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Circulating tryptophan metabolites and risk of colon cancer: Results from case-control and prospective cohort studies

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Abbreviations: 95% CI, 95% confidence interval; BMI, body mass index; CORSA, Colorectal Cancer Study of Austria; EPIC, European Prospective Investigation into Cancer and Nutrition study; IDO, indoleamine 2,3-dioxygenase; LLOQ, lower limit of quantification; OR, odds ratio; TDO, tryptophan 2,3-dioxygenase; TPH1, tryptophan hydroxylase 1; ULOQ, upper limit of quantification.

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Abstract

Dysregulation of tryptophan metabolism has been linked to colorectal tumorigenesis; however, epidemiological studies investigating tryptophan metabolites in relation to colorectal cancer risk are limited. We studied associations of plasma tryptophan, serotonin and kynurenine with colon cancer risk in two studies with cancer patients and controls, and in one prospective cohort: ColoCare Study (110 patients/153 controls), the Colorectal Cancer Study of Austria (CORSA; 46 patients/390 controls) and the European Prospective Investigation into Cancer and Nutrition (EPIC; 456 matched case-control pairs). Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for colon cancer risk. Tryptophan was inversely associated with colon cancer risk in ColoCare (OR per 1-SD = 0.44; 95% CI, 0.31-0.64) and EPIC (OR per 1-SD = 0.86; 95% CI, 0.74-0.99). Comparing detectable vs nondetectable levels, serotonin was positively associated with colon cancer in CORSA (OR = 6.39; 95% CI, 3.61-11.3) and EPIC (OR = 2.03; 95% CI, 1.20-3.40). Kynurenine was inversely associated with colon cancer in ColoCare (OR per 1-SD = 0.74; 95% CI, 0.55-0.98), positively associated in CORSA (OR per 1-SD = 1.79; 95% CI, 1.27-2.52), while no association was observed in EPIC. The kynurenine-to-tryptophan ratio was positively associated with colon cancer in ColoCare (OR per 1-SD = 1.38; 95% CI, 1.03-1.84) and CORSA (OR per 1-SD = 1.44; 95% CI, 1.06-1.96), but not in EPIC. These results suggest that higher plasma tryptophan may be associated with lower colon cancer risk, while increased serotonin may be associated with a higher risk of colon cancer. The kynurenine-to-tryptophan ratio may also reflect altered tryptophan catabolism during colon cancer development.

KEYWORDS
colon cancer, kynurenine, plasma, serotonin, tryptophan

What's new?

Previous studies have suggested that altered metabolism of the amino acid tryptophan may play a role in the development of colorectal cancer. In this study, the authors found that higher plasma levels of tryptophan may be associated with a decreased risk of colon cancer, while higher plasma serotonin (a metabolite of tryptophan) may be associated with an increased risk. These results support a potential role for the tryptophan pathway in colon cancer development.

1 | INTRODUCTION

Globally, cancers of the colon and rectum were responsible for approximately 1.8 million new cases and over 800 000 cancer deaths in 2018.[1] Established risk factors for colorectal cancer include obesity, diabetes, consumption of alcohol, red and processed meats and tobacco smoking. Being physically active and consuming dairy products, wholegrains and dietary fibers have been associated with lower risk.[2-4] The mechanisms linking many of these factors to colorectal cancer development are currently not well understood.

There is accumulating evidence from experimental studies that tryptophan metabolism may play a central role in the development of several chronic diseases including colorectal cancer. Tryptophan is an essential amino acid, and humans rely on its supply from diet and protein degradation. In addition to its role in protein synthesis, tryptophan is a precursor for metabolites involved in several central and peripheral functions,[5] and is metabolized through three pathways: (a) direct metabolism by the gut microbiota into indole derivatives[5,6]; (b) via the serotonin pathway, leading to serotonin and its metabolites through tryptophan hydroxylase 1 (TPH1) and (c) the kynurenine

pathway, leading to kynurenine and its downstream products via tryptophan 2,3-dioxygenase (TDO; hepatic) and indoleamine 2,3-dioxygenase (IDO; extrahepatic). Quantitatively the most important catabolic pathway is via TDO to kynurenine in the liver, but the flux via IDO has been linked with tumor growth through the suppression of antitumor immune responses.[7,8] In addition to normal tissues, TDO and IDO are expressed in cancer cells and are studied as targets for cancer immunotherapeutic interventions.[9] The role of serotonin in the promotion of colorectal carcinogenesis is supported by experimental evidence, but there is also evidence for its intestinal protective role, possibly explaining why in some studies participants receiving selective serotonin reuptake inhibitors have lower risk of colorectal cancer.[10] Some recent studies have shown colorectal cancer patients to have higher plasma serotonin than the cancer-free controls[11] and elevated plasma serotonin to be a predictor for colorectal cancer recurrence and poor prognosis.[12] Despite this, epidemiological studies investigating tryptophan metabolism in relation to colorectal cancer are limited. To our knowledge, studies that examine the associations between circulating levels of tryptophan, serotonin and kynurenine with colon cancer risk, both prior and after the onset of the disease, have not been conducted.

We aimed to investigate the associations of circulating tryptophan and its two major metabolites, serotonin and kynurenine, with colon cancer risk in three different studies including colon cancer patients, cancer-free controls and participants in a prospective cohort. In particular, our aim was to study whether the increase in serotonin, and the changes in kynurenine production reflecting IDO activation in cancer, could be seen many years prior to diagnosis, and whether differences could be observed in the risk estimates between the prospective cohort and the studies including colon cancer patients.

2 | MATERIALS AND METHODS

2.1 | Study populations

2.1.1 | Colon cancer patients and controls

Two European studies of both colon cancer patients and cancer-free controls were used: (a) the ColoCare Study, a prospective study of colorectal cancer patients[13] (NCT02328677) with parallel recruitment of cancer-free controls (PRÄVENT)[14] and (b) the Colorectal Cancer Study of Austria (CORSA), a case-control study nested within a two-stage colorectal cancer screening program.[15]

From ColoCare, we identified 110 patients with newly diagnosed colon cancer, recruited at the University Hospital of Heidelberg and the National Center for Tumor Diseases in Heidelberg, Germany.[13] Cancer-free controls (n = 153) were from the PRÄVENT cohort, with participants recruited in Heidelberg, Germany.[14] All participants were free of colitis ulcerosa, except for two cases and one control. In our study, we will refer the combined ColoCare and PRÄVENT studies as ColoCare.

For CORSA, participants with a positive result in fecal occult blood testing were recruited within the colorectal cancer screening program B-PREDICT (Burgenland Prevention Trial of Colorectal Disease with Immunological Testing). Additional colon cancer patients were recruited from four hospitals in Vienna.[16] In total, 46 patients with histologically confirmed colon cancer and 390 population-based controls, known to be free of polyps and colorectal cancer as confirmed by colonoscopy, were included in the present study. Samples were matched for age, sex and smoking status. All participants were free of colitis, diverticulitis and any prior cancer.

Lifestyle data were collected using study-specific questionnaires, which were harmonized.[16]

2.1.2 | European Prospective Investigation into Cancer and Nutrition study

The European Prospective Investigation into Cancer and Nutrition (EPIC) study is a large multicenter prospective cohort study of 521 448 participants recruited between 1992 and 2000.[17,18] Country-specific questionnaires were used to collect information on a large number of exposures.[18] Incident cancer cases were identified using cancer registries in Italy, the Netherlands, Spain and the United Kingdom. In France and Germany, cancer cases were identified during follow-up from a combination of sources, including health insurance records, cancer and pathology registries and active follow-up. The follow-up time for cancer cases was derived from the dates of cancer diagnosis and recruitment. Controls were selected from the full cohort of individuals who were alive and free of cancer (except nonmelanoma skin cancer) at the time of diagnosis of the cases, using incidence density sampling. The controls were matched to cases by age (±6 months at recruitment), sex, study center, follow-up time since blood collection, fasting status, menopausal status, phase of menstrual cycle and time of day (±4 hours) at blood collection. Information on colitis status was not available in EPIC. In total, 456 incident colon cancer cases and 456 matched controls were included in the current analysis.

2.2 | Sample collection and analysis

In ColoCare and CORSA, nonfasting EDTA plasma samples were collected at recruitment and stored at −80°C until their shipment on dry ice.[16] Blood samples from cancer patients were collected prior to any treatment or surgery. In EPIC, nonfasting plasma samples were collected at baseline and stored in liquid nitrogen in the IARC. All samples used in the present study were analyzed at IARC between February and October 2016 by liquid chromatography-mass spectrometry (1290 Infinity LC system, Agilent Technologies, Santa Clara, CA; Triple Quad 4500 mass spectrometer, AB Sciex, Framingham, MA) using the AbsoluteIDQ p180 kit (Biocrates Life Sciences AG, Innsbruck, Austria).[19,20]

2.3 | Cancer definition

Colon cancer was defined using the tenth revision of the International Classification of Diseases (ICD-10) and the second revision of the

TABLE 1 Baseline characteristics for the ColoCare and CORSA studies

	ColoCare		CORSA	
	Cancer-free controls	Colon cancer cases	Cancer-free controls	Colon cancer cases
Sample size	153	110	390	46
Sex				
Men	59 (39.0%)	67 (61.0%)	255 (65.0%)	33 (72.0%)
Women	94 (61.0%)	43 (39.0%)	135 (35.0%)	13 (28.0%)
Age (mean; SD)	51; 15	65; 13	63; 13	69; 14
BMI (kg/m^2)				
<18.5	3 (1.9%)	1 (0.9%)	3 (0.8%)	0 (0%)
18.5-25	91 (59.5%)	33 (30.0%)	88 (22.5%)	13 (28.3%)
25-30	35 (22.9%)	52 (47.3%)	181 (46.4%)	20 (43.5%)
≥30	19 (12.4%)	24 (21.8%)	97 (24.9%)	7 (15.2%)
Missing	5 (3.3%)	0 (0%)	21 (5.4%)	6 (13.0%)
Continuous (mean; SD)	24.7; 4.6	27.1; 4.3	27.9; 4.7	27.0; 3.8
Smoking status				
Never	75 (49%)	40 (36.4%)	202 (51.8%)	24 (52.2%)
Former	47 (30.7%)	47 (42.7%)	127 (32.6%)	14 (30.4%)
Current	25 (16.4%)	15 (13.6%)	51 (13.1%)	8 (17.4%)
Missing	6 (3.9%)	8 (7.3%)	10 (2.5%)	0 (0%)
Education				
Low	16 (10.5%)	52 (47.3%)	124 (31.8%)	9 (19.6%)
Intermediate	30 (19.6%)	19 (17.3%)	192 (49.2%)	12 (26.1%)
High	98 (64%)	29 (26.3%)	60 (15.4%)	2 (4.3%))
Other	5 (3.3%)	1 (0.9%)	0 (0%)	0 (0%)
Missing	4 (2.6%)	9 (8.2%)	14 (3.6%)	23 (50.0%))
Diabetes				
No	144 (94.1%)	85 (77.3%)	NA	NA
Yes	3 (2%)	15 (13.6%)		
Missing	6 (3.9%)	10 (9.1%)		
Meat, sausage and fat				
Much	NA	NA	95 (24.3%)	9 (19.6%)
Some			162 (41.5%)	20 (43.5%)
Little			117 (30%)	14 (30.4%)
None			8 (2.1%)	2 (4.3%)
Missing			8 (2.1%)	1 (2.2%)
Fruit, vegetables, salad				
Much	NA	NA	140 (35.9%)	18 (39.1%)
Some			172 (44.1%)	24 (52.2%)
Little			65 (16.7%)	3 (6.5%)
None			0 (0%)	0 (0%)
Missing			13 (3.3%)	1 (2.2%)
Sweets and pastry				
Much	NA	NA	72 (18.5%)	12 (26.1%)
Some			163 (41.8%)	21 (45.6%)
Little			133 (34.1%)	12 (26.1%)
None			11 (2.8%)	0 (0%)
Missing			11 (2.8%)	1 (2.2%)

TABLE 1 (Continued)

	ColoCare		CORSA	
	Cancer-free controls	Colon cancer cases	Cancer-free controls	Colon cancer cases
Tryptophan (μM): Median (IQR)				
Overall	60 (52, 68)	48 (37, 61)	52 (45, 59)	51 (46, 60)
Men	62 (51, 73)	54 (38, 64)	53 (47, 62)	53 (47, 61)
Women	60 (52, 67)	43 (35, 52)	49 (43, 55)	51 (45, 56)
Below LLOQ	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Above LLOQ	153 (100%)	110 (100%)	390 (100%)	46 (100%)
Serotonin (μM):				
Below LLOQ	NA	NA	308 (79%)	24 (52%)
Men			205 (80.4%)	19 (57.6%)
Women			103 (76.3%)	5 (38.5%)
Above LLOQ			82 (21%)	22 (48%)
Men			50 (19.6%)	14 (42.4%)
Women			32 (23.7%)	8 (61.5%)
Kynurenine (μM): Median (IQR)				
Overall	2.5 (1.9, 2.9)	2.3 (2.0, 3.1)	2.3 (1.9, 2.8)	2.8 (2.3, 3.5)
Men	2.6 (2.2, 3.1)	2.5 (2.1, 3.3)	2.4 (2.0, 2.9)	2.8 (2.5, 3.6)
Women	2.4 (1.9, 2.7)	2.1 (1.7, 2.8)	2.2 (1.7, 2.7)	2.9 (2.0, 3.1)
Below LLOQ	0 (0%)	1 (1%)	1 (0.3%)	0 (0%)
Above LLOQ	153 (100%)	109 (99%)	389 (99.7%)	46 (100%)
Kynurenine-to-tryptophan ratio: Median (IQR)				
Overall	0.04 (0.03, 0.05)	0.05 (0.04, 0.07)	0.04 (0.04, 0.05)	0.05 (0.04, 0.07)
Men	0.04 (0.03, 0.05)	0.05 (0.04, 0.06)	0.04 (0.04, 0.05)	0.05 (0.04, 0.07)
Women	0.04 (0.03, 0.05)	0.05 (0.04, 0.07)	0.04 (0.03, 0.06)	0.05 (0.05, 0.06)

Abbreviations: BMI, body mass index; CORSA, Colorectal Cancer Study of Austria; IQR, interquartile range; LLOQ, lower limit of quantification; NA, not available.

International Classification of Diseases for Oncology (ICDO-2). Cancer of the colon included cancers within the caecum, appendix, ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon, sigmoid colon, overlapping sites of colon and unspecified sites within the colon (C18.0-18.9).

2.4 | Statistical analysis

Concentrations of kynurenine were log (ln) transformed to reduce skewness. All metabolites were standardized by dividing by their SD to make the results comparable. Concentrations below the lower limit of quantification (LLOQ) were replaced with half the LLOQ. There were no values above the upper limit of quantification (ULOQ). The LLOQ and ULOQ were the following: kynurenine (1, 80 μM), serotonin (0.1, 8 μM) and tryptophan (5, 400 μM). Overall, there was one participant in ColoCare, one in CORSA and nine in EPIC with kynurenine concentration below the LLOQ. All the observations for tryptophan were within the quantification limits (Tables 1 and 2). The concentration of serotonin was below LLOQ in ColoCare for most participants (97%), likely due to its lower levels in blood plasma than serum.[21] Serotonin was therefore not analyzed in ColoCare. A high proportion of serotonin was below LLOQ also in CORSA (76%) and EPIC (84%), and serotonin was, therefore, analyzed as a binary variable (below/above LLOQ). We also calculated the kynurenine-to-tryptophan ratio, which is often used to indirectly estimate IDO activity,[22] by dividing the original value of kynurenine with that of tryptophan, before applying the same log transformation and standardization as for the individual metabolites.

In ColoCare and CORSA, multivariable logistic regression models were applied adjusting for sex (male, female), age at recruitment (continuous), body mass index (BMI; continuous), smoking status (never, former, current) and education (low, intermediate, high, other). For ColoCare, the models were further adjusted for diabetes (no, yes). For CORSA, further adjustments were performed for dietary categories: "fruit, vegetables, salads," "meat, sausage, fat" and "sweets, pastry" (in all: much, some, little, none).

In EPIC, conditional logistic regression models were used due to the nested case-control design, stratified by matched case-control pair, and adjusted for smoking status (never, former, current), physical activity (inactive, moderately inactive, moderately active, active), diabetes (self-reported at baseline), waist circumference (continuous), height (continuous), alcohol consumption at recruitment (continuous), consumption of red and processed meat (continuous), consumption of fiber (continuous) and

TABLE 2 Baseline characteristics for the EPIC study

	Cancer-free controls	Colon cancer cases
Sample size	456	456
Age at recruitment: Mean (SD)	56 (7.7)	56 (7.8)
Time to diagnosis: Mean (SD)	NA	6.6 (3.5)
Waist circumference (cm): Mean (SD)		
Overall	87.5 (11.5)	89.2 (13.6)
Men	95.4 (8.4)	98.0 (10.3)
Women	82.8 (10.5)	84.0 (12.4)
Height (cm): Mean (SD)		
Overall	163.5 (9.3)	164.4 (8.9)
Men	172.1 (6.5)	171.9 (7.1)
Women	158.4 (6.5)	159.9 (6.6)
Physical activity		
Inactive	136 (29.8%)	142 (31.1%)
Moderately inactive	186 (40.8%)	199 (43.7%)
Moderately active	74 (16.2%)	68 (14.9%)
Active	59 (12.9%)	47 (10.3%)
Missing	1 (0.3%)	0 (0%)
Smoking status		
Never	245 (53.7%)	214 (46.9%)
Former	127 (27.9%)	141 (30.9%)
Smoker	82 (18%)	8 (21.5%)
Unknown	2 (0.4%)	3 (0.7%)
Education levels		
None/primary school	233 (51.1%)	226 (49.6%)
Technical/Prof/ secondary school	142 (31.1%)	149 (32.7%)
Longer education incl. university	72 (15.8%)	69 (15.1%)
Not specified	9 (2%)	12 (2.6%)
Diabetes		
No	404 (88.6%)	405 (88.8%)
Yes	19 (4.2%)	19 (4.2%)
Unknown	33 (7.2%)	32 (7%)
Consumption of alcohol (g/d): Mean (SD)		
Overall	13.2 (17.5)	15.2 (19.5)
Men	22.8 (21.4)	27.8 (23.4)
Women	7.13 (11.2)	7.6 (11.4)
Consumption of red and processed meat (g/d): Mean (SD)		
Overall	77.5 (44.5)	76.3 (79.5)
Men	87.2 (47.4)	97.7 (117.7)
Women	71.6 (41.6)	63.6 (37.9)
Consumption of dietary fiber (g/d): Mean (SD)		
Overall	23.3 (8.0)	22.8 (7.5)
Men	24.9 (8.9)	23.8 (8.6)
Women	22.3 (7.2)	22.1 (6.8)
Tryptophan (μM): Median (IQR)		
Overall	50 (43.9, 56.1)	47.8 (42.7, 55.2)
Men	52.2 (47.4, 59.2)	51.6 (45.9, 58.5)
Women	48.3 (42.7, 53.4)	46.2 (41.2, 52.8)
Below LLOQ	0 (0%)	0 (0%)
Above LLOQ	456 (100%)	456 (100%)
Serotonin (μM)		
Below LLOQ	393 (86%)	370 (81%)
Men	146 (85.4%)	139 (81.3%)
Women	247 (86.7%)	231 (81.1%)
Above LLOQ	63 (14%)	86 (19%)
Men	25 (14.6%)	32 (18.7%)
Women	38 (13.3%)	54 (18.9%)
Kynunerine (μM): Median (IQR)		
Overall	2 (1.7, 2.3)	2 (1.7, 2.4)
Men	2.1 (1.8, 2.4)	2 (1.8, 2.4)
Women	1.9 (1.6, 2.3)	1.9 (1.6, 2.3)
Below LLOQ	0 (0%)	9 (2%)
Above LLOQ	456 (100%)	447 (98%)
Kynurenine-tryptophan ratio: Median (IQR)		
Overall	0.04 (0.03, 0.05)	0.04 (0.03, 0.05)
Men	0.04 (0.03, 0.05)	0.04 (0.03, 0.05)
Women	0.04 (0.03, 0.05)	0.04 (0.03, 0.05)

Abbreviations: EPIC, European Prospective Investigation into Cancer and Nutrition study; IQR, interquartile range; LLOQ, lower limit of quantification; NA, not applicable.

education level (none/primary school, technical/professional/secondary school, longer education including university). The analysis plan for ColoCare, CORSA and EPIC is summarized in Figure 1.

The main results were derived from a multiple imputation approach of the missing values for the covariates in the models in order to increase the statistical power.[23,24] Since the models for kynurenine-to-tryptophan ratio may be influenced by dietary intake of tryptophan, we performed additional analysis adjusted for estimated dietary tryptophan intake, but since the results were very similar, the unadjusted model was used. In EPIC, the analysis was repeated after excluding participants with less than 2 years of follow-up, to take into account potential bias due to underlying subclinical cancer. The analysis was performed using R (version 3.6.3) and Stata (version 15).

3 | RESULTS

3.1 | Colon cancer patients and controls

On average, participants' age was over 60 years, except for the controls in ColoCare, where the average age was 51 years. The BMI was also

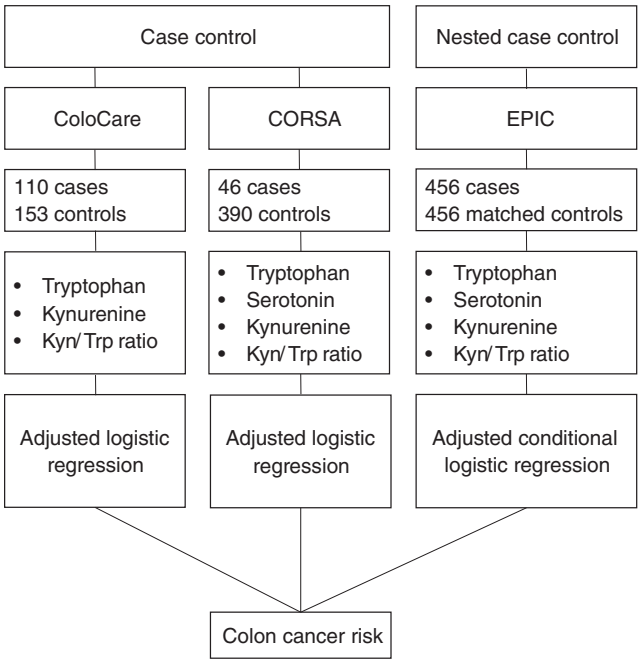

FIGURE 1 Graphical representation of the analysis plan of the present study, showing the number of samples, plasma analytes measured and statistical models used for each of the three study populations included. CORSA, Colorectal Cancer Study of Austria; EPIC, European Prospective Investigation into Cancer and Nutrition study; Kyn, kynurenine; Trp, tryptophan

lower for the controls in ColoCare than those in CORSA (Table 1). Approximately 80% of the controls and cases in both studies were categorized as former or current smokers. Median tryptophan levels were higher in controls compared with cases, in both studies, while serotonin (CORSA only) and kynurenine-to-tryptophan ratio were lower among controls. However, the median levels of kynurenine were higher in controls than in cases in ColoCare (2.5 μM vs 2.3 μM) but lower in controls than in cases in CORSA (2.3 μM vs 2.8 μM). In ColoCare, two cases (male and female) and one control (female) had colitis, and for these the tryptophan concentrations were 66.3 μM, 35.2 μM and 56.7 μM, with kynurenine levels at 3.3 μM, 3.1 μM and 2.8 μM, respectively. Serotonin was below LLOQ in all the three samples.

Figure 2 shows the results of the multivariable logistic regression models. For tryptophan, high heterogeneity was found between ColoCare and CORSA, with a statistically significant inverse association observed only for ColoCare (odds ratio [OR] per 1-SD = 0.44; 95% confidence interval [CI], 0.31-0.64). Serotonin was positively associated with colon cancer in CORSA (OR [binary] = 4.67; 95% CI, 2.31-9.43). In ColoCare, serotonin data were missing. For kynurenine, results in ColoCare and CORSA were opposite (Figure 2). In ColoCare, an inverse association was observed (OR per 1-SD = 0.74; 95% CI, 0.55-0.98), while in CORSA the association was positive (OR per 1-SD = 1.79; 95% CI, 1.27-2.52). For the kynurenine-to-tryptophan ratio, the results were consistent, with higher levels associated with higher colon cancer risk in both ColoCare (OR per 1-SD = 1.38; 95% CI, 1.03-1.84) and CORSA (OR per 1-SD = 1.44; 95% CI, 1.06-1.96), with no evidence of heterogeneity.

3.2 | European Prospective Investigation into Cancer and Nutrition study

In EPIC, participants with future colon cancer were slightly taller than controls and had larger waist circumference (Table 2). Furthermore, most of the participants in both groups were physically inactive while 48% of the controls and 52% of the cases were former or current smokers at the time of recruitment. Higher circulating levels of tryptophan were associated with a lower colon cancer risk (OR per 1-SD = 0.86; 95% CI, 0.74-0.99), while a higher risk was observed for higher levels of serotonin (OR [binary] = 2.03; 95% CI, 1.20-3.40) (Figure 2). Kynurenine and the kynurenine-to-tryptophan ratio were not associated with colon cancer risk, although there was a suggestive inverse association for kynurenine, in the continuous analysis only (OR per 1-SD = 0.88; 95% CI, 0.77-1.00) (Figure 2). Restricting the analysis to participants with two or more years of follow-up showed similar results with the main analysis, with slightly stronger inverse association for kynurenine (OR per 1-SD increment: 0.86; 95% CI, 0.75-0.98) (Figure S1).

4 | DISCUSSION

In three different studies, where two included colon cancer patients and cancer-free controls (ColoCare, CORSA), and one was a prospective cohort with prediagnostic samples (EPIC), we found plasma tryptophan levels inversely associated with colon cancer in both types of studies (ColoCare, EPIC). Serotonin could not be detected in the samples from ColoCare, but was positively associated with colon cancer in CORSA, as well as in EPIC. For kynurenine, the associations were opposite in the ColoCare and CORSA, and not significant in EPIC. However, the positive association of the kynurenine-to-tryptophan ratio with colon cancer was consistent across both studies that included cancer patients (ColoCare and CORSA), while there was no association with colon cancer in EPIC, where samples were collected years before the diagnosis.

The observed inverse association between plasma tryptophan and colon cancer risk is concordant with earlier case-control studies that have shown lower serum or plasma tryptophan in colorectal cancer patients compared to cancer-free controls.[25-28] In the present study, we also found this association to exist several years prior to the diagnosis. A number of studies also suggest some bacterial metabolites of tryptophan, such as indole and indole-3-propionic acid, to have beneficial effects on gut permeability, reducing gut leakiness that can increase the risk of colorectal cancer.[6,29-31] Moreover, serum levels of indole-3-propionic acid have been associated with better insulin secretion and lower risk of type 2 diabetes, with a positive correlation with dietary fiber intake and inverse correlation with inflammatory markers.[32,33] In the current study,

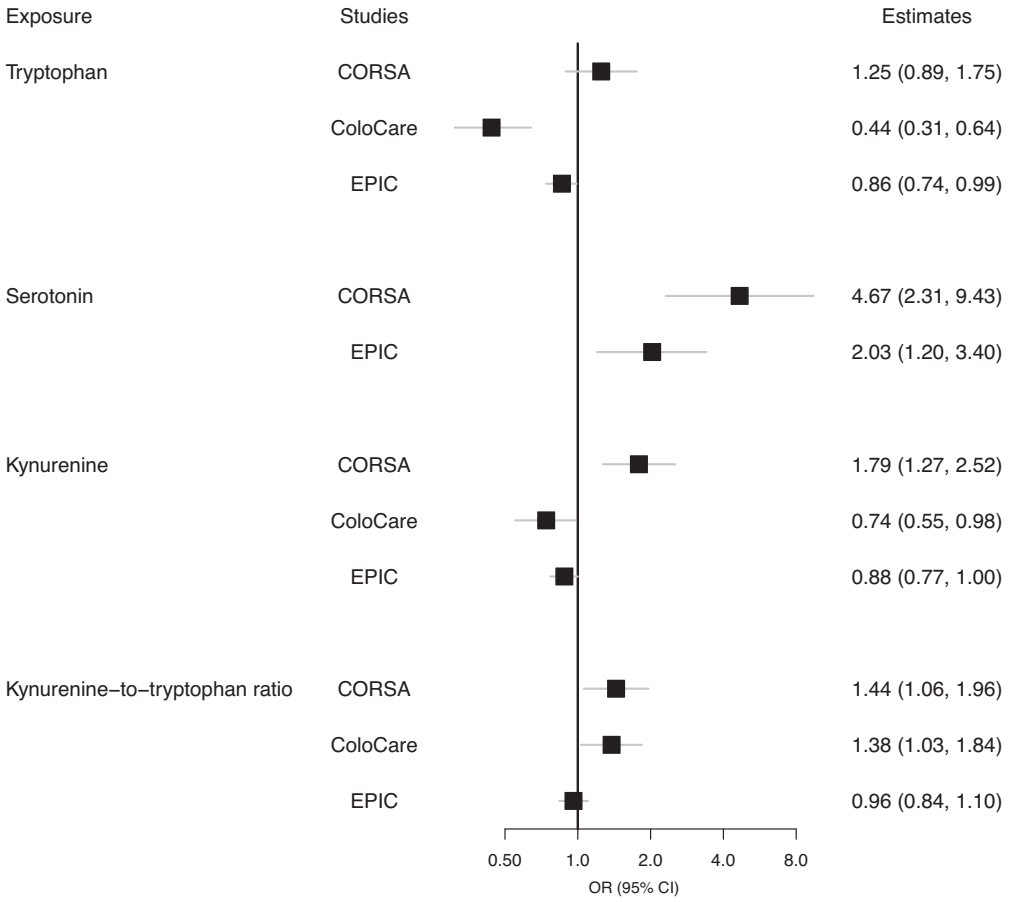

FIGURE 2 Associations of tryptophan, serotonin, kynurenine and kynurenine-to-tryptophan ratio with colon cancer in ColoCare, CORSA and EPIC studies. The ORs correspond to 1-SD difference in concentration levels of the biomarkers except for serotonin where the comparison was done between detectable and undetectable concentration levels. CI, confidence interval; CORSA, Colorectal Cancer Study of Austria; EPIC, European Prospective Investigation into Cancer and Nutrition study; OR, odds ratio

our analyses did not cover the microbiota-driven indole pathway, but the inverse association of their precursor tryptophan with colon cancer in samples collected both at diagnosis and years before the cancer occurrence, warrants further studies of the role of this pathway in colon cancer development. In addition to being a substrate of indole metabolites, tryptophan can also have potential direct effects on colon cancer development. Its depletion activates protein kinase GCN2, a key metabolic component under conditions of amino acid deprivation, leading to inhibition of T cell-mediated immune response, which has been linked to a higher risk of colorectal cancer.[7,8,34,35]

In contrast to the results for tryptophan, we observed positive associations between serotonin and colon cancer risk in CORSA and EPIC, studies where serotonin data were available. Most of the body's serotonin is synthesized in the gut from tryptophan by enterochromaffin cells that line the gut epithelium, particularly in the colon where the number of these cells is highest.[36] A fraction of the gut-derived serotonin enters circulation and is transported by platelets in blood.[37,38] The enterochromaffin cells are sensitive to luminal stimuli and can release serotonin as a response to nutrient exposure, direct or chemical signaling by bacteria and mechanical stimuli, although the exact mechanisms are not well understood.[37] However, recent studies have linked gut-derived serotonin with regulation of lipid metabolism, glucose homeostasis and diseases associated with metabolic syndrome, including obesity and type 2 diabetes.[37,39] Moreover, serotonin can suppress the release of adiponectin, an adipokine associated with improved insulin sensitivity and known for its anti-inflammatory characteristics.[37,40-43] This evidence suggests that elevated gut-derived serotonin may play a role in obesity and type 2 diabetes, two well-established risk factors for colorectal cancer.[2,4,44,45] Recently, we showed that consumption of wholegrain rye was associated with lower levels of plasma serotonin in an experimental study,[46] which is interesting given that wholegrain consumption has also been linked with lower colorectal cancer risk as well as lower body fatness.[2,47-49] In the present study, higher plasma serotonin was associated with increased risk of colon cancer, which supports earlier results.

For kynurenine, we observed opposite results in ColoCare and CORSA, while a nonsignificant inverse association was observed for EPIC. Experimental evidence suggests that kynurenine contributes to carcinogenesis by mediating the differentiation of CD4+ T cells into regulatory T cells, and by assisting immune surveillance evasion by the tumor cells.[34] Recently, kynurenine has been proposed as an oncometabolite and biologically active in colon cancer, and inhibition

of its production as an efficient approach to limit the growth of colon cancer cells.[50] In our study, kynurenine was positively associated with colon cancer in CORSA, supporting the earlier studies, but not in ColoCare. Some of this disagreement may be due to the complexity of kynurenine metabolism we may have not been able to take into account,[51,52] but most likely due to the differences in the cancer-free controls used for CORSA and ColoCare including age, BMI and sex (Table 1). In addition, it should be noted that the controls in CORSA were identified from a screening program with a positive test result. Although these participants did not have polyps or cancer, they may have had other gastrointestinal abnormalities. However, for the kynurenine-to-tryptophan ratio, our analyses yielded consistent results for CORSA and ColoCare, with a clear difference to EPIC, which included only prediagnostic samples. Although kynurenine-to-tryptophan ratio is not an accurate measure of the IDO activity in vivo,[52] there is some evidence for its prognostic utility,[53] and our results suggest it may more robustly reflect the altered tryptophan catabolism in colon cancer than either tryptophan or kynurenine alone. Kynurenine-to-tryptophan ratio was also notably different between EPIC and the studies including colon cancer patients. As shown in Tables 1 and 2, interquartile range (IQR) for the participants in EPIC (measured before cancer diagnosis) was 0.03 to 0.05, while in the two other studies the IQR for the cases, measured at diagnosis, was 0.04 to 0.07. These results suggest increased tryptophan catabolism through the kynurenine pathway after colon cancer occurrence.

To our knowledge, this is the first study to combine and compare data from studies with colon cancer patients and cancer-free controls, and from a prospective cohort to examine the role of tryptophan and its major metabolites in colon cancer development. Importantly, all blood samples used in the current study were analyzed in the same laboratory with the same analytical instrumentation, diminishing any potential bias due to analytic methods or laboratories.

Nevertheless, we also note several limitations. We measured total plasma tryptophan (a sum of free and albumin-bound), which does not enable excluding the effect of albumin concentration from the tryptophan results.[54] The serotonin concentrations were below LLOQ in almost all participants from ColoCare, while the rates for CORSA and EPIC were 76% and 84%, respectively. This may be due to differences in sample handling and plasma preparation. It is known that serotonin can leak from platelets into plasma during plasma processing, requiring careful and consistent sample handling to avoid variability and bias.[21] Clotting releases serotonin from platelets, and thus serum samples can provide better measures of circulating serotonin compared to the platelet-poor plasma used in our study.[21,38,55] Plasma concentration of serotonin is also linked to platelet biology, and thus we should be conservative regarding its association with cancer risk.[56] Moreover, we cannot exclude potential study effects in ColoCare and CORSA, which might contribute the large heterogeneity in their results. For example, the controls in ColoCare were on average 12 years younger than the controls in CORSA, while the average age of cases was similar. We also used the kynurenine-to-tryptophan ratio as an indirect method to assess IDO activity and estimate the role of tryptophan catabolism via kynurenine pathway. However, this pathway is responsive to unspecified inflammation as well, and there are additional determinants of kynurenine-to-tryptophan ratio aside IDO.[7,52] Finally, we cannot exclude potential residual confounding in our results especially in ColoCare and CORSA, where information on confounders such as alcohol consumption was not available.

In summary, we found total plasma concentration of tryptophan inversely associated with colon cancer risk and positive associations for serotonin. We also observed consistent differences for kynurenine-to-tryptophan ratio between studies that included colon cancer patients and those with prospectively collected samples, which suggest altered tryptophan catabolism during colon cancer development. Taken together, our results support earlier studies on the role of tryptophan metabolism in colon cancer and offer new insights into changes in the metabolic flux of tryptophan prior and after colon cancer diagnosis. Future prospective studies of tryptophan metabolism including additional tryptophan metabolites and samples enabling separation of platelet and albumin bound fractions are merited.

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CONFLICT OF INTEREST

As Executive Director of the Comprehensive Cancer Center at Huntsman Cancer Institute (Salt Lake City, Utah), Cornelia M. Ulrich formally oversees research funded by several pharmaceutical companies. However, she does not direct those research efforts and has not received funding directly herself that would constitute a conflict to the current work. The other authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Written informed consent was obtained from all participants. The ColoCare Study has been approved by the ethics committee of the Medical Faculty at the University of Heidelberg. The CORSA Study was approved by the ethical review committee of the Medical University of Vienna (1160/2016), by the "Ethikkommission der Stadt Wien" (06-150-VK) and by the institutional review board "Ethikkommission Burgenland" (33/2010). The study was also approved by the International Agency for Research on Cancer ethics committee. For EPIC, informed consent was obtained for each center and the study was approved by the Institutional Review Board at the International Agency for Research on Cancer (IARC) and by the local ethical committees.

DISCLAIMER

The funders had no role in the design and conduct of the study, the collection, analysis and interpretation of the data, or the preparation, review and approval of the manuscript or in the decision to submit the manuscript for publication. Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer/World Health Organization.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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