis**

126 We calculated plasma levels of homocysteine, folate, vitamin B-12, vitamin B-6, and riboflavin for
127 each case and control as the mean of the values from the EPIC and ORDET samples. Coefficients
128 of variation (CV) for each, considering the ORDET and EPIC samples as replicates of a single
129 sample were as follows: 13% for homocysteine, 16% for folate, 14% for vitamin B-12, 31% for
130 vitamin B-6, and 23% for riboflavin. Plasma levels were grouped into quartiles based on the
131 distribution in controls. Baseline characteristics of study participants, according to quartiles of
132 plasma vitamin B-6, were summarized as means and standard deviations (continuous variables) or
133 frequencies (categorical variables). Unconditional logistic regression models were used to estimate
134 relative risks (RRs) for breast cancer with 95% confidence intervals (CIs), with lowest quartile as
135 reference; the significance of linear trends was assessed by treating each quartile as a continuous
136 variable in the model and performing the Wald test. RRs were also calculated for 1 standard
137 deviation increments of micronutrient concentration as a continuous variable. We ran a minimally

138 adjusted model, with the matching variables – age (continuous), date of recruitment (continuous),
139 time between ORDET and EPIC recruitment (continuous), and menopausal status
140 (premenopausal/postmenopausal in EPIC) – as covariates. Was also ran fully-adjusted models, with
141 the following additional covariates: family history of breast cancer in first degree relatives (yes, no),
142 age at menarche (<15 years, ≥15 years), parity (nulliparous, 1-2 children, >2 children), oral
143 contraceptive use (never, sometime), education (≤8 years, >8 years), smoking status (never, former,
144 current), alcohol consumption (continuous), and BMI (continuous).

145 We analyzed all women, and postmenopausal and premenopausal women separately. *P* values for
146 interaction between plasma micronutrient with menopausal status were estimated by adding the
147 product of quartile of plasma micronutrient with menopausal status to the model and applying the
148 Wald test. We also analyzed risk of developing breast cancer subtypes defined by receptor status.
149 Heterogeneity was investigated by the Wald test. We analyzed subgroups defined by alcohol intake
150 (abstainers, <7 g/d, ≥7 g/d) with *P* interaction calculated by treating alcohol intake as a
151 dichotomous variable (abstainer, consumer) and multiplying this by the analyte value (continuous).
152 We excluded one case and two controls because confounder variables were missing; the analyses
153 were thus performed on 514 women, 258 cases and 256 controls. All statistical tests were two-
154 sided, differences were considered significant for *P* <0.05. The analyses were performed with Stata
155 version 11.2 (College Station, TX, USA).

156 **RESULTS**

157 Baseline characteristics of study participants by quartiles of plasma vitamin B-6 are shown in Table
158 1. Women in the highest vitamin B-6 quartile tended to have lower BMI, lower plasma
159 homocysteine, and higher levels of other B vitamins. They were also better educated, and less likely
160 to be smokers, to have used oral contraceptives, and to have a family history of breast cancer. Table
161 2 shows the risks (RRs) of developing breast cancer by quartiles of plasma homocysteine and B
162 vitamins for all study women. High levels of vitamin B-6 in the continuous model were associated
163 with decreased breast cancer risk [RR: 0.78; 95% CI: 0.63, 0.96 (fully-adjusted model)], however

164 no reduction in risk was found in the analysis based on quartiles. None of the other micronutrients
165 was significantly associated with risk (P trend ≥ 0.31).

166 Table 3 shows risk estimates by menopausal status at baseline. Among postmenopausal women,
167 none of the micronutrients was significantly associated with breast cancer risk (P trend ≥ 0.29).

168 Among premenopausal women, high levels of vitamin B-6 were associated with significantly
169 lowered breast cancer risk in the continuous model (RR: 0.66; 95% CI: 0.48, 0.92), however no
170 reduction in risk was found in the analysis based on quartiles. The highest quartile of plasma
171 riboflavin (compared to the lowest) was also associated with significantly lowered breast cancer
172 risk [RR: 0.45; 95% CI: 0.21, 0.94, P trend 0.021 (fully adjusted model)]; there was a significant
173 interaction between menopausal status and plasma riboflavin ($P=0.021$). Levels of homocysteine
174 and the other B vitamins were not significantly associated with premenopausal breast cancer risk (P
175 trend ≥ 0.22). However risk in the third quartile of vitamin B-12 concentration was significantly
176 lower than reference (RR: 0.41; 95% CI: 0.19, 0.92). No interaction was found between menopausal
177 status and plasma levels of homocysteine ($P=0.44$), folate ($P=0.46$), vitamin B-12 ($P=0.45$) or
178 vitamin B-6 ($P=0.42$).

179 Associations of plasma homocysteine and B vitamins with breast cancer subtypes defined by
180 hormonal receptor status are shown in Supplemental Tables 1, 2, and 3. The second quartile of
181 plasma homocysteine was associated with significantly decreased risk of ER+ disease (RR: 0.54;
182 95% CI: 0.31, 0.96) compared to the lowest. Significant heterogeneity depending on ER status was
183 found for plasma folate (P heterogeneity 0.045), however no significant association of folate with
184 either ER+ (P trend=0.13) or ER- (P trend=0.24) disease was found. Significant heterogeneity
185 depending on ER status was found for B-12 in the continuous model (P heterogeneity 0.032), again
186 however no significant association was found with either ER+ or ER- disease. High vitamin B-6
187 was associated with lowered risk (borderline significance) of ER+ disease in the continuous model
188 (RR: 0.79; 95% CI: 0.63, 1.00) (Supplemental Table 1). Vitamin B-6 in the continuous model was
189 associated with a significantly lowered risk of PR+ disease (RR: 0.72; 95% CI: 0.55, 0.95). The
190 second quartile of plasma homocysteine was associated with a significantly lowered (by 49%) risk

191 of PR+ disease, and the second quartile of vitamin B-12 was associated with a significantly
192 decreased (by 61%) risk of PR- disease compared to the first quartile. No significant heterogeneity
193 in relation to PR status was found ($P \geq 0.22$) (Supplemental Table 2). None of the micronutrients
194 analyzed was associated with the risk of developing either HER2+ (P trend ≥ 0.07) or HER2- (P
195 trend ≥ 0.30) disease, and no significant heterogeneity in relation to HER2 status was found (P
196 ≥ 0.09) (Supplemental Table 3).

197 When the analyses were stratified by alcohol intake, plasma vitamin B-12 was associated with
198 significantly increased breast cancer risk among abstainers (RR: 4.88; 95% CI: 1.16, 20.55 for the
199 highest vs. lowest quartile), however few cases were available for this sub-analysis; P for
200 interaction between plasma vitamin B-12 and alcohol intake was not significant. High vitamin B-6
201 was associated with a significantly lowered breast cancer risk among women who drank >7 g/d of
202 alcohol (RR: 0.71; 95% CI: 0.51, 0.99 in the continuous model); no association was found for
203 abstainers or women who drank ≤ 7 g/d of alcohol. P for interaction between plasma vitamin B-6 and
204 alcohol intake was not significant (0.87) (data not shown).

205

206 **DISCUSSION**

207 In this nested case-control study, considering all women, breast cancer risk decreased with
208 increasing plasma vitamin B-6 levels. None of the other micronutrients was associated with breast
209 cancer risk overall. However, when women were separated by menopausal status, high vitamin B-6
210 and riboflavin were associated with significantly decreased breast cancer risk among
211 premenopausal women. Although plasma folate and vitamin B-12 were not significantly associated
212 with the risk of breast cancer subtype defined by ER status, there was significant heterogeneity,
213 with a non-significantly decreased risk of ER+ disease for high folate and vitamin B-12, and non-
214 significantly increased risk of ER- disease for high folate and vitamin B-12. For increasing levels of
215 vitamin B-6, the risks of ER+ and PR+ breast cancer decreased significantly, but heterogeneity
216 between receptor positive and negative status was not significant. Finally, increasing vitamin B-6

217 was associated with decreasing breast cancer risk among women who drank >7g/day of alcohol
218 compared to those who drank less or abstained.

219 Previous studies on associations of plasma levels of nutrients involved in one-carbon metabolism
220 with breast cancer risk focused mainly on homocysteine and folate. Of five studies concerned with
221 homocysteine, two found no association (30,32), two case-control studies found that higher
222 homocysteine levels were associated with increased breast cancer risk (29,31), and a case-control
223 study nested in the Women's Health Study found that homocysteine levels were not associated with
224 overall breast cancer risk, but among women with low folate status the risk was increased if
225 homocysteine was high (21). We found no evidence that homocysteine influenced breast cancer
226 risk.

227 Our finding of a null association between plasma folate and breast cancer risk is in agreement with:
228 a nested case-control study in the Washington County serum bank (30); a case-control study
229 conducted in Taiwan (29); and a case-control study on postmenopausal women nested in the Malmö
230 Diet and Cancer cohort (34). Other studies found a decreased breast cancer risk for increasing blood
231 folate levels (36,37), especially in women whose alcohol intake was high (≥ 15 g/d) (32). Finally, a
232 case-control study nested in the Women's Health Study found no association of folate with overall
233 breast cancer risk, but that high folate was unexpectedly associated with increased risks of
234 premenopausal, ER-positive, and PR-positive breast cancer (33).

235 To our knowledge, only three studies have investigated plasma vitamin B-12 and breast cancer risk.
236 One found no association (33), as did our study; another found that for high B-12 risks of overall
237 and premenopausal breast cancer decreased (32); and the other found that risks decreased especially
238 among women postmenopausal at recruitment (30).

239 Our finding that high vitamin B-6 was associated with significantly lowered overall breast cancer
240 risk is not completely in line with the results of the nested case-control study of Zhang et al. (32)
241 which found that among postmenopausal women the association was of borderline significance, but
242 was significant among women who drank less than 15 g/d of alcohol. Lurie et al (38) also found that
243 postmenopausal women with high vitamin B-6 had lowered breast cancer risk, which appeared

244 limited to women with ER+, PR+ and ER+PR+ cancers. Finally, two nested case-control studies
245 found no association between plasma vitamin B-6 and overall breast cancer risk (30,33).

246 Our finding that breast cancer risk was significantly lowered among premenopausal women with
247 high compared to low plasma riboflavin appears unique, as we are aware of no other study to
248 investigate riboflavin/breast cancer associations. The fact that the association was confined to
249 premenopausal women is unexpected – but not too unexpected, given the numerous differences in
250 terms of risk factors, prognosis and molecular biology, between breast cancer in pre- and post-
251 menopausal women; it suggests the need for further research.

252 Vitamin B-6 and riboflavin may lower breast cancer risk through mechanisms other than one-
253 carbon metabolism, since they are essential cofactors in numerous reactions central to human
254 metabolism (39,40). In addition, vitamin B-6 has been shown to decrease oxidative stress, cell
255 proliferation and angiogenesis, and to enhance immune function (39,41); while low vitamin B-6
256 concentrations have been associated with high levels of inflammatory markers (42). Certain
257 carcinogens are metabolized by flavin-dependent enzymes, and the resulting metabolites may have
258 either increased or decreased carcinogenicity (43). Some studies (reviewed in (44)) suggest that the
259 risk of certain cancers is increased when riboflavin is deficient.

260 Furthermore, both riboflavin and vitamin B-6 are cofactors in the pathway by which tryptophan is
261 degraded to kynurenines, and many products of this pathway are neuroactive compounds with
262 immunomodulatory effects (45). The same pathway is stimulated by inflammatory molecules and is
263 often systematically up-regulated during an active immune response (45,46). Since inflammation
264 has been linked to increased overall and premenopausal breast cancer risk (47,48), vitamin B-6 and
265 riboflavin status might be linked to breast cancer risk by inflammation-related mechanisms, perhaps
266 involving the kynurenine pathway. However few other data are available to suggest mechanisms by
267 which low riboflavin status can increase the risk of breast cancer – an association we found in
268 premenopausal women.

269 Study strengths include: prospective design which rendered reverse causation unlikely as an
270 explanation for the associations found; the availability of detailed information on lifestyle, dietary,

271 and anthropometric variables, which made it possible to control for potential confounders; and
272 the availability of two plasma samples taken approximately five years apart, which made it possible
273 to analyze plasma micronutrient levels twice, providing estimates that are likely to be more reliable
274 than a single measurement. A possible study limitation is that samples were collected, stored at -
275 196°C (EPIC samples) or -80°C (ORDET samples), and analyzed up to 25 years later. There may
276 have been differential decay of the analytes over that period. However unless analyte decay varied
277 with initial concentration (which seems unlikely) this will not bias analyte-risk associations. A
278 stability study conducted on vitamin B-6, vitamin B-12, and folic acid in plasma with EDTA, and
279 riboflavin in whole blood, found that no large decline had occurred after 4 years of storage at -20°C
280 (49). Moreover, the small number of breast cancers diagnosed will have decreased the power to
281 detect associations, especially in subgroup analyses. Another limitation is that we performed
282 multiple statistical comparisons that were not corrected for, thereby increasing the risk of
283 erroneously rejecting null hypotheses. Finally, little data is available on biological mechanisms that
284 could explain associations between low vitamin B-6 and riboflavin status and increased breast
285 cancer risk, especially among premenopausal women.

286 To conclude, the findings of this case-control study nested in the EPIC-Varese cohort suggest that
287 high plasma concentrations of vitamin B-6 may decrease the risk of breast cancer and particularly
288 of ER+ breast cancer, and may also lower the risk in moderate-to-heavy drinkers (>7g/d alcohol).
289 High plasma levels of riboflavin may decrease the risk of breast cancer in premenopausal but not
290 postmenopausal women. Homocysteine and other the B vitamins investigated do not seem to
291 influence breast cancer risk. Further research is required to elucidate the mechanisms by which B
292 vitamins can influence the etiology of breast cancer.

293

294 **ACKNOWLEDGEMENTS**

295 We thank A. Evangelista and D. Del Sette for technical support, and Don Ward for help with the
296 English. The authors' contributions were as follows: VK and SS designed the study; SP, AA, and
297 GM developed the methodology; CA, SG, and SS analyzed and interpreted the data; CA, SG, VP,

298 VK, and SS wrote the manuscript; IC, AC, and GG provided essential materials. CA had primary
299 responsibility for final content. All authors have read and approved the final manuscript.

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Table 1. Baseline characteristics of study participants by quartiles of plasma vitamin B-6 among women of the EPIC-Varese study¹.

Characteristic	1: 1.998-6.723 ng/mL (n=136)	2: 6.724-9.438 ng/mL (n=132)	3: 9.439-13.13 ng/mL (n=127)	4: 13.14-109.3 ng/mL (n=119)
Age, years	53.1 ± 7.4	53.7 ± 7.7	53.5 ± 8.5	53.1 ± 8.6
Body mass index, kg/m ²	27.0 ± 4.7	25.6 ± 4.0	26.4 ± 5.0	25.5 ± 5.8
Alcohol consumption, g/d	7.4 ± 10.1	9.7 ± 11.7	9.6 ± 13.5	7.5 ± 11.5
Plasma homocysteine, mmol/L	11.5 ± 3.9	11.7 ± 4.5	11.8 ± 7.0	10.9 ± 3.0
Plasma folate, ng/mL	6.6 ± 1.7	7.1 ± 1.9	7.7 ± 2.1	8.0 ± 2.3
Plasma vitamin B-12, pg/mL	539.1 ± 177.7	602.8 ± 236.4	630.4 ± 337.2	605.2 ± 223.5
Plasma riboflavin, ng/mL	7.4 ± 5.8	8.1 ± 7.2	8.0 ± 6.7	9.6 ± 12.1
Family history of breast cancer, n (%)				
No	121 (89.0)	123 (93.2)	115 (90.5)	109 (91.6)
Yes	15 (11.0)	9 (6.8)	12 (9.5)	10 (8.4)
Age at menarche, n(%)				
<15 years	123 (90.4)	117 (88.6)	112 (88.2)	109 (91.6)
≥15 years	13 (9.6)	15 (11.4)	15 (11.8)	10 (8.4)
Menopausal status, n(%)				
Postmenopausal	69 (50.7)	75 (56.8)	72 (56.7)	62 (52.1)
Premenopausal	67 (49.3)	57 (43.2)	55 (43.3)	57 (47.9)
Parity, n(%)				
Nulliparous	6 (4.4)	17 (12.9)	17 (13.4)	11 (9.2)
1-2 children	92 (67.7)	87 (65.9)	77 (60.6)	84 (70.6)
>2 children	38 (27.9)	28 (21.2)	33 (26.0)	24 (20.2)
Oral contraceptive use, n(%)				
Never	88 (64.7)	92 (69.7)	89 (70.1)	70 (58.8)
Sometime	48 (35.3)	40 (30.3)	38 (29.9)	49 (41.2)
Education, n(%)				
≤ 8 years	66 (48.5)	62 (47.0)	55 (43.3)	43 (36.1)
>8 years	70 (51.5)	70 (53.0)	72 (56.7)	76 (63.9)
Smoking status, n(%)				
Current smoker	29 (21.32)	20 (15.2)	17 (13.4)	14 (11.8)
Ex-smoker	24 (17.7)	19 (14.4)	16 (12.6)	17 (14.3)
Never smoker	83 (61.0)	93 (70.4)	94 (74.0)	88 (73.9)

¹Values are means ± SD, or n (%).

Table 2. RRs of developing breast cancer by quartiles of plasma homocysteine, folate, vitamin B-12, vitamin B-6, and riboflavin among women of the EPIC-Varese study.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	<i>P</i> trend	Continuous (for each 1 SD increase)
Homocysteine						
Range, mmol/L	5.971-9.541	9.542-10.44	10.45-12.21	12.22-82.70		
Cases/Controls	74/64	47/64	74/65	63/63		
RR (95% CI) ¹	1	0.61 (0.37-1.02)	0.97 (0.59-1.59)	0.82 (0.49-1.38)	0.81	1.11 (0.90-1.36)
RR (95% CI) ²	1	0.62 (0.37-1.05)	0.95 (0.58-1.56)	0.79 (0.46-1.34)	0.68	1.10 (0.89-1.36)
Folate						
Range, ng/mL	2.609-5.968	5.969-7.218	7.219-8.599	8.600-15.35		
Cases/Controls	79/64	64/64	59/64	56/64		
RR (95% CI) ¹	1	0.80 (0.50-1.30)	0.79 (0.48-1.28)	0.73 (0.44-1.19)	0.21	0.89 (0.75-1.06)
RR (95% CI) ²	1	0.80 (0.49-1.31)	0.86 (0.52-1.44)	0.74 (0.45-1.23)	0.31	0.89 (0.75-1.07)
Vitamin B-12						
Range, pg/mL	175.4-443.2	443.3-545.4	545.5-687.8	687.9-2310		
Cases/Controls	76/64	55/64	60/64	67/64		
RR (95% CI) ¹	1	0.72 (0.44-1.18)	0.77 (0.47-1.26)	0.90 (0.55-1.46)	0.72	1.03 (0.86-1.22)
RR (95% CI) ²	1	0.67 (0.40-1.11)	0.69 (0.42-1.15)	0.88 (0.53-1.45)	0.62	1.04 (0.86-1.25)
Vitamin B-6						
Range, ng/mL	1.998-6.723	6.724-9.438	9.439-13.13	13.14-109.3		
Cases/Controls	72/64	68/64	63/64	55/64		
RR (95% CI) ¹	1	0.93 (0.56-1.51)	0.86 (0.53-1.40)	0.77 (0.47-1.27)	0.29	0.80 (0.65-0.99)
RR (95% CI) ²	1	0.93 (0.57-1.549)	0.87 (0.52-1.43)	0.76 (0.45-1.27)	0.28	0.78 (0.63-0.96)
Riboflavin						
Range, ng/mL	1.465-4.512	4.513-6.405	6.406-9.052	9.053-122.7		
Cases/Controls	74/64	66/64	56/65	62/63		
RR (95% CI) ¹	1	0.92 (0.56-1.49)	0.74 (0.45-1.21)	0.87 (0.53-1.42)	0.41	1.08 (0.89-1.30)
RR (95% CI) ²	1	0.88 (0.54-1.45)	0.73 (0.44-1.21)	0.83 (0.50-1.39)	0.36	1.08 (0.88-1.33)

¹ Adjusted for age, menopausal status, recruitment date, and distance between ORDET and EPIC recruitment.

² Further adjusted for family history of breast cancer, age at menarche, parity, oral contraceptive use, smoking status, education, alcohol consumption, and BMI.

Table 3. RRs of developing breast cancer by quartiles of plasma homocysteine, folate, vitamin B-12, vitamin B-6, and riboflavin according to menopausal status among women of the EPIC-Varese study.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	<i>P</i> trend	Continuous (for each 1 SD increase)
Postmenopausal women						
Homocysteine						
Range, mmol/L	5.971-9.541	9.542-10.44	10.45-12.21	12.22-82.70		
Cases/Controls	27/21	24/30	44/42	43/47		
RR (95%CI) ¹	1	0.58 (0.26-1.29)	0.80 (0.38-1.65)	0.66 (0.31-1.39)	0.47	1.04 (0.77-1.39)
RR (95%CI) ²	1	0.63 (0.27-1.45)	0.77 (0.37-1.64)	0.66 (0.30-1.44)	0.43	1.05 (0.77-1.41)
Folate						
Range, ng/mL	2.609-5.968	5.969-7.218	7.219-8.599	8.600-15.35		
Cases/Controls	37/33	32/33	35/35	34/39		
RR (95%CI) ¹	1	0.85 (0.43-1.69)	0.93 (0.48-1.82)	0.79 (0.41-1.55)	0.58	0.95 (0.74-1.21)
RR (95%CI) ²	1	0.85 (0.41-1.75)	1.15 (0.56-2.37)	0.82 (0.40-1.66)	0.77	0.95 (0.73-1.23)
Vitamin B-12						
Range, pg/mL	175.4-443.2	443.3-545.4	545.5-687.8	687.9-2310		
Cases/Controls	42/44	29/34	34/27	33/35		
RR (95%CI) ¹	1	0.89 (0.46-1.71)	1.27 (0.65-2.48)	1.02 (0.54-1.93)	0.44	1.11 (0.86-1.43)
RR (95%CI) ²	1	0.83 (0.42-1.65)	1.13 (0.56-2.27)	0.88 (0.45-1.72)	0.91	1.04 (0.79-1.36)
Vitamin B-6						
Range, ng/mL	1.998-6.723	6.724-9.438	9.439-13.13	13.14-109.3		
Cases/Controls	36/33	36/39	37/35	29/33		
RR (95%CI) ¹	1	0.82 (0.43-1.60)	0.94 (0.48-1.83)	0.83 (0.42-1.67)	0.72	0.89 (0.69-1.14)
RR (95%CI) ²	1	0.82 (0.41-1.64)	0.94 (0.47-1.88)	0.91 (0.43-1.89)	0.90	0.87 (0.68-1.13)
Riboflavin						
Range, ng/mL	1.465-4.512	4.513-6.405	6.406-9.052	9.053-122.7		
Cases/Controls	34/37	36/40	33/38	35/25		
RR (95%CI) ¹	1	1.02 (0.53-1.97)	0.95 (0.49-1.84)	1.56 (0.78-3.14)	0.29	1.33 (0.98-1.79)
RR (95%CI) ²	1	0.97 (0.49-1.93)	0.92 (0.45-1.85)	1.59 (0.76-3.33)	0.29	1.36 (0.98-1.87)
Premenopausal women						
Homocysteine						
Range, mmol/L	5.971-9.541	9.542-10.44	10.45-12.21	12.22-82.70		
Cases/Controls	47/43	23/34	30/23	20/16		

RR (95% CI) ¹	1	0.61 (0.31-1.20)	1.13 (0.56-2.26)	1.05 (0.48-2.32)	0.71	1.20 (0.84-1.71)
RR (95% CI) ²	1	0.59 (0.29-1.19)	1.12 (0.55-2.28)	1.05 (0.46-2.39)	0.72	1.25 (0.85-1.83)
Folate						
Range, ng/mL	2.609-5.968	5.969-7.218	7.219-8.599	8.600-15.35		
Cases/Controls	42/31	32/31	24/29	22/25		
RR (95% CI) ¹	1	0.76 (0.38-1.51)	0.65 (0.31-1.33)	0.67 (0.32-1.40)	0.22	0.83 (0.64-1.08)
RR (95% CI) ²	1	0.70 (0.34-1.42)	0.61 (0.28-1.30)	0.66 (0.30-1.46)	0.24	0.83 (0.63-1.09)
Vitamin B-12						
Range, pg/mL	175.4-443.2	443.3-545.4	545.5-687.8	687.9-2310		
Cases/Controls	34/20	26/30	26/37	34/29		
RR (95% CI) ¹	1	0.51 (0.24-1.11)	0.42 (0.20-0.89)	0.70 (0.33-1.49)	0.34	0.96 (0.75-1.22)
RR (95% CI) ²	1	0.49 (0.22-1.10)	0.41 (0.19-0.92)	0.81 (0.36-1.82)	0.57	1.06 (0.81-1.39)
Vitamin B-6						
Range, ng/mL	1.998-6.723	6.724-9.438	9.439-13.13	13.14-109.3		
Cases/Controls	36/31	32/25	26/29	26/31		
RR (95% CI) ¹	1	1.10 (0.54-2.26)	0.77 (0.37-1.60)	0.69 (0.33-1.46)	0.24	0.70 (0.51-0.97)
RR (95% CI) ²	1	1.07 (0.50-2.27)	0.80 (0.36-1.74)	0.65 (0.30-1.42)	0.22	0.66 (0.48-0.92)
Riboflavin						
Range, ng/mL	1.465-4.512	4.513-6.405	6.406-9.052	9.053-122.7		
Cases/Controls	40/27	30/24	23/27	27/38		
RR (95% CI) ¹	1	0.85 (0.41-1.77)	0.56 (0.26-1.18)	0.48 (0.24-0.97)	0.025	0.93 (0.74-1.17)
RR (95% CI) ²	1	0.85 (0.40-1.83)	0.58 (0.27-1.26)	0.45 (0.21-0.94)	0.021	0.92 (0.73-1.17)

¹ Adjusted for age, recruitment date, and distance between ORDET and EPIC recruitment.

² Further adjusted for family history of breast cancer, age at menarche, parity, oral contraceptive use, smoking status, education, alcohol consumption, and BMI.

Supplemental Table 1. RRs of developing breast cancer subtypes defined by ER status according to quartiles of plasma homocysteine, folate, vitamin B-12, PLP vitamin B-6 and riboflavin, among women recruited to the EPIC-Varese study

	Cases/Controls	ER+ RR (95%CI) ¹	RR (95%CI) ²	Cases/Controls	ER- RR (95%CI) ¹	RR (95%CI) ²
Homocysteine						
Quartile 1 (low)	59/64	1	1	8/64	1	1
Quartile 2	33/64	0.54 (0.31-0.94)	0.54 (0.31-0.96)	12/64	1.39 (0.53-3.69)	1.43 (0.53-3.84)
Quartile 3	56/65	0.90 (0.54-1.52)	0.90 (0.53-1.53)	10/65	1.19 (0.43-3.32)	1.12 (0.40-3.15)
Quartile 4 (high)	50/63	0.81 (0.46-1.40)	0.79 (0.45-1.40)	11/63	1.30 (0.46-3.67)	1.21 (0.42-3.44)
<i>P</i> trend		0.77	0.71		0.75	0.89
<i>P</i> heterogeneity	0.63 ¹ /0.73 ²					
Continuous		1.09 (0.85-1.41)	1.10 (0.85-1.43)		1.08 (0.69-1.69)	1.05 (0.67-1.66)
<i>P</i> heterogeneity	0.95 ¹ /0.85 ²					
Folate						
Quartile 1 (low)	65/64	1	1	6/64	1	1
Quartile 2	49/64	0.75 (0.45-1.25)	0.72 (0.43-1.22)	11/64	1.83 (0.63-5.30)	1.95 (0.66-5.73)
Quartile 3	43/64	0.69 (0.41-1.16)	0.73 (0.42-1.27)	14/64	2.60 (0.93-7.27)	2.92 (1.01-8.48)
Quartile 4 (high)	41/64	0.65 (0.38-1.10)	0.65 (0.37-1.12)	10/64	1.75 (0.59-5.16)	1.83 (0.61-5.53)
<i>P</i> trend		0.09	0.13		0.26	0.24
<i>P</i> heterogeneity	0.043 ¹ /0.045 ²					
Continuous		0.86 (0.71-1.04)	0.86 (0.70-1.05)		1.06 (0.77-1.47)	1.08 (0.77-1.51)
<i>P</i> heterogeneity	0.22 ¹ /0.19 ²					
Vitamin B-12						
Quartile 1 (low)	60/64	1	1	12/64	1	1
Quartile 2	44/64	0.73 (0.43-1.24)	0.68 (0.40-1.17)	6/64	0.51 (0.18-1.45)	0.48 (0.17-1.40)
Quartile 3	45/64	0.75 (0.44-1.27)	0.68 (0.39-1.17)	12/64	0.95 (0.39-2.31)	0.97 (0.39-2.43)
Quartile 4 (high)	49/64	0.84 (0.50-1.41)	0.82 (0.48-1.41)	11/64	0.97 (0.39-2.38)	1.00 (0.39-2.55)
<i>P</i> trend		0.52	0.45		0.81	0.74
<i>P</i> heterogeneity	0.55 ¹ /0.46 ²					
Continuous		0.92 (0.74-1.13)	0.91 (0.73-1.14)		1.26 (0.93-1.71)	1.35 (0.96-1.88)
<i>P</i> heterogeneity	0.05 ¹ /0.032 ²					
Vitamin B-6						
Quartile 1 (low)	54/64	1	1	11/64	1	1
Quartile 2	55/64	1.01 (0.60-1.69)	1.01 (0.59-1.73)	10/64	0.88 (0.35-2.24)	0.88 (0.34-2.29)

Quartile 3	46/64	0.84 (0.49-1.42)	0.84 (0.48-1.45)	11/64	0.94 (0.38-2.36)	0.96 (0.37-2.49)
Quartile 4 (high)	43/64	0.80 (0.47-1.36)	0.77 (0.44-1.34)	9/64	0.82 (0.31-2.14)	0.83 (0.30-2.28)
<i>P</i> trend		0.32	0.27		0.73	0.28
<i>P</i> heterogeneity	0.83 ¹ /0.74 ²					
Continuous		0.82 (0.66-1.02)	0.79 (0.63-1.00)		0.77 (0.50-1.19)	0.74 (0.48-1.14)
<i>P</i> heterogeneity	0.79 ¹ /0.76 ²					
Riboflavin						
Quartile 1 (low)	55/64	1	1	15/64	1	1
Quartile 2	49/64	0.91 (0.54-1.54)	0.86 (0.50-1.47)	9/64	0.63 (0.25-1.56)	0.66 (0.26-1.65)
Quartile 3	40/65	0.72 (0.42-1.24)	0.69 (0.40-1.20)	12/65	0.76 (0.32-1.77)	0.80 (0.33-1.89)
Quartile 4 (high)	54/63	1.02 (0.61-1.71)	0.93 (0.55-1.60)	5/63	0.34 (0.12-1.01)	0.38 (0.13-1.15)
<i>P</i> trend		0.86	0.65		0.09	0.15
<i>P</i> heterogeneity	0.11 ¹ /0.24 ²					
Continuous		1.10 (0.90-1.34)	1.09 (0.88-1.36)		1.09 (0.81-1.47)	1.13 (0.83-1.54)
<i>P</i> heterogeneity	0.92 ¹ /0.80 ²					

¹ Adjusted for age, menopausal status, recruitment date, and distance between ORDET and EPIC recruitment.

² Further adjusted for family history of breast cancer, age at menarche, parity, oral contraceptive use, smoking status, education, alcohol consumption, and BMI.

Supplemental Table 2. RRs of developing breast cancer subtypes defined by PR status according to quartiles of plasma homocysteine, folate, vitamin B-12, PLP vitamin B-6 and riboflavin, among women recruited to the EPIC-Varese study

	Cases/Controls	PR+ RR (95%CI) ¹	RR (95%CI) ²	Cases/Controls	PR- RR (95%CI) ¹	RR (95%CI) ²
Homocysteine						
Quartile 1 (low)	49/64	1	1	19/64	1	1
Quartile 2	25/64	0.50 (0.28-0.91)	0.51 (0.28-0.94)	21/64	1.01 (0.49-2.08)	1.02 (0.49-2.13)
Quartile 3	45/65	0.90 (0.52-1.56)	0.90 (0.51-1.58)	19/65	0.87 (0.41-1.84)	0.83 (0.39-1.78)
Quartile 4 (high)	39/63	0.80 (0.44-1.43)	0.80 (0.44-1.47)	20/63	0.87 (0.40-1.90)	0.80 (0.36-1.76)
<i>P</i> trend		0.77	0.78		0.65	0.49
<i>P</i> heterogeneity	0.83 ¹ /0.66 ²					
Continuous		1.10 (0.85-1.44)	1.13 (0.86-1.49)		1.02 (0.71-1.46)	0.99 (0.68-1.42)
<i>P</i> heterogeneity	0.66 ¹ /0.47 ²					
Folate						
Quartile 1 (low)	52/64	1	1	19/64	1	1
Quartile 2	38/64	0.73 (0.42-1.25)	0.70 (0.40-1.22)	22/64	1.14 (0.56-2.33)	1.15 (0.55-2.38)
Quartile 3	37/64	0.75 (0.43-1.29)	0.80 (0.45-1.42)	19/64	1.07 (0.51-2.23)	1.14 (0.53-2.44)
Quartile 4 (high)	31/64	0.62 (0.35-1.09)	0.60 (0.33-1.08)	19/64	1.00 (0.48-2.10)	1.04 (0.49-2.20)
<i>P</i> trend		0.11	0.13		0.97	0.93
<i>P</i> heterogeneity	0.26 ¹ /0.23 ²					
Continuous		0.84 (0.68-1.03)	0.83 (0.67-1.03)		1.00 (0.78-1.29)	1.02 (0.79-1.33)
<i>P</i> heterogeneity	0.21 ¹ /0.14 ²					
Vitamin B-12						
Quartile 1 (low)	45/64	1	1	26/64	1	1
Quartile 2	40/64	0.89 (0.51-1.54)	0.83 (0.47-1.47)	11/64	0.43 (0.19-0.95)	0.39 (0.18-0.88)
Quartile 3	36/64	0.79 (0.45-1.40)	0.71 (0.40-1.27)	20/64	0.77 (0.39-1.55)	0.74 (0.36-1.52)
Quartile 4 (high)	37/64	0.83 (0.47-1.46)	0.80 (0.45-1.43)	22/64	0.91 (0.46-1.79)	0.91 (0.45-1.84)
<i>P</i> trend		0.47	0.37		0.97	0.97
<i>P</i> heterogeneity	0.57 ¹ /0.48 ²					
Continuous		0.93 (0.74-1.15)	0.92 (0.73-1.16)		1.09 (0.84-1.42)	1.15 (0.87-1.52)
<i>P</i> heterogeneity	0.26 ¹ /0.16 ²					
Vitamin B-6						
Quartile 1 (low)	47/64	1	1	18/64	1	1
Quartile 2	43/64	0.91 (0.53-1.56)	0.90 (0.51-1.58)	21/64	1.12 (0.54-2.33)	1.08 (0.51-2.28)

Quartile 3	34/64	0.72 (0.41-1.26)	0.70 (0.39-1.26)	22/64	1.18 (0.57-2.44)	1.18 (0.56-2.46)
Quartile 4 (high)	34/64	0.72 (0.41-1.26)	0.67 (0.37-1.21)	18/64	1.03 (0.49-2.18)	1.02 (0.47-2.24)
<i>P</i> trend		0.17	0.13		0.90	0.89
<i>P</i> heterogeneity	0.27 ¹ /0.22 ²					
Continuous		0.76 (0.58-0.99)	0.72 (0.55-0.95)		0.90 (0.70-1.15)	0.86 (0.66-1.12)
<i>P</i> heterogeneity	0.31 ¹ /0.31 ²					
Riboflavin						
Quartile 1 (low)	46/64	1	1	22/64	1	1
Quartile 2	35/64	0.78 (0.45-1.38)	0.72 (0.40-1.28)	23/64	1.07 (0.54-2.14)	1.12 (0.55-2.26)
Quartile 3	32/65	0.70 (0.39-1.24)	0.65 (0.36-1.18)	20/65	0.85 (0.42-1.73)	0.90 (0.43-1.86)
Quartile 4 (high)	45/63	1.01 (0.59-1.74)	0.91 (0.51-1.60)	14/63	0.66 (0.31-1.41)	0.70 (0.32-1.54)
<i>P</i> trend		0.94	0.70		0.25	0.35
<i>P</i> heterogeneity	0.31 ¹ /0.55 ²					
Continuous		1.19 (0.96-1.47)	1.18 (0.94-1.48)		0.83 (0.56-1.23)	0.88 (0.59-1.30)
<i>P</i> heterogeneity	0.08 ¹ /0.14 ²					

¹ Adjusted for age, menopausal status, recruitment date, and distance between ORDET and EPIC recruitment.

² Further adjusted for family history of breast cancer, age at menarche, parity, oral contraceptive use, smoking status, education, alcohol consumption, and BMI.

Supplemental Table 3. RRs of developing breast cancer by HER2 subtype by quartiles of plasma homocysteine, folate, vitamin B-12, vitamin B-6 and riboflavin among women recruited to the EPIC-Varese study.

	Cases/Controls	HER2+ RR (95%CI) ¹	RR (95%CI) ²	Cases/Controls	HER2- RR (95%CI) ¹	RR (95%CI) ²
Homocysteine						
Quartile 1 (low)	15/64	1	1	46/64	1	1
Quartile 2	7/64	0.43 (0.16-1.13)	0.44 (0.16-1.19)	32/64	0.67 (0.38-1.20)	0.67 (0.37-1.21)
Quartile 3	7/65	0.46 (0.17-1.24)	0.40 (0.15-1.10)	46/65	0.96 (0.55-1.67)	0.94 (0.53-1.66)
Quartile 4 (high)	7/63	0.46 (0.16-1.28)	0.40 (0.14-1.17)	42/63	0.88 (0.49-1.58)	0.86 (0.47-1.57)
<i>P</i> trend		0.12	0.07		0.92	0.85
<i>P</i> heterogeneity	0.15 ¹ /0.09 ²					
Continuous		1.06 (0.66-1.71)	1.08 (0.65-1.79)		1.00 (0.75-1.33)	1.00 (0.74-1.34)
<i>P</i> heterogeneity	0.80 ¹ /0.77 ²					
Folate						
Quartile 1 (low)	10/64	1	1	47/64	1	1
Quartile 2	5/64	0.51 (0.16-1.59)	0.51 (0.16-1.64)	46/64	0.96 (0.56-1.65)	0.91 (0.53-1.59)
Quartile 3	10/64	1.11 (0.43-2.89)	1.30 (0.48-3.58)	38/64	0.86 (0.49-1.50)	0.91 (0.51-1.62)
Quartile 4 (high)	11/64	1.17 (0.46-2.98)	1.26 (0.47-3.35)	35/64	0.77 (0.44-1.36)	0.77 (0.43-1.38)
<i>P</i> trend		0.49	0.38		0.33	0.41
<i>P</i> heterogeneity	0.23 ¹ /0.19 ²					
Continuous		1.11 (0.80-1.55)	1.13 (0.80-1.58)		0.88 (0.72-1.08)	0.88 (0.71-1.08)
<i>P</i> heterogeneity	0.18 ¹ /0.17 ²					
Vitamin B-12						
Quartile 1 (low)	11/64	1	1	50/64	1	1
Quartile 2	7/64	0.67 (0.24-1.84)	0.61 (0.21-1.74)	36/64	0.72 (0.41-1.26)	0.65 (0.37-1.15)
Quartile 3	8/64	0.69 (0.25-1.84)	0.54 (0.19-1.53)	38/64	0.76 (0.43-1.32)	0.66 (0.37-1.17)
Quartile 4 (high)	10/64	0.92 (0.36-2.35)	0.89 (0.33-2.39)	42/64	0.86 (0.50-1.48)	0.81 (0.46-1.42)
<i>P</i> trend		0.87	0.74		0.63	0.45
<i>P</i> heterogeneity	0.92 ¹ /0.94 ²					
Continuous		1.08 (0.76-1.54)	1.11 (0.75-1.65)		0.98 (0.79-1.21)	0.98 (0.78-1.22)
<i>P</i> heterogeneity	0.59 ¹ /0.53 ²					
Vitamin B-6						
Quartile 1 (low)	12/64	1	1	45/64	1	1
Quartile 2	9/64	0.72 (0.28-1.83)	0.71 (0.27-1.88)	45/64	1.00 (0.58-1.72)	1.02 (0.58-1.79)

Quartile 3	9/64	0.70 (0.27-1.80)	0.67 (0.25-1.81)	39/64	0.86 (0.49-1.50)	0.88 (0.49-1.56)
Quartile 4 (high)	6/64	0.49 (0.17-1.42)	0.43 (0.14-1.30)	37/64	0.84 (0.48-1.47)	0.84 (0.47-1.50)
<i>P</i> trend		0.20	0.14		0.45	0.47
<i>P</i> heterogeneity	0.40 ¹ /0.29 ²					
Continuous		0.77 (0.48-1.23)	0.68 (0.41-1.11)		0.81 (0.64-1.03)	0.81 (0.63-1.02)
<i>P</i> heterogeneity	0.81 ¹ /0.51 ²					
Riboflavin						
Quartile 1 (low)	10/64	1	1	48/64	1	1
Quartile 2	9/64	0.94 (0.35-2.49)	0.88 (0.32-2.40)	44/64	0.95 (0.55-1.63)	0.90 (0.51-1.57)
Quartile 3	10/65	0.97 (0.38-2.53)	0.98 (0.36-2.63)	32/65	0.66 (0.37-1.17)	0.62 (0.34-1.12)
Quartile 4 (high)	7/63	0.72 (0.26-2.02)	0.76 (0.26-2.23)	42/63	0.91 (0.52-1.56)	0.82 (0.46-1.44)
<i>P</i> trend		0.59	0.71		0.47	0.30
<i>P</i> heterogeneity	0.90 ¹ /0.85 ²					
Continuous		0.95 (0.61-1.49)	0.99 (0.67-1.48)		1.14 (0.93-1.40)	1.13 (0.91-1.41)
<i>P</i> heterogeneity	0.42 ¹ /0.50 ²					

¹Adjusted for age, menopausal status, recruitment date, and distance between ORDET and EPIC recruitment.

²Further adjusted for family history of breast cancer, age at menarche, parity, oral contraceptive use, smoking status, education, alcohol consumption, and BMI.