

Blood polyphenol concentrations and differentiated thyroid carcinoma in women from the European Prospective Investigation into Cancer and Nutrition (EPIC) study.

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SHORT TITLE: Polyphenol biomarkers & thyroid cancer

KEYWORDS: polyphenol, biomarkers, thyroid cancer, EPIC, nested case–control study

ABBREVIATIONS: EPIC, European Prospective Investigation into Cancer and Nutrition; IARC, International Agency for Research on Cancer; LOQ, limit of quantification; TC, thyroid cancer; TNM, tumor-node-metastasis

1 **ABSTRACT:**

2 Background. Polyphenols are natural compounds with anticarcinogenic properties
3 in cellular and animal models, but epidemiological evidence investigating the
4 associations of these compounds with thyroid cancer (TC) is lacking.

5 Objective. The aim of this study was to evaluate the relationships between blood
6 concentrations of 36 polyphenols and TC risk in the European Prospective
7 Investigations into Cancer and Nutrition (EPIC).

8 Methods. A nested case-control study was conducted on 273 female cases (210
9 papillary, 45 follicular, and 18 not otherwise specified TC tumors) and 512 strictly
10 matched controls. Blood polyphenol levels were analyzed by high pressure liquid
11 chromatography coupled to tandem mass spectrometry after enzymatic hydrolysis.

12 Results. Using multivariable adjusted conditional logistic regression models, caffeic
13 acid ($OR_{\log_2}=0.55$, 95% CI: 0.33, 0.93) and its dehydrogenated metabolite, 3,4-
14 dihydroxyphenylpropionic acid ($OR_{\log_2}=0.84$, 95% CI: 0.71, 0.99) were inversely
15 associated with differentiated TC risk. Similar results were observed for papillary
16 TC, but not for follicular TC. Ferulic acid was also inversely associated only with
17 papillary TC ($OR_{\log_2}=0.68$, 95% CI: 0.51, 0.91). However, none of these
18 relationships was significant after Bonferroni correction for multiple testing. No
19 association was observed with any of the remaining polyphenols with total
20 differentiated, papillary or follicular TC.

21 Conclusions. Blood polyphenol levels were mostly not associated with
22 differentiated TC risk in women, although our study raises the possibility that high
23 blood concentrations of caffeic, 3,4-dihydroxyphenylpropionic, and ferulic acids
24 may be related to a lower papillary TC risk.

25 INTRODUCTION

26 Thyroid cancer (TC) is the most common endocrine cancer and is classified into
27 two main groups: differentiated (mostly papillary and follicular) and non-
28 differentiated (e.g. anaplastic) carcinomas (1). TC is more frequent in women than
29 in men, and its incidence has been increasing over the last three decades (2),
30 partially attributable to overdiagnosis (3). To date, only few risk factors have been
31 established (i.e. benign thyroid disease, radiation exposure, and body size) (4, 5).
32 However, the role of dietary factors in TC carcinogenesis is not clearly understood
33 (1).

34 Polyphenols are bioactive phytochemicals, abundant in the human diet and
35 showing a high variability in their chemical structure. Over 500 individual
36 polyphenols have been identified from dietary sources, almost exclusively plant-
37 based foods (6). Once ingested, polyphenols are partially absorbed and
38 conjugated in both the gut mucosa and liver. Many of the non-absorbed
39 compounds reach the colon, undergo extensive catabolism reactions by
40 microbiota, and finally can be absorbed as simple phenolic acids (7, 8).

41 Established biological properties of polyphenols include antioxidant, anti-
42 inflammatory and chemo-preventive effects (9). Polyphenols have been shown to
43 induce apoptosis, inhibit cell proliferation and invasion in TC cells (10). However,
44 epidemiological evidence on the association between polyphenol intake and TC
45 risk is scarce and inconclusive. In a US cohort, dietary flavan-3-ol intake was
46 negatively and flavanones were positively related to TC risk (11). In a previous
47 analysis of dietary polyphenol intake and differentiated TC risk in the European
48 Prospective Investigation into Cancer and Nutrition (EPIC) cohort the results were

49 null, except in subjects with BMI \geq 25, where inverse associations with intake of
50 phenolic acids were detected (12). However, the assessment of polyphenol
51 exposures using dietary questionnaires and food composition databases has well-
52 known limitations. Polyphenol biomarkers constitute an alternative and objective
53 way for estimating polyphenol exposures, which take into account inter-individual
54 variations in bioavailability (13, 14).

55 We hypothesized that polyphenols may have a preventive role in differentiated TC
56 and polyphenol biomarkers may capture dietary exposure better than
57 questionnaires. Therefore, our aim was to explore the associations between 36
58 blood polyphenol concentrations and differentiated TC risk, and the difference
59 between TC histological subtypes, in women in a nested case-control study within
60 the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort.

61

62 **MATERIAL AND METHODS**

63 **Study population, sample and data collection**

64 The EPIC is an on-going multicenter prospective cohort study that enrolled
65 521,324 men and women, mainly between the ages of 35 and 70 years,
66 predominantly from the general population of 10 European countries in the nineties
67 (15). All participants gave written informed consent, and the study was approved
68 by the Ethics Review Committee of the International Agency for Research on
69 Cancer (IARC) and by the local ethical committee of individual EPIC centers.

70 At baseline, habitual food and nutrient intake over the previous year was assessed
71 through a validated center/country-specific dietary questionnaire (15) and the
72 standardized EPIC Nutrient Database (16). Anthropometric data were measured,

73 except in EPIC-Oxford, Norway and France, where they were self-reported (15).
74 Blood samples, from approximately 80% of the EPIC cohort, were collected at
75 recruitment according to standardized procedures and stored at IARC under liquid-
76 nitrogen (-196°C) for all countries, except at Denmark under nitrogen-vapor ($-$
77 150°C) (15).

78 **Endpoint assessments**

79 Primary incident TC cases were identified through record linkage with regional
80 cancer registries in most of the centers, except in France, Germany, Greece and
81 Naples (Italy), where follow-up was based on a combination of methods, including
82 health insurance records, cancer and pathology registries and active follow-up
83 evaluation of study participants and their next-of-kin. TC was defined as code C73
84 in the 10th Revision of the International Classification of Diseases (ICD-10). This
85 analysis focused on differentiated TC, i.e., papillary (morphologic codes: 8050,
86 8130, 8260, 8340–8344 and 8350), follicular carcinomas (8290, 8330–8335), and
87 not otherwise specified, which are likely to also be papillary (8000, 8010, 8140). TC
88 cases with rare or missing histological types (medullary, anaplastic, lymphoma,
89 other morphologies) were not included. For each EPIC center, closure dates of the
90 study period were defined as the latest dates of complete follow-up for both cancer
91 incidence and vital status (between February 2011 and December 2015).

92 **Nested case–control design**

93 Only incident female TC cases were selected among participants with available
94 blood sample at baseline, because the number of TC in men is very low in EPIC
95 ($n=76$) (17). Female controls were selected by incidence density sampling from all

96 cohort members alive and free of cancer (except non-melanoma skin cancer) at
97 the time of diagnosis of the corresponding case and were also matched by study
98 center, duration of follow-up, age (± 1 year), date of blood collection (± 3 months),
99 time of blood collection (± 1 hour), and fasting status at the time of blood collection
100 (< 3 hours (not fasting), 3–6 hours (in between), or > 6 hours (fasting)). For every
101 case, 2 matched controls were identified.

102 **Laboratory measurements**

103 Samples, cases and matched controls, were left-overs of a previous EPIC study
104 (17). No samples from Sweden remained for the analysis. Therefore, all samples
105 experienced one freeze-thaw cycle before polyphenol analyses at IARC.
106 Polyphenols concentrations in biological samples are generally stable after freeze-
107 thaw cycles (18, 19). Citrated plasma was used for laboratory analyses except for
108 samples from Denmark (serum). The list of 36 polyphenols measured was
109 tabulated in **Table 1**. Blood polyphenols were measured by differential isotope
110 labeling and liquid chromatography electrospray ionization tandem mass
111 spectrometry. Detailed information of the method was published elsewhere (20).
112 Limits of quantification (LOQ) for the polyphenols varied between 0.11 nmol/L for
113 daidzein and 44.4 nmol/L for quercetin and isorhamnetin. Blood polyphenol
114 concentrations that fell below the LOQ were set to values corresponding to half the
115 limit of quantification. All intra-batch coefficients of variation were $<10\%$; while all
116 inter-batch coefficients of variations were $<20\%$ (except for phloretin and enterodiol
117 for which CVs were 22.0% and 21.5%, respectively). Samples from cases and
118 matched controls were analyzed together, within the same analytical batch.

119 **Statistical Analyses**

120 Medians and percentiles (25th and 75th) of blood polyphenol concentrations of
121 cases and controls were calculated and compared using Wilcoxon tests.
122 Spearman's correlation coefficients were calculated to assess the correlations
123 among blood polyphenol concentrations in the controls. Means with standard
124 deviations, medians with interquartile ranges or frequencies (where appropriate) of
125 baseline characteristics were computed and compared between cases and
126 controls. Baseline characteristic differences between cases and controls were
127 tested by conditional logistic regression.

128 In our power analysis calculations, a total of 273 cases and matched controls (1:2)
129 will allow us to detect an exposure-disease association with a $\beta=0.80$ for an
130 OR=0.6 for highest vs. lowest quartiles of exposure in the control population,
131 assuming $\alpha=0.05$ (21). The estimated disease prevalence is 0.2% (12).

132 Multivariable conditional logistic regression, stratified by case-control set, was
133 used to compute ORs and the corresponding 95% CI for the associations between
134 blood polyphenol concentrations and differentiated TC risk. The quality of the
135 models was checked using graphical methods and a goodness-of-fit test. Blood
136 polyphenol concentrations were categorized into quartiles based on the distribution
137 of blood levels in controls. Tests for linear trend were performed by assigning the
138 medians of each quartile as scores and entered this variable as a continuous term
139 in the logistic regression models. Blood polyphenol concentrations were also
140 analyzed as continuous variables, after \log_2 transformation. OR_{\log_2} estimates can
141 be interpreted as the relative risk associated with a doubling in blood polyphenol
142 concentration. Possible nonlinear associations were tested using restricted cubic
143 spline models. The basic model was conditioned on matching factors only, while

144 the multivariable model was additionally adjusted for BMI (kg/m^2), alcohol
145 consumption (g/d), and age of menarche (y). Other lifestyle, anthropometric, and
146 reproductive variables such as smoking status (never, current, former, unknown),
147 physical activity using the Cambridge index (inactive and moderately inactive,
148 moderately active and active, unknown) (22), education level (none, primary,
149 technical/professional, secondary, higher education, unknown), menopausal status
150 [premenopausal, postmenopausal, perimenopausal, surgical postmenopausal
151 (bilateral oophorectomy)], parity (no, yes, unknown), number of full-term
152 pregnancies (nulliparous, 1, 2, 3, ≥ 4 , unknown), breastfeeding (no, yes, unknown),
153 ever oral contraceptives (OC) use (no, yes, unknown), ever hormonal replace
154 therapy (HRT) use (no, yes, unknown), and prevalent diabetes (no, yes, unknown)
155 were evaluated as potential confounders, but were not included in the final model
156 because they were not different ($P\text{-value} > 0.1$) between cases and controls in the
157 logistic regressions conditional on matching variables. Missing values were
158 retained by creating a separate category (unknown) for categorical variables.

159 Similar conditional logistic regression models were conducted for polyphenols
160 (caffeic acid, and 3,4-dihydroxyphenylpropionic acid) which were significantly
161 associated with differentiated TC risk by tumor-node-metastasis (TNM) stage (low:
162 T1-T2 vs. high: T3-T4), and histological type (papillary vs. follicular), and
163 heterogeneity by subgroups was tested using the Wald test assessed with the SAS
164 macro *%subtype* (23). Moreover, modification of the ORs was evaluated by age at
165 blood collection (<48, 48-55, >55 y), education level (primary or lower vs.
166 secondary or higher), smoking status (never vs. ever), physical activity (inactive or
167 moderately inactive vs. moderately active or active), BMI (<25 vs. ≥ 25 kg/m^2),

168 menopausal status (premenopausal, perimenopausal, postmenopausal), alcohol
169 consumption (≤ 5 g/d vs. >5 g/d), time to diagnosis (<4 , 4-7, >7 years), and
170 countries (high vs. low incidence for differentiated TC) using a likelihood ratio test.
171 EPIC countries with TC incidence rates per year of $>1/10,000$ in women (i.e.,
172 France, Germany, Greece, Italy, and Spain) were considered to have high TC
173 incidence, while UK, the Netherlands, Denmark, and Norway were considered to
174 have low TC incidence.

175 To account for multiple comparisons, the Bonferroni correction was applied giving
176 a stricter P value threshold for statistical significance at 0.0015, based on the 33
177 polyphenols analyzed (P value $< 0.05/33 = 0.0015$). Blood polyphenol levels
178 associated with differentiated TC risk at P value between < 0.05 and 0.0015 were
179 selected as candidates for independent validation studies. All analyses were
180 performed using SAS Software (version 9.3, SAS Institute Inc, Cary, NC, USA).

181

182 **RESULTS**

183 The current study included 273 incident differentiated TC cases (210 papillary, 45
184 follicular, and 18 not otherwise specified TC tumors) and 512 matched controls
185 after a median follow-up time of 12.6 years. (**Supplementary Figure 1**). All cases
186 and controls were women with a mean age at blood collection of 50 years. At
187 baseline, controls tended to have a lower BMI and to consume more alcohol than
188 cases (**Table 2**). Moreover, controls were more likely to have experienced
189 menarche at an older age than cases, although the difference was not significant.
190 The rest of baseline characteristics were comparable in cases and controls.

191 Thirty six polyphenols were measured in blood samples from cases and controls.
192 Three of them (epigallocatechin, gallic acid ethyl ester) were
193 excluded from the association analyses because the numbers of samples <LOQ
194 were higher than 75% (Table 1). Most polyphenols showed similar blood
195 concentrations in cases and controls; except caffeic acid found in slightly lower
196 concentrations in differentiated TC cases when compared with controls (Table 1).
197 Moderate correlations were observed between caffeic and ferulic acids (mainly
198 originating from coffee intake) (24) and coffee intake (respectively $r=0.39$ and
199 $r=0.50$), and between 3,4-dihydroxyphenylpropionic acid (a metabolite of caffeic
200 acid formed in the gut) and coffee intake ($r=0.38$).

201 Several strong correlations were observed between polyphenol concentrations in
202 blood, such as between 3,5-dihydroxybenzoic acid and 3,5-
203 dihydroxyphenylpropionic acid ($r=0.85$), genistein and daidzein ($r=0.77$), naringenin
204 and hesperetin ($r=0.72$), caffeic acid and 3,4-dihydroxyphenylpropionic acid
205 ($r=0.64$), and caffeic acid and ferulic acid ($r=0.68$) reflecting co-occurrence in their
206 main food sources or biotransformation reflecting co-occurrence in their main food
207 sources or biotransformation (**Supplementary Table 2**).

208 In the multivariable models, blood concentrations of caffeic acid ($OR_{\log 2}=0.55$, 95%
209 CI: 0.33, 0.93) and 3,4-dihydroxyphenylpropionic acid ($OR_{\log 2}=0.84$, 95% CI: 0.71,
210 0.99) were inversely associated with differentiated TC risk (**Table 3**), although they
211 did not reach the Bonferroni threshold. In the restricted cubic spline model, no
212 evidence of non-linearity for the relationships between both caffeic acid and 3,4-
213 dihydroxyphenylpropionic acid and differentiated TC risk was observed (data not

214 shown). All other polyphenol concentrations were not related to differentiated TC
215 risk.

216 In the results divided by TC histological subtype, inverse associations were
217 observed between blood concentrations of caffeic acid ($OR_{\log 2}=0.36$, 95% CI: 0.19,
218 0.68; P for heterogeneity = 0.048), 3,4-dihydroxyphenylpropionic acid ($OR_{\log 2}=0.74$,
219 95% CI: 0.61, 0.90; P for heterogeneity = 0.030) (**Table 4**), and ferulic acid
220 ($OR_{\log 2}=0.68$, 95% CI: 0.51, 0.91; P for heterogeneity = 0.062) and papillary TC
221 tumors; but no associations were detected with follicular TC tumors. None of the
222 other blood polyphenols were associated with either papillary or follicular TC
223 tumors (data not shown). In the subgroup analyses, an inverse association was
224 observed with blood concentrations of caffeic and 3,4-dihydroxyphenylpropionic
225 acids in countries with low TC incidence, but not in countries with high TC
226 incidence (P for heterogeneity < 0.05). However, none of these results reached the
227 Bonferroni threshold ($p=0.0015$). Similar inverse associations were observed for
228 the relation between either caffeic acid or 3,4-dihydroxyphenylpropionic acid and
229 differentiated TC risk across strata of age at blood collection, education level,
230 smoking status, physical activity, BMI, menopausal status, alcohol intake, and
231 years between blood draw and diagnosis denoting no effect modification (Table 4).

232

233 **DISCUSSION**

234 In the current prospective nested case-control study, inverse trends were observed
235 between blood concentrations of both caffeic acid and its dihydrogenated
236 metabolite, 3,4-dihydroxyphenylpropionic acid (also called dihydrocaffeic acid) and
237 total differentiated TC risk, but they did not reach the Bonferroni threshold for

238 statistically significant associations when corrected for multiple comparisons. The
239 remaining blood polyphenol levels were not associated with total differentiated TC
240 risk. Interestingly, the two inverse associations were restricted to papillary TC and
241 were more striking in countries with low incidence of TC. For 3,4-
242 dihydroxyphenylpropionic acid, the negative association was also stronger in stage
243 T1-T2 than in T3-T4 carcinomas. Papillary TC and low stage thyroid tumors are
244 more likely to be related to over-diagnosis than high stage TCs in countries with
245 high incidence. However, over-diagnosis is not related with these TC tumor types
246 in countries with low incidence (3).

247 To our knowledge, this is the first study evaluating the relations between blood
248 polyphenol levels and TC risk. Although no results were statistically significant after
249 Bonferroni correction, concentrations of caffeic, 3,4-dihydroxyphenylpropionic and
250 ferulic acids might be inversely associated with papillary TC risk, but not with
251 follicular TC risk. Caffeic and ferulic acids are abundant in human diets, and are
252 mostly present in an esterified form as chlorogenic and feruloylquinic acids (esters
253 of caffeic or ferulic acids and quinic acid) (25). They contribute to 78% and 19% of
254 total hydroxycinnamic acid intake (mean intake in Europe = 541.2mg/d) (26).
255 Caffeic acid in blood mainly originates from the hydrolysis of chlorogenic acid by
256 the gut microbiota and from the absorption in the gut of the free form of caffeic acid
257 (27). Ferulic acid in blood results from both the hydrolysis of feruloylquinic acid and
258 from the *O*-methylation of caffeic acid in the liver. Dihydrocaffeic acid is only
259 present in the diet in very low amounts (26). Dihydrocaffeic acid in blood is mainly
260 formed by microbial hydrogenation of caffeic acid in the gut (27). All three
261 compounds in both blood, in the current study, and in urine, in a previous analysis

262 including 475 subjects from the EPIC study (24), showed moderate-to-high
263 correlations with coffee intake and poor or no correlations with any other tested
264 food groups, except for ferulic acid and cereals (24). Indeed, a urinary metabolite
265 of caffeic acid (caffeic acid sulphate) was correlated to whole-grain rye intake
266 ($r=0.58$) in a free-living Swedish population (28); while urinary ferulic
267 concentrations were increased after an intervention with rye bran bread in humans
268 (29) and with rye bran in mice (30). Unfortunately, data on coffee consumption was
269 not available in these analyses, so the potential confounding effect of coffee on
270 whole-grain cereal was not measured.

271 In three previous EPIC studies, intakes of either phenolic acids (mainly
272 hydroxycinnamic acids) (12), or coffee (31), or total fiber (32) were not related to
273 the risk of either overall TC or its histological subtypes (papillary and follicular
274 tumors). Moreover, no differences in the coffee consumption between differentiated
275 TC cases and controls were observed in our study (Supplementary table 1).
276 Furthermore, the consumption of either whole grain cereals or total grains was not
277 associated with TC risk in a series of hospital-based case-control studies (33) or in
278 a meta-analysis (34). Differences between results obtained with the intake
279 measurement, and those obtained here with biomarkers might be explained by a
280 more limited accuracy of exposure measurements when relying on intake data
281 rather than biomarker data (9, 13). In fact, it is difficult to accurately estimate
282 polyphenol intake by dietary questionnaires, due to the variability of polyphenol
283 content within same or similar foods, such as the heterogeneity of polyphenol
284 composition on the different coffee types according to brewing methods (espresso
285 vs. diluted coffee) and cultivars (Arabica vs. Robusta) (35, 36). Thus, dietary

286 biomarkers should be more accurate and objective measurements than dietary
287 questionnaires, accounting for inter-individual variability in phenolic acid
288 bioavailability (14).

289 Although the associations were not statistically significant after Bonferroni
290 correction, they were biologically plausible. The underlying potential mechanisms
291 of action of caffeic, ferulic and 3,4-dihydroxyphenylpropionic acids in thyroid
292 carcinogenesis could be directly associated with their anticarcinogenic properties
293 (37). In particular, ferulic acid has been shown to modulate cell cycle arrest,
294 apoptosis, invasion, migration, and colony formation on TT medullary TC cells (38).
295 Moreover, they have been indirectly associated with their anti-diabetic, anti-obesity,
296 indirect antioxidant and anti-inflammatory properties (9). It is important to bear in
297 mind that obesity (5), type 2 diabetes (39) and inflammation (17) are potential risk
298 factors of TC risk. Plasma concentrations of total and several individual
299 polyphenols (i.e. daidzein, 3,5-dihydroxyphenylpropionic acid, 3,4-
300 dihydroxyphenylpropionic acid, ferulic acid, caffeic acid, and hydroxytyrosol) were
301 inversely associated with levels of high-sensitivity C-reactive protein in a previous
302 cross-sectional analysis in an EPIC subsample (40), suggesting that these
303 polyphenols may protect against harmful health effects related to inflammation.
304 Moreover, plasma and urinary concentrations of caffeic acid and other coffee
305 polyphenols were associated with a lower risk of type 2 diabetes in two cohorts
306 (41, 42). Indeed, caffeic and dihydrocaffeic acids inhibit amyloid formation of
307 human islet amyloid polypeptide *in vitro* (43), and decrease glucose uptake and the
308 detrimental effects of high glucose concentrations in endothelial cells (44). In
309 addition, caffeic and ferulic acids modulates the activity of several transcriptional

310 regulatory factors [e.g. AMP-activated protein kinase (AMPK), peroxisome
311 proliferator activator protein- γ (PPAR- γ), and peroxisome proliferator activator
312 protein- γ co-activator-1 α (PGC-1 α)] and enzymatic pathways (e.g. fatty acid
313 synthase, 3-hydroxy-3-methylglutaryl CoA reductase, and acyl-CoA cholesterol
314 acyltransferase) to control obesity (45).

315 Caffeic, ferulic, and 3,4-dihydroxyphenylpropionic acids are compounds of food
316 origin, but they also come from the microbiota catabolism (27). Polyphenols can
317 modulate the gut microbiota towards a more healthy composition (46). Indeed
318 dietary chlorogenic acid supplementation improves gut health in weaned piglets
319 (47). Dysbiosis, an alteration of gut microbiota, is associated with intestinal and
320 extra-intestinal diseases, including cancer and metabolic disorders such as obesity
321 and type 2 diabetes (48, 49). Both TC and thyroid nodules were associated with
322 the composition of gut microbiome in two observational studies in Chinese
323 populations (50, 51).

324 Major strengths of this study are its prospective design, its long follow-up, its
325 relatively large size for a TC study, and the coverage of several European
326 countries with a wide polyphenol exposure heterogeneity. Moreover, the direct
327 analysis of 36 polyphenols in blood provides a valid measurement of the
328 endogenous exposure. However, several limitations of this study also warrant
329 mention. 1) Half-lives of polyphenols are short to moderate, suggesting that a
330 single measurement of these biomarkers is more likely to reflect relatively short-
331 term levels, except for polyphenols regularly consumed that tend to maintain
332 relatively similar concentrations in blood during the entire day. The three phenolic
333 acids inversely associated with TC risk in the present work mainly originate from

334 coffee, a beverage most often consumed on a daily basis. 2) Fasting status affects
335 blood levels of polyphenols, particularly polyphenols coming from food and quickly
336 absorbed. However, TC cases were matched with controls by fasting status and
337 time of blood collection to minimize this limitation. 3) We measured blood
338 polyphenols only once for each individual, so we cannot account for intra-variability
339 and changes in the exposure along the study follow-up. This issue could be
340 particularly relevant for few polyphenols, because they have a relatively poor ICC
341 (0.3-0.4), but not for others (ICC >0.5) ([http://exposome-](http://exposome-explorer.iarc.fr/reproducibilities)
342 [explorer.iarc.fr/reproducibilities](http://exposome-explorer.iarc.fr/reproducibilities)). Therefore, our results on few blood flavonoids
343 may have been attenuated by partial misclassification. 4) Information on history of
344 benign thyroid diseases, thyroidectomy among control subjects and use of drugs
345 that could interfere with thyroid function was not available in the EPIC study. 5)
346 Although we controlled for a wide range of established TC risk factors, the
347 possibility of residual confounding still exists, though the findings were all little
348 affected by adjustment in our study. 6) We cannot exclude the possibility that our
349 findings were due to chance, because they did not reach the Bonferroni threshold.
350 However, it is often considered to be overly conservative and might have over-
351 corrected the model. Moreover, the findings were similar in both general and
352 subgroup analyses (except for the risk of follicular TC and high TNM stage
353 differentiated TC) and are biologically plausible. 7) Generalizability of the results
354 should be done cautiously, because our study only analyzed European women and
355 other populations may show different genetic background (e.g. non-European
356 ancestry), and microbiota composition with possible consequences on phenolic
357 acid bioavailability.

358 In summary, this prospective investigation conducted in a relatively large nested
359 case-control study in women within the EPIC, a European multi-country cohort,
360 shows that blood polyphenol concentrations are mostly not associated with TC risk.
361 However, our study raises the possibility that high blood levels of caffeic, 3,4-
362 dihydroxyphenylpropionic and ferulic acids may be related to a lower risk of
363 papillary TC. These three compounds are, therefore, interesting candidates for
364 validation in independent studies on papillary TC.

365

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368 **CONFLICT OF INTEREST**

369 The authors are not aware of any conflicts of interest.

370 **AUTHOR CONTRIBUTION:**

371 R.Z.-R. designed the research; D.A. performed the laboratory analysis; L.L.-B.
372 performed the statistical analysis; R.Z.-R. drafted the manuscript; S.F., A.S., S.R.,
373 A.A. had primary responsibility for final content; S.F., C.K., K.O., A. Tjønneland,
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375 V.P., S.P., R.T., F.R., G.S.,JR.Q., M.R.-B., P.A., M.-D. C., E.A., M.A., J.H., R.V.,
376 N.J.W., T.Y.N.T., D.A., G.B., E.W., A.S., S.R., A.A. contributed to the design of the
377 study, data collection, and quality control and analysis. All authors read, critically
378 reviewed and approved the final manuscript.

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Table 1. Medians (25th and 75th percentiles) and number of samples with concentrations below the limit of detection (LOD) of plasma polyphenol levels among differentiated thyroid cancer cases and controls.

Plasma concentrations (nmol/L)	Cases (N=273)		Controls (N=512)		P for differences ²
	N <LOD	Median (p25-p75)	N <LOD	Median (p25-p75)	
Flavonoids					
Apigenin	1 (0.2%)	10.9 (10.1-12.4)	0	11.2 (10.0-12.7)	0.26
Catechin	112 (41%)	12.0 (5.6-18.2)	215 (41%)	12.2 (5.6-16.9)	0.61
Daidzein	0	7.9 (5.2-17.5)	2 (0.4%)	8.0 (5.6-16.9)	0.61
Epicatechin	132 (48%)	11.4 (5.6-15.4)	292 (55%)	5.6 (5.6-15.0)	0.11
Epigallocatechin ¹	252 (91%)	-	496 (94%)	-	-
Equol	41 (15%)	0.4 (0.2- 0.7)	58 (11%)	0.4 (0.2- 0.7)	0.61
Galocatechin ¹	274 (99%)	-	523 (99%)	-	-
Genistein	0	4.3 (2.0-11.4)	3 (1.1%)	4.1 (2.2-10.3)	0.97
Hesperetin	68 (25%)	2.3 (1.1-19.3)	142 (27%)	2.2 (1.1- 15.2)	0.67
Kaempferol	0	84.0 (73.0-97.0)	0	84.0 (74.0-94.5)	0.91
Naringenin	8 (1.6%)	3.1 (1.3-11.9)	6 (2.2%)	3.4 (1.6-9.4)	0.84
Phloretin	179 (65%)	1.1 (1.1-2.6)	334 (63%)	1.1 (1.1-2.8)	0.59
Quercetin	0	142.0 (123.0-161.0)	0	142.0 (123.0-165.0)	0.61
Phenolic acids					
3-Hydroxybenzoic acid	2 (0.4%)	17.3 (10.8-30.9)	2 (0.7%)	16.7 (10.9-26.3)	0.53
4-Hydroxybenzoic acid	0	348.0 (313.0-399.0)	0	346.0 (314.5-392.5)	0.71
3,5-Dihydroxybenzoic acid	3 (0.6%)	21.2 (12.3-40.7)	1 (0.4%)	19.1 (11.6-41.3)	0.70
3-Hydroxyphenylacetic acid	17 (3.3%)	53.0 (20.8-101.8)	35 (13%)	56.5 (21.5-108.3)	0.67
4-Hydroxyphenylacetic acid	3 (0.6%)	249.0 (178.0-341.0)	27 (10%)	233.5 (182.0-306.0)	0.22
3,4-Dihydroxyphenylacetic acid	1 (0.2%)	21.8 (16.8-28.4)	2 (0.4%)	21.9 (16.9-28.0)	0.75
3,4-Dihydroxyphenylpropionic acid	13 (2.5%)	18.0 (14.3-26.4)	17 (6.2%)	19.3 (14.6-30.4)	0.053
3,5-Dihydroxyphenylpropionic acid	2 (0.4%)	27.1 (17.0-48.8)	7 (2.6%)	26.5 (17.0-53.5)	0.73

Caffeic acid	0	131.0 (116.0-151.0)	0	135.0 (118.0-157.0)	0.054
m-Coumaric acid	40 (15%)	5.7 (2.6-10.9)	77 (15%)	5.0 (2.1-12.2)	0.63
p-Coumaric acid	0	25.4 (21.2-31.1)	1 (0.4%)	25.6 (21.5- 31.5)	0.50
Ferulic acid	0	104.0 (71.0-183.0)	0	110.5 (71.0-206.5)	0.38
Gallic acid	16 (3.1%)	16.2 (13.7-20.3)	26 (9.5%)	16.1 (13.6-19.9)	0.76
Gallic acid ethyl ester ¹	235 (85%)	-	415 (79%)	-	-
Homovanillic acid	0	82.0 (65.0-106.0)	1 (0.4%)	79.0 (64.0-106.0)	0.59
Isorhamnetin	4 (0.8%)	65.0 (57.0-76.0)	1 (0.4%)	66.0 (57.0-76.0)	0.77
Protocatechuic acid	0	232.0 (215.0-255.0)	2 (0.7%)	230.5 (214.0-257.0)	0.88
Vanillic acid	0	197.0 (178.0-225.0)	2 (0.4%)	195.0 (176.0-230.0)	0.97
Stilbenes					
Resveratrol	106 (38%)	2.5 (1.1-3.9)	199 (38%)	2.5 (1.1- 3.8)	0.91
Lignans					
Enterodiol	62 (23%)	1.0 (0.5-2.0)	110 (21%)	1.0 (0.5- 2.1)	0.55
Enterolactone	4 (0.8%)	8.6 (3.7-15.4)	5 (1.8%)	8.3 (3.8- 15.8)	0.98
Tyrosols					
Hydroxytyrosol	117 (42%)	12.0 (5.6-15.2)	222 (42%)	12.2 (5.6-15.5)	0.37
Tyrosol	0	3.5 (2.7-5.1)	3 (1.1%)	3.7 (2.70-5.3)	0.25

¹Limit of quantification (LOQ) = 11.1 nmol/L for epigallocatechin and gallic acid ethyl ester, LOQ = 1.11 nmol/L for gallic acid ethyl ester.

²From Wilcoxon tests.

Table 2. Baseline characteristics among differentiated thyroid cancer cases and controls.

Characteristic	Cases (N=273)	Controls (N=512)	P-value ¹
Age at blood collection (y) ²	50.0 (8.6)	50.0 (8.7)	Matched
Body mass index (kg/m ²) ²	26.4 (4.7)	25.6 (4.6)	0.007
Alcohol intake (g/d) ³	1.4 (0.1- 8.1)	2.6 (0.2- 11.2)	0.019
Coffee intake (g/d) ³	120 (41- 296)	129 (60- 300)	0.82
Age at menarche (y) ²	12.7 (1.5)	12.9 (1.5)	0.069
Physical activity ⁴			0.14
Inactive or Moderately inactive	192 (70.3)	341 (66.6)	
Moderately active or active	80 (29.3)	167 (32.6)	
Smoking status ⁴			0.77
Never	162 (59.3)	311 (60.7)	
Former	53 (19.4)	98 (19.1)	
Smoker	53 (19.4)	99 (19.3)	
Highest educational level ⁴			0.26
None	28 (10.3)	46 (9.0)	
Primary school completed	98 (35.9)	180 (35.2)	
Technical/professional school	54 (19.8)	86 (16.8)	
Secondary school	38 (13.9)	92 (18.0)	
Longer education	49 (18.0)	103 (20.1)	
Menopausal status ⁴			0.47
Premenopausal	128 (46.9)	242 (47.3)	
Postmenopausal	100 (36.6)	194 (37.9)	
Perimenopausal	35 (12.8)	64 (12.5)	
Surgical postmenopause	10 (3.7)	12 (2.3)	
Full term pregnancies (yes) ⁴	239 (88.5)	440 (86.4)	0.48
Number of full term pregnancies ⁴			0.84
0	31 (11.5)	69 (13.6)	
1	46 (17.1)	85 (16.8)	
2	122 (45.4)	214 (42.2)	
3	48 (17.8)	96 (18.9)	
≥4	22 (8.2)	43 (8.5)	
Breastfeeding (yes) ⁴	191 (71.3)	377 (74.8)	0.25
Ever use of OC (yes) ⁴	127 (46.5)	242 (47.3)	0.62
Ever use of HRT (yes) ⁴	34 (12.8)	69 (13.9)	0.71
Fasting status ⁴			Matched
<3h	105 (38.5)	187 (36.5)	
3-6h	41 (15.0)	82 (16.0)	
>6h	125 (45.8)	240 (46.9)	
Prevalent diabetes (yes) ⁴	10 (2.1)	5 (1.9)	1.00

OC oral contraceptives; HRT hormone replace therapy

¹From logistic regression conditional on matching variables.

²Mean (SD)

³Median (25th and 75th percentiles)

⁴N(%).Numbers may not sum to totals due to missing values.

Table 3. Odds ratio (ORs) and 95% confidence intervals (CI) of differentiated thyroid cancer for log₂-transformed polyphenol concentrations (nmol/L).

Polyphenols	Basic model ¹		Multivariable model ²	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Flavonoids				
Apigenin	0.84 (0.59, 1.20)	0.34	0.83 (0.58, 1.19)	0.32
Catechin	1.06 (0.90, 1.26)	0.47	1.13 (0.95, 1.35)	0.17
Daidzein	0.96 (0.85, 1.09)	0.56	0.96 (0.84, 1.09)	0.54
Epicatechin	1.11 (0.93, 1.33)	0.27	1.13 (0.95, 1.36)	0.17
Epigallocatechin				
Equol	0.95 (0.85, 1.05)	0.29	0.95 (0.85, 1.05)	0.32
Gallocatechin				
Genistein	1.01 (0.91, 1.11)	0.92	1.00 (0.91, 1.10)	0.98
Hesperetin	1.03 (0.96, 1.09)	0.43	1.02 (0.95, 1.08)	0.62
Kaempferol	1.07 (0.57, 1.98)	0.84	1.05 (0.56; 1.96)	0.89
Naringenin	1.02 (0.95, 1.10)	0.59	1.01 (0.94, 1.10)	0.71
Phloretin	0.96 (0.82, 1.11)	0.56	0.94 (0.81, 1.09)	0.41
Quercetin	0.73 (0.40, 1.35)	0.32	0.81 (0.44, 1.51)	0.51
Phenolic acids				
3-Hydroxybenzoic acid	1.05 (0.90, 1.23)	0.55	1.08 (0.92, 1.27)	0.34
4-Hydroxybenzoic acid	1.24 (0.66, 2.34)	0.50	1.25 (0.65, 2.37)	0.50
3,5-Dihydroxybenzoic acid	0.99 (0.86, 1.14)	0.87	0.99 (0.86, 1.14)	0.88
3-Hydroxyphenylacetic acid	0.99 (0.91, 1.09)	0.91	1.01 (0.92, 1.11)	0.85
4-Hydroxyphenylacetic acid	1.08 (0.86, 1.36)	0.49	1.08 (0.86, 1.36)	0.52
3,4-Dihydroxyphenylacetic acid	0.82 (0.60, 1.10)	0.19	0.83 (0.61, 1.14)	0.25
3,4-Dihydroxyphenylpropionic acid	0.84 (0.71, 0.99)	0.032	0.84 (0.71, 0.99)	0.039
3,5-Dihydroxyphenylpropionic acid	0.99 (0.85, 1.17)	0.94	1.00 (0.85, 1.18)	0.96
Caffeic acid	0.52 (0.31, 0.86)	0.011	0.55 (0.33, 0.93)	0.025
m-Coumaric acid	1.01 (0.93, 1.09)	0.89	1.01 (0.93, 1.10)	0.76
p-Coumaric acid	0.88 (0.62, 1.26)	0.49	0.93 (0.64, 1.34)	0.68
Ferulic acid	0.82 (0.64, 1.04)	0.10	0.82 (0.64, 1.04)	0.10
Gallic acid	0.98 (0.73, 1.32)	0.91	1.06 (0.79, 1.43)	0.71
Gallic acid ethyl ester				
Homovanillic acid	1.02 (0.76, 1.38)	0.88	1.07 (0.79, 1.45)	0.67
Isorhamnetin	0.76 (0.37, 1.57)	0.47	0.71 (0.34, 1.47)	0.36
Protocatechuic acid	0.69 (0.20, 2.40)	0.56	0.76 (0.22, 2.66)	0.66
Vanillic acid	1.05 (0.72, 1.53)	0.81	1.02 (0.70, 1.50)	0.90
Stilbenes				
Resveratrol	0.98 (0.86, 1.11)	0.74	1.03 (0.90, 1.19)	0.63
Lignans				
Enterodiol	0.98 (0.90, 1.08)	0.71	1.00 (0.91, 1.09)	0.93
Enterolactone	0.98 (0.89, 1.06)	0.57	0.99 (0.91, 1.09)	0.87
Tyrosols				
Hydroxytyrosol	0.85 (0.67, 1.08)	0.19	0.90 (0.70, 1.14)	0.37
Tyrosol	0.88 (0.71, 1.09)	0.24	0.92 (0.74, 1.14)	0.44

¹From conditional logistic regressions, conditioned on matching factors only (basic model).

²From multivariable conditional logistic regressions, conditioned on matching factors with additional adjustment for BMI, alcohol consumption, and age of menarche.

No associations exceed the Bonferroni threshold ($P < 0.05/33$) = 0.0015.

Table 4. Odds ratios (ORs) and 95% confidence intervals (CI) of differentiated thyroid cancer of caffeic acid and 3,4-dihydroxyphenylpropionic acid (log₂-transformed) blood concentrations (nmol/L) stratified by selected variables.

	Cases	Controls	Caffeic acid		3,4-dihydroxyphenylpropionic acid	
			OR (95% CI)	<i>P</i> -value	OR (95% CI)	<i>P</i> -value
Histological type						
Papillary	210	396	0.36 (0.19, 0.69)	0.048 ¹	0.74 (0.61, 0.90)	0.030 ¹
Follicular	45	82	1.52 (0.52, 4.49)		1.26 (0.79, 2.01)	
TNM stage						
Low: T1-T2	118	218	0.54 (0.26, 1.10)	0.15 ¹	0.73 (0.57, 0.95)	0.040 ¹
High: T3-T4	29	56	2.16 (0.46, 10.00)		1.44 (0.90, 2.30)	
Thyroid Cancer Incidence						
High incident countries ³	219	415	0.86 (0.48, 1.56)	0.010 ²	0.92 (0.77, 1.11)	0.040 ²
Low incident countries ⁴	54	97	0.15 (0.04, 0.54)		0.55 (0.34, 0.89)	
Age at blood collection (y)						
<48	114	211	0.43 (0.16, 1.18)	0.57 ²	0.78 (0.58, 1.06)	0.75 ²
48-45	75	135	0.45 (0.13, 1.57)		0.84 (0.59, 1.20)	
≥46	84	166	0.64 (0.31, 1.33)		0.90 (0.69, 1.18)	
Education						
Primary or less	126	226	0.39 (0.14, 1.14)	0.34 ²	0.82 (0.62, 1.10)	0.98 ²
Secondary or more	147	286	0.59 (0.27, 1.31)		0.86 (0.64, 1.17)	
Smoking						
Never	162	311	0.60 (0.24, 1.50)	0.45 ²	0.79 (0.60, 1.04)	0.99 ²
Ever	106	197	0.48 (0.17, 1.32)		1.04 (0.74, 1.47)	
Physical activity						
Inactive or moderately inactive	192	341	0.40 (0.18, 0.89)	0.87 ²	0.71 (0.56, 0.91)	0.31 ²
Moderately active or active	80	167	0.19 (0.03, 1.12)		0.72 (0.41, 1.25)	

Body mass index (kg/m²)						
<25	119	264	0.56 (0.24, 1.31)	0.28 ²	1.02 (0.78, 1.33)	0.54 ²
≥25	154	248	0.56 (0.24, 1.34)		0.89 (0.69, 1.16)	
Menopausal status at blood collection						
Premenopausal	128	242	0.31 (0.13, 0.78)	0.19 ²	0.78 (0.58, 1.04)	0.60 ²
Perimenopausal	35	64	1.33 (0.16, 10.76)		0.84 (0.52, 1.37)	
Postmenopausal (natural and surgical)	110	206	0.69 (0.35, 1.34)		0.92 (0.72, 1.16)	
Alcohol intake (g/d)						
≤5	176	300	0.90 (0.40, 2.03)	0.23 ²	0.89 (0.70, 1.13)	0.61 ²
>5	96	212	0.42 (0.15, 1.12)		0.83 (0.59, 1.16)	
Years between blood draw and diagnosis						
<4	49	86	1.14 (0.40, 3.31)	0.29 ¹	1.02 (0.69, 1.53)	0.17 ²
4-7	56	108	0.38 (0.10, 1.43)		1.01 (0.71, 1.43)	
>7	168	318	0.45 (0.23, 0.89)		0.73 (0.58, 0.91)	

TNM tumour-node-metastasis

¹P for heterogeneity based on the Wald test.

²P for interaction based on the likelihood ratio test.

³High incident countries for differentiated thyroid cancer: France, Germany, Greece, Italy, and Spain.

⁴Low incident countries for differentiated thyroid cancer: UK, the Netherlands, Denmark, and Norway.