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

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ABSTRACT

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

Objectives: The aim of this study was to evaluate the relations between blood concentrations of 36 polyphenols and TC risk in EPIC (the European Prospective Investigation into Cancer and Nutrition).

Methods: A nested case–control study was conducted on 273 female cases (210 papillary, 45 follicular, and 18 not otherwise specified TC tumors) and 512 strictly matched controls. Blood polyphenol concentrations were analyzed by HPLC coupled to tandem MS after enzymatic hydrolysis.

Results: Using multivariable-adjusted conditional logistic regression models, caffeic acid (OR_{log2}: 0.55; 95% CI: 0.33, 0.93) and its dehydrogenated metabolite, 3,4-dihydroxyphenylpropionic acid (OR_{log2}: 0.84; 95% CI: 0.71, 0.99), were inversely associated with differentiated TC risk. Similar results were observed for papillary TC, but not for follicular TC. Ferulic acid was also inversely associated only with papillary TC (OR_{log2}: 0.68; 95% CI: 0.51, 0.91). However, none of these relations was significant after Bonferroni correction for multiple testing. No association was observed for any of the remaining polyphenols with total differentiated, papillary, or follicular TC.

Conclusions: Blood polyphenol concentrations were mostly not associated with differentiated TC risk in women, although our study

raises the possibility that high blood concentrations of caffeic, 3,4-dihydroxyphenylpropionic, and ferulic acids may be related to a lower papillary TC risk. *Am J Clin Nutr* 2021;113:162–171.

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

Introduction

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

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

Established biological properties of polyphenols include antioxidant, anti-inflammatory, and chemopreventive effects (9). Polyphenols have been shown to induce apoptosis and inhibit cell proliferation and invasion in TC cells (10). However, epidemiological evidence on the association between polyphenol intake and TC risk is scarce and inconclusive. In a US cohort, dietary flavan-3-ol intake was negatively, whereas flavanones were positively, related to TC risk (11). In a previous analysis of dietary polyphenol intake and differentiated TC risk in the EPIC (European Prospective Investigation into Cancer and Nutrition) cohort the results were null, except in subjects with BMI (in kg/m^2) $\geq$25, where inverse associations with intake of phenolic acids were detected (12). However, the assessment of polyphenol exposures using dietary questionnaires and food composition databases has well-known limitations. Polyphenol biomarkers constitute an alternative and objective way to estimate polyphenol exposures, taking into account interindividual variations in bioavailability (13, 14).

We hypothesized that polyphenols may have a preventive role in differentiated TC and polyphenol biomarkers may capture dietary exposure better than questionnaires. Therefore, our aim was to explore the associations between 36 blood polyphenol concentrations and differentiated TC risk, and the difference between TC histological subtypes, in women in a nested case–control study within the EPIC cohort.

Methods

Study population, sample, and data collection

EPIC is an ongoing multicenter prospective cohort study that enrolled 521,324 men and women, mainly between the ages of 35 and 70 y, predominantly from the general population of 10 European countries in the 1990s (15). All participants gave written informed consent, and the study was approved by the Ethics Review Committee of the International Agency for Research on Cancer (IARC) and by the local ethical committees of the individual EPIC centers.

At baseline, habitual food and nutrient intake over the previous year was assessed via a validated center/country-specific dietary questionnaire (15) and the standardized EPIC Nutrient Database (16). Anthropometric data were measured, except in EPIC-Oxford, Norway, and France, where they were self-reported (15). Blood samples, from ~80% of the EPIC cohort, were collected at recruitment according to standardized procedures and stored at the IARC under liquid-nitrogen (−196°C) for all countries, except in Denmark where they were stored under nitrogen-vapor (−150°C) (15).

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Supplemental Figure 1 and Supplemental Table 1 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

For information on how to submit an application for gaining access to EPIC data and/or biospecimens, please follow the instructions at http://epic.iarc.fr/access/index.php.

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Abbreviations used: EPIC, the European Prospective Investigation into Cancer and Nutrition; ICC, Intraclass Correlation Coefficient; IARC, International Agency for Research on Cancer; LOQ, limit of quantification; TC, thyroid cancer; TNM, tumor-node-metastasis.

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Endpoint assessments

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

Nested case–control design

Only incident female TC cases were selected among participants with available blood samples at baseline, because the number of TC cases in men is very low in EPIC ($n = 76$) (17). Female controls were selected by incidence density sampling from all cohort members alive and free of cancer (except nonmelanoma skin cancer) at the time of diagnosis of the corresponding case and were also matched by study center, duration of follow-up, age (±1 y), date of blood collection (±3 mo), time of blood collection (±1 h), and fasting status at the time of blood collection [<3 h (not fasting), 3–6 h (in between), or >6 h (fasting)]. For every case, 2 matched controls were identified. For some controls, there was not sample available as they were leftovers from a previous study (17).

Laboratory measurements

Samples—cases and matched controls—were leftovers of a previous EPIC study (17). No samples from Sweden remained for the analysis. Therefore, all samples experienced 1 freeze–thaw cycle before polyphenol analyses at the IARC. Polyphenol concentrations in biological samples are generally stable after freeze–thaw cycles (18, 19). Citrated plasma was used for the laboratory analyses except for the samples from Denmark (serum). **Table 1** lists the 36 polyphenols measured. Blood polyphenols were measured by differential isotope labeling and LC electrospray ionization tandem MS. Detailed information on the method has been published elsewhere (20). Limits of quantification (LOQs) for the polyphenols varied between 0.11 nmol/L for daidzein and 44.4 nmol/L for quercetin and isorhamnetin. Blood polyphenol concentrations that fell below the LOQ were set to values corresponding to half the LOQ. All intrabatch CVs were <10%; whereas, all interbatch CVs were <20% (except for phloretin and enterodiol, for which the CVs were 22.0% and 21.5%, respectively). Samples from cases and matched controls were analyzed together, within the same analytical batch.

Statistical analyses

Medians [IQRs] of blood polyphenol concentrations of cases and controls were calculated and compared using the Wilcoxon Signed Rank test. Spearman rank correlation coefficients were calculated to assess the correlations among blood polyphenol concentrations in the controls. Means ± SDs, medians [IQRs], or frequencies (where appropriate) of baseline characteristics were computed and compared between cases and controls. Baseline characteristic differences between cases and controls were tested by conditional logistic regression.

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

Multivariable conditional logistic regression, stratified by case–control set, was used to compute ORs and the corresponding 95% CIs for the associations between blood polyphenol concentrations and differentiated TC risk. The quality of the models was checked using graphical methods and a goodness-of-fit test. Blood polyphenol concentrations were categorized into quartiles based on the distribution of blood concentrations in controls. Tests for linear trend were performed by assigning the medians of each quartile as scores and entering this variable as a continuous term in the logistic regression models. Blood polyphenol concentrations were also analyzed as continuous variables, after $\log_2$ transformation. OR_{log2} estimates can be interpreted as the RR associated with a doubling in the blood polyphenol concentration. Possible nonlinear associations were tested using restricted cubic spline models. The basic model was conditioned on matching factors only, whereas the multivariable model was further adjusted for BMI, alcohol consumption (g/d), and age of menarche (y). Other lifestyle, anthropometric, and reproductive variables such as smoking status (never, current, former, unknown), physical activity using the Cambridge index (inactive and moderately inactive, moderately active and active, unknown) (22), education level (none, primary, technical/professional, secondary, higher education, unknown), menopausal status [premenopausal, postmenopausal, perimenopausal, surgical postmenopausal (bilateral oophorectomy)], parity (no, yes, unknown), number of full-term pregnancies (nulliparous, 1, 2, 3, ≥4, unknown), breastfeeding (no, yes, unknown), ever oral contraceptives use (no, yes, unknown), ever hormonal replacement therapy use (no, yes, unknown), and prevalent diabetes (no, yes, unknown) were evaluated as potential confounders, but were not included in the final model because they were not different (P value > 0.1) between cases and controls in the logistic regressions conditional on matching variables. Missing values were retained by creating a separate category (unknown) for categorical variables.

Similar conditional logistic regression models were conducted for polyphenols (caffeic acid and 3,4-dihydroxyphenylpropionic acid) which were significantly associated with differentiated TC risk by tumor-node-metastasis (TNM) stage (low: T1–T2 compared with high: T3–T4) and histological type (papillary compared with follicular), and heterogeneity by subgroups was tested using the Wald test assessed with the SAS macro *%subtype* (23). Moreover, modification of the ORs by age at blood collection (<48, 48–55, >55 y), education level (primary or

TABLE 1 Median [IQR] plasma polyphenol concentrations and numbers of samples with concentrations below the LOQ among differentiated thyroid cancer cases and controls[1]

	Cases ($n = 273$)		Controls ($n = 512$)		P for differences[2]
Plasma concentrations, nmol/L	n (%) < LOQ	Median [IQR]	n (%) < LOQ	Median [IQR]	
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[3]	252 (91)	—	496 (94)	—	—
Equol	41 (15)	0.4 [0.2–0.7]	58 (11)	0.4 [0.2–0.7]	0.61
Gallocatechin[3]	271 (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[3]	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 [2.7–5.3]	0.25

[1]LOQ, limit of quantification.
[2]From Wilcoxon Signed Rank tests.
[3]LOQ = 11.1 nmol/L for epigallocatechin and gallocatechin; LOQ = 1.11 nmol/L for gallic acid ethyl ester.

lower compared with secondary or higher), smoking status (never compared with ever), physical activity (inactive or moderately inactive compared with moderately active or active), BMI (<25 compared with ≥25), menopausal status (premenopausal, perimenopausal, postmenopausal), alcohol consumption (≤5 g/d compared with >5 g/d), time to diagnosis (<4, 4–7, >7 y), and countries (high compared with low incidence for differentiated TC) was evaluated using a likelihood ratio test. EPIC countries with TC incidence rates per year of >1 in 10,000 in women (i.e., France, Germany, Greece, Italy, and Spain) were considered to have high TC incidence, whereas the United Kingdom, Netherlands, Denmark, and Norway were considered to have low TC incidence.

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

Results

The current study included 273 incident differentiated TC cases (210 papillary, 45 follicular, and 18 not otherwise specified

TABLE 2 Baseline characteristics among differentiated thyroid cancer cases and controls[1]

Characteristic	Cases ($n = 273$)	Controls ($n = 512$)	P value[2]
Age at blood collection, y	50.0 ± 8.6	50.0 ± 8.7	Matched
BMI, kg/m^2	26.4 ± 4.7	25.6 ± 4.6	0.007
Alcohol intake, g/d	1.4 [0.1–8.1]	2.6 [0.2–11.2]	0.019
Coffee intake, g/d	120 [41–296]	129 [60–300]	0.82
Age at menarche, y	12.7 ± 1.5	12.9 ± 1.5	0.069
Physical activity			0.14
Inactive or moderately inactive	192 (70.3)	341 (66.6)	
Moderately active or active	80 (29.3)	167 (32.6)	
Smoking status			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			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			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	239 (88.5)	440 (86.4)	0.48
Full-term pregnancies, *n*			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	191 (71.3)	377 (74.8)	0.25
Ever use of OCs	127 (46.5)	242 (47.3)	0.62
Ever use of HRT	34 (12.8)	69 (13.9)	0.71
Fasting status			Matched
<3 h	105 (38.5)	187 (36.5)	
3–6 h	41 (15.0)	82 (16.0)	
>6 h	125 (45.8)	240 (46.9)	
Prevalent diabetes	10 (2.1)	5 (1.9)	1.00

[1] Values are means ± SDs, medians [IQRs], or *n* (%) unless otherwise indicated. Numbers may not sum to totals owing to missing values. HRT, hormone replacement therapy; OC, oral contraceptive.

[2] From logistic regression conditional on matching variables.

TC tumors) and 512 matched controls after a median follow-up time of 12.6 y (**Supplemental Figure 1**). All cases and controls were women with a mean age at blood collection of 50 y. At baseline, controls tended to have a lower BMI and to consume more alcohol than cases (**Table 2**). Moreover, controls were more likely to have experienced menarche at an older age than cases, although the difference was not significant. The rest of the baseline characteristics were comparable in cases and controls.

Thirty-six polyphenols were measured in blood samples from cases and controls. Three of them (epigallocatechin, gallocatechin, and gallic acid ethyl ester) were excluded from the association analyses because >75% of samples were below the LOQ (Table 1). Most polyphenols showed similar blood concentrations in cases and controls, except caffeic acid was found in slightly lower concentrations in differentiated TC cases than in controls (Table 1). Moderate correlations were observed between caffeic and ferulic acids (mainly originating from coffee intake) (24) and coffee intake ($r = 0.39$ and $r = 0.50$, respectively) and between 3,4-dihydroxyphenylpropionic acid (a metabolite of caffeic acid formed in the gut) and coffee intake ($r = 0.38$).

Several strong correlations were observed between polyphenol concentrations in blood, such as between 3,5-dihydroxybenzoic acid and 3,5-dihydroxyphenylpropionic acid ($r = 0.85$), genistein and daidzein ($r = 0.77$), naringenin and hesperetin ($r = 0.72$), caffeic acid and 3,4-dihydroxyphenylpropionic acid ($r = 0.64$), and caffeic acid and ferulic acid ($r = 0.68$), reflecting co-occurrence in their main food sources or biotransformation (**Supplemental Table 1**).

In the multivariable models, blood concentrations of caffeic acid (OR_{log2}: 0.55; 95% CI: 0.33, 0.93) and 3,4-dihydroxyphenylpropionic acid (OR_{log2}: 0.84; 95% CI: 0.71, 0.99) were inversely associated with differentiated TC risk (**Table 3**), although they did not reach the Bonferroni threshold.

TABLE 3 ORs and 95% CIs of differentiated thyroid cancer for $\log_2$-transformed polyphenol concentrations[1]

	Basic model[2]		Multivariable model[3]	
Polyphenols, nmol/L	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

[1]No associations exceeded the Bonferroni threshold ($P < 0.05/33) = 0.0015$.
[2]From conditional logistic regressions, conditioned on matching factors only (basic model).
[3]From multivariable conditional logistic regressions, conditioned on matching factors with additional adjustment for BMI, alcohol consumption, and age of menarche.

In the restricted cubic spline model, no evidence of nonlinearity was observed for the relations between both caffeic acid and 3,4-dihydroxyphenylpropionic acid and differentiated TC risk (data not shown). All other polyphenol concentrations were not related to differentiated TC risk.

In the results divided by TC histological subtype, inverse associations were observed between blood concentrations of caffeic acid (OR_{log2}: 0.36; 95% CI: 0.19, 0.68; *P*-heterogeneity = 0.048), 3,4-dihydroxyphenylpropionic acid (OR_{log2}: 0.74; 95% CI: 0.61, 0.90; *P*-heterogeneity = 0.030) (**Table 4**), and ferulic acid (OR_{log2}: 0.68; 95% CI: 0.51, 0.91; *P*-heterogeneity = 0.062) and papillary TC tumors; but no associations were detected with follicular TC tumors. None of the other blood polyphenols were associated with either papillary or follicular TC tumors (data not shown). In the subgroup analyses, an inverse association was observed with blood concentrations of caffeic and 3,4-dihydroxyphenylpropionic acids in countries with low TC incidence, but not in countries with high TC incidence (*P*-heterogeneity < 0.05). However, none of these results reached the Bonferroni threshold ($P = 0.0015$). Similar inverse associations were observed for the relation between either caffeic acid or 3,4-dihydroxyphenylpropionic acid and differentiated TC risk across strata of age at blood collection, education level, smoking status, physical activity, BMI, menopausal status, alcohol intake,

TABLE 4 ORs and 95% CIs of differentiated thyroid cancer for log_2-transformed caffeic acid and 3,4-dihydroxyphenylpropionic acid blood concentrations stratified by selected variables[1]

			Caffeic acid		3,4-dihydroxyphenylpropionic acid	
	Cases, *n*	Controls, *n*	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Histological type						
Papillary	210	396	0.36 (0.19, 0.69)	0.048[2]	0.74 (0.61, 0.90)	0.030[2]
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[2]	0.73 (0.57, 0.95)	0.040[2]
High: T3–T4	29	56	2.16 (0.46, 10.00)		1.44 (0.90, 2.30)	
Thyroid cancer incidence						
High-incidence countries[3]	219	415	0.86 (0.48, 1.56)	0.010[4]	0.92 (0.77, 1.11)	0.040[4]
Low-incidence countries[5]	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[4]	0.78 (0.58, 1.06)	0.75[4]
48–55	75	135	0.45 (0.13, 1.57)		0.84 (0.59, 1.20)	
>55	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[4]	0.82 (0.62, 1.10)	0.98[4]
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[4]	0.79 (0.60, 1.04)	0.99[4]
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[4]	0.71 (0.56, 0.91)	0.31[4]
Moderately active or active	80	167	0.19 (0.03, 1.12)		0.72 (0.41, 1.25)	
BMI, kg/m^2						
<25	119	264	0.56 (0.24, 1.31)	0.28[4]	1.02 (0.78, 1.33)	0.54[4]
≥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[4]	0.78 (0.58, 1.04)	0.60[4]
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[4]	0.89 (0.70, 1.13)	0.61[4]
>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[2]	1.02 (0.69, 1.53)	0.17[4]
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)	

[1] TNM, tumor-node-metastasis.
[2] *P*-heterogeneity based on the Wald test.
[3] High-incidence countries for differentiated thyroid cancer: France, Germany, Greece, Italy, and Spain.
[4] *P*-interaction based on the likelihood ratio test.
[5] Low-incidence countries for differentiated thyroid cancer: United Kingdom, Netherlands, Denmark, and Norway.

and years between blood draw and diagnosis, denoting no effect modification (Table 4).

Discussion

In the current prospective nested case–control study, inverse trends were observed between blood concentrations of both caffeic acid and its dehydrogenated metabolite, 3,4-dihydroxyphenylpropionic acid (also called dihydrocaffeic acid), and total differentiated TC risk, but they did not reach the Bonferroni threshold for statistically significant associations when corrected for multiple comparisons. The remaining blood polyphenol concentrations were not associated with total differentiated TC risk. Interestingly, the 2 inverse associations were restricted to papillary TC and were more striking in countries with low incidence of TC. For 3,4-dihydroxyphenylpropionic acid, the negative association was also stronger in stage T1–T2 than in stage T3–T4 carcinomas. Papillary TC and low-stage thyroid tumors are more likely to be related to overdiagnosis than are high-stage TCs in countries with high incidence. However, overdiagnosis is not related with these TC tumor types in countries with low incidence (3).

To our knowledge, this is the first study evaluating the relations between blood polyphenol concentrations and TC risk. Although no results were statistically significant after Bonferroni correction, concentrations of caffeic, 3,4-dihydroxyphenylpropionic, and ferulic acids might be inversely associated with papillary TC risk, but not with follicular TC risk. Caffeic and ferulic

acids are abundant in human diets, and are mostly present in an esterified form as chlorogenic and feruloylquinic acids (esters of caffeic or ferulic acids and quinic acid) (25). They contribute 78% and 19% of total hydroxycinnamic acid intake (mean intake in Europe = 541.2 mg/d) (26). Caffeic acid in blood mainly originates from the hydrolysis of chlorogenic acid by the gut microbiota and from the absorption in the gut of the free form of caffeic acid (27). Ferulic acid in blood results from both the hydrolysis of feruloylquinic acid and the *O*-methylation of caffeic acid in the liver. Dihydrocaffeic acid is only present in the diet in very low amounts (26). Dihydrocaffeic acid in blood is mainly formed by microbial hydrogenation of caffeic acid in the gut (27). All 3 compounds in both blood, in the current study, and urine, in a previous analysis including 475 subjects from the EPIC study (24), showed moderate-to-high correlations with coffee intake and poor or no correlations with any other tested food groups, except for ferulic acid and cereals (24). Indeed, a urinary metabolite of caffeic acid (caffeic acid sulfate) was correlated to whole-grain rye intake ($r = 0.58$) in a free-living Swedish population (28), whereas urinary ferulic concentrations were increased after an intervention with rye bran bread in humans (29) and with rye bran in mice (30). Unfortunately, data on coffee consumption were not available in these analyses, so the potential confounding effect of coffee on whole-grain cereal was not measured.

In 3 previous EPIC studies, intakes of phenolic acids (mainly hydroxycinnamic acids) (12), coffee (31), or total fiber (32) were not related to the risk of either overall TC or its histological subtypes (papillary and follicular tumors). Moreover, no differences in coffee consumption between differentiated TC cases and controls were observed in our study (**Table 2**). Furthermore, the consumption of either whole-grain cereals or total grains was not associated with TC risk in a series of hospital-based case–control studies (33) or in a meta-analysis (34). Differences between results obtained with the measurement of intake, and those obtained here with biomarkers might be explained by a more limited accuracy of exposure measurements when relying on intake data rather than biomarker data (9, 13). In fact, it is difficult to accurately estimate polyphenol intake via dietary questionnaires owing to the variability of polyphenol content within the same or similar foods, such as the heterogeneity of polyphenol composition in the different coffee types according to brewing methods (espresso compared with diluted coffee) and cultivars (arabica compared with robusta) (35, 36). Thus, dietary biomarkers should be more accurate and objective measurements than dietary questionnaires, accounting for interindividual variability in phenolic acid bioavailability (14).

Although the associations were not statistically significant after Bonferroni correction, they were biologically plausible. The underlying potential mechanisms of action of caffeic, ferulic, and 3,4-dihydroxyphenylpropionic acids in thyroid carcinogenesis could be directly associated with their anticarcinogenic properties (37). In particular, ferulic acid has been shown to modulate cell cycle arrest, apoptosis, invasion, migration, and colony formation on TT medullary TC cells (38). Moreover, they have been indirectly associated with antidiabetic, antiobesity, antioxidant, and anti-inflammatory properties (9). It is important to bear in mind that obesity (5), type 2 diabetes (39), and inflammation (17) are potential risk factors for TC. Plasma concentrations of total and several individual polyphenols (i.e., daidzein, 3,5-dihydroxyphenylpropionic acid, 3,4-dihydroxyphenylpropionic acid, ferulic acid, caffeic acid, and hydroxytyrosol) were inversely associated with concentrations of high-sensitivity C-reactive protein in a previous cross-sectional analysis in an EPIC subsample (40), suggesting that these polyphenols may protect against harmful health effects related to inflammation. Moreover, plasma and urinary concentrations of caffeic acid and other coffee polyphenols were associated with a lower risk of type 2 diabetes in 2 cohorts (41, 42). Indeed, caffeic and dihydrocaffeic acids inhibit amyloid formation of human islet amyloid polypeptide in vitro (43), and decrease glucose uptake and the detrimental effects of high glucose concentrations in endothelial cells (44). In addition, caffeic and ferulic acids modulate the activity of several transcriptional regulatory factors (e.g., AMP-activated protein kinase, peroxisome proliferator–activated receptor-γ, and peroxisome proliferator–activated receptor-γ co-activator-1α) and enzymatic pathways (e.g., fatty acid synthase, 3-hydroxy-3-methylglutaryl CoA reductase, and acyl-CoA cholesterol acyltransferase) to control obesity (45).

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

Major strengths of this study are its prospective design, its long follow-up, its relatively large size for a TC study, and the coverage of several European countries with a wide heterogeneity in polyphenol exposure. Moreover, the direct analysis of 36 polyphenols in blood provides a valid measurement of the endogenous exposure. However, several limitations of this study also warrant mention. *1*) Half-lives of polyphenols are short to moderate, suggesting that a single measurement of these biomarkers is more likely to reflect relatively short-term concentrations, except for polyphenols regularly consumed that tend to maintain relatively similar concentrations in blood during the entire day. The 3 phenolic acids inversely associated with TC risk in the present work mainly originate from coffee, a beverage most often consumed on a daily basis. *2*) Fasting status affects blood concentrations of polyphenols, particularly polyphenols coming from food and quickly absorbed. However, TC cases were matched with controls by fasting status and time of blood collection to minimize this limitation. *3*) We measured blood polyphenols only once for each individual, so we cannot account for intraindividual variability and changes in the exposure along the study follow-up. This issue could be particularly relevant for a few polyphenols, because they have a relatively poor intraclass correlation coefficient (ICC) (0.3–0.4), but not for others (ICC > 0.5) (http://exposome-explorer.iarc.fr/reproducibilities). Therefore, our results on a few blood flavonoids may have been attenuated by partial misclassification. *4*) Information on history of benign thyroid diseases, thyroidectomy among control subjects, and use of drugs that could interfere with thyroid function was not available in the EPIC study. *5*) Although

we controlled for a wide range of established TC risk factors, the possibility of residual confounding still exists, although the findings were all little affected by adjustment in our study. *6*) We cannot exclude the possibility that our findings were due to chance, because they did not reach the Bonferroni threshold. However, it is often considered to be overly conservative and might have overcorrected the model. Moreover, the findings were similar in both the general and subgroup analyses (except for the risk of follicular TC and high TNM stage differentiated TC) and are biologically plausible. *7*) Generalization of the results should be done cautiously, because our study only analyzed European women and other populations may show different genetic backgrounds (e.g., non-European ancestry) and microbiota composition with possible consequences for phenolic acid bioavailability.

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

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