Heterocyclic aromatic amine intake increases colorectal adenoma risk: findings from a prospective European cohort study[1–3]

Sabine Rohrmann, Silke Hermann, and Jakob Linseisen

ABSTRACT

Background: Heterocyclic aromatic amines (HCAs), which arise from cooking meat and fish at high temperatures, may increase the risk of colorectal adenomas. Conversely, flavonoids might counteract the negative effects of HCAs.

Objective: The association between dietary HCA intake and colorectal adenoma incidence was investigated in a prospective cohort study.

Design: At recruitment (1994–1998), detailed information on diet, anthropometric measures, lifestyle, and medication use was assessed in 25,540 participants of the European Prospective Investigation into Cancer and Nutrition–Heidelberg cohort study. Dietary HCA intake was estimated by using information from food-frequency questionnaires on meat consumption, applied cooking methods, and preferred degree of browning. Until June 2007, 516 verified incident colorectal adenomas were identified. Participants with negative colonoscopy ($n = 3966$) were also included in the analytic cohort. Multivariate Cox proportional hazards regression was used to examine the association between colorectal adenoma risk and intake of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), and 2-amino-3,4,8-dimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx).

Results: In multivariate analyses, the intake of PhIP as the most abundant dietary HCA was associated with an increased risk of colorectal adenoma (relative risk: 1.47; 95% CI: 1.13, 1.93; quartile 4 compared with quartile 1; P for trend $= 0.002$), but no statistically significant associations were observed for MeIQx and DiMeIQx intakes. In addition, adenoma risk also increased with the consumption of strongly or extremely browned meat (P for trend $= 0.04$). The association of PhIP intake with adenoma risk was most pronounced for small adenomas (P for trend $= 0.01$) and adenomas localized in the distal colon (P for trend $= 0.002$).

Conclusion: The results of this first European cohort study support data from case-control studies of a positive association between HCA intake and colorectal adenoma risk. *Am J Clin Nutr* 2009;89:1418–24.

INTRODUCTION

Colorectal adenomas are thought to be premalignant precursors that frequently develop into cancer (1). Meat consumption has been shown to be associated with the risk of colorectal adenoma (2, 3), and one underlying cause might be the formation of heterocyclic aromatic amines (HCA) during cooking of meat at high temperatures. Whereas the carcinogenicity of different HCAs has been proven in animal studies (4, 5), the influence on adenoma development in humans has not been sufficiently explored. To date, only 6 studies [5 population-based case-control studies (3, 6–9) and 1 cohort study], all conducted in the United States, have evaluated the association between intake of HCAs and adenoma risk (10). The outcome of the research has been inconsistent. Some (6–8), but not all (3, 9), studies observed a positive association between increased intake of HCAs and the risk of developing colorectal adenomas. The cohort study (10) reported a positive association between a higher intake of a calculated sum of meat-derived mutagens and the risk of distal colon adenomas; however, HCA intake alone was not responsible for this finding.

Flavonoids are a heterogeneous group of secondary plant components, many of which have anticarcinogenic properties such as radical scavenging, modification of enzyme activities involved in carcinogen activation and inactivation, cellular signaling, cell cycle regulation, and induction of apoptosis (11). For example, in vitro studies have shown that flavonoids can inhibit the bioactivation of HCAs (12, 13). Thus, it is hypothesized that polyphenolic compounds can affect HCA metabolism in humans (14), which could in turn modify HCA-related health risks.

It was the aim of this study to examine the association between dietary HCA intake and the incidence of colorectal adenoma. In addition, we evaluated whether this association is modified by dietary flavonoid intake.

[1] From the Unit of Nutritional Epidemiology, Division of Cancer Epidemiology, German Cancer Research Center, Heidelberg, Germany.

[2] Supported by the Kurt-Eberhard-Bode-Foundation; the Environmental Cancer Risk, Nutrition and Individual Susceptibility, a Network of Excellence of the European Commission (contract no 513943); "Europe Against Cancer" Programme of the European Commission (S12.195579, S12.296584, S13.26938, SPC.2002.332, and FP6-006438); and German Cancer Aid.

[3] Reprints not available. Address correspondence to S Rohrmann, Division Cancer Epidemiology (C020), Im Neuenheimer Feld 280, 69120 Heidelberg. Germany. E-mail: s.rohrmann@dkfz-heidelberg.de.

Received July 8, 2008. Accepted for publication December 22, 2008.
First published online March 4, 2009; doi: 10.3945/ajcn.2008.26658.

SUBJECTS AND METHODS

Study participants and questionnaires

The European Prospective Investigation into Cancer and Nutrition (EPIC)–Heidelberg Study is a cohort study that started in 1994 as part of EPIC (15). Between 1994 and 1998, 25,540 participants aged 35–65 y were recruited from the general population of Heidelberg and surrounding communities. Detailed information on diet, lifestyle, anthropometric measures, and medical history were obtained by means of questionnaires and face-to-face interviews (15).

Diet was assessed by using a validated food-frequency questionnaire (16). During the second follow-up of the cohort (2002–2004), the same food-frequency questionnaire was administered again but including detailed questions on meat preparation methods and preferred degree of browning (17), which was completed and sent back by 21,452 participants. We computed the average daily consumption of red and processed meat as well as white meat. In addition, we calculated meat consumption prepared by hazardous cooking methods (broiling, frying, and grilling/barbecuing, which are strongly related to the formation of HCAs in meat) as well as meat consumption by preferred degree of browning (lightly browned, moderately browned, strongly browned, or extremely browned). Mean daily dietary intake of HCAs from meat was calculated by using published data of the HCA content in different types of meat (18–23) in combination with information on degree of browning, cooking method, and habitual meat consumption. Further details have been published elsewhere (24).

On the basis of a database established for the estimation of flavonoid intake through consumption of plant-derived foodstuffs in Germany (25, 26) and on the US database on flavonoid content of food (www.nal.usda.gov/fnic/foodcomp/Data/Flav/flav.html Date 05/12/2005), an updated database on the food content of flavonoids was used to calculate mean daily intake of flavonoids, ie, intake of flavonols, flavones, flavan-3-ols (catechins), flavanones, anthocyanidins, and isoflavones. For the earlier version of the database, a comparison between short-term intake data and fasting plasma concentrations of some flavonols and flavanones yielded correlation coefficients between 0.42 and 0.64 ($P < 0.01$) (27).

Self-reports of adenomas between recruitment and June 2007 were verified by a trained physician. Incident adenoma cases were coded per the second version of the International Classification of Diseases for Oncology (ICD-O2). Mortality data were coded according to the 10th revision of the *International Classification of Diseases*, Injuries and Causes of Deaths (ICD-10).

In the 3 follow-ups, 960 participants reported having had a colorectal adenoma. Of the self-reported adenomas, for which information on food preparation methods and preferred degree of browning was available, 516 were confirmed to be incident colorectal adenomas and 167 were hyperplastic polyps. For a subanalysis, the colon was divided based on ICD-10 into the proximal colon (C180, C181, C182, C183, C184, C1841, C1842, C1843, and C185), distal colon (C186 and C187), and rectum (C199, C209, C2091, C2092, and C2093).

Because of the fact that colorectal adenomas develop frequently without symptoms and thus are often not diagnosed, only participants who underwent a colonoscopy were included in the analyses. During follow-up, 5064 participants had reported a colonoscopy. Of these participants, we excluded all prevalent cancer cases other than nonmelanoma skin cancer ($n = 371$), subjects with hyperplastic polyps ($n = 167$), as well as 44 persons who did not return the detailed questionnaire on meat preparation methods and preferred degree of browning, which left 3966 participants with a negative result from colonoscopy in the analytic cohort.

All participants gave informed consent at study entry. In addition, approval for this study was given by the ethical committee of Heidelberg University Medical School.

Statistical analyses

Cox proportional hazards regression was used to examine the association of colorectal adenoma incidence with 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), and 2-amino-3,4,8-dimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx), modeling daily HCA intake as a categorical variable based on quartiles of intake of the cohort. We also examined the associations between total intake of red and processed meat, total intake of white meat, total intake of meat that has been fried, broiled, or grilled/barbecued, and total intake of meat that was consumed strongly or extremely browned (by quartiles of intake). Age was used as the primary time variable in the Cox models. Time at entry was the age at recruitment, and the time of exit was the age at which participants received a diagnosis of adenoma or cancer, died, were lost to follow-up, or were censored at the end of the follow-up period, whichever came first. The analyses were stratified by sex and age at recruitment in 1-y categories. In multivariate regression models, we adjusted for energy intake without energy from alcohol (in quartiles), ethanol intake (in quartiles), consumption of milk and milk product (in quartiles), fiber intake (in quartiles), body mass index (in kg/m^2; <25, 25–30, or >30), family history of colorectal cancer (yes or no), vigorous physical activity (none, <2 h/wk, or ≥ 2 h/wk), intake of nonsteroidal antiinflammatory drugs (NSAIDs; yes or no), smoking (never, former, or current), pack-years of smoking (≤ 5, >5–10, >10–15, or >15), and education (none or primary, technical or professional school, secondary school, or university degree). Additional adjustment for folate intake or fruit and vegetable consumption did not appreciably alter our results (data not shown). The results are given as relative risks (RRs) and 95% CIs. We also examined whether the observed associations changed when total red and processed meat intake was controlled for in the Cox models. Trend tests were performed by using the median of the respective HCA intake categories to create a continuous variable. We performed subanalyses by adenoma site [proximal colon (including colon transversum), distal colon, and rectum], adenoma size (≤ 1 and >1 cm), morphology (tubular and tubulovillous), sex, and flavonoid intake. We tested for interactive effects by including a cross-product term along with the main-effect terms in the Cox regression model. Statistical significance of the cross-product term was evaluated with the Wald test. All analyses were conducted by using SAS, version 9.1 (SAS Institute Inc, Cary, NC).

RESULTS

In our cohort, 516 colorectal adenomas were identified within 34,769 person-years of follow-up. The distribution of adenomas by site, size, and morphology is shown in **Table 1**. Compared

TABLE 1
Baseline characteristics of colorectal adenoma cases and cohort participants with a negative colonoscopy in the European Prospective Investigation into Cancer and Nutrition (EPIC)–Heidelberg Study[1]

	Cases (n = 516)	Cohort participants with negative colonoscopy (n = 3699)	P value[2]
Adenoma location [n (%)]			
Right colon[3]	146 (28.3)	—	
Left colon[3]	183 (35.5)	—	
Rectum[3]	115 (22.3)	—	
Missing	72 (14.0)		
Adenoma morphology [n (%)]			
Tubulovillous	211 (40.9)	—	
Tubular	294 (57.0)	—	
Others	11 (0.1)	—	
Adenoma size [n (%)]			
≤1cm	224 (43.4)	—	
>1 cm	165 (32.0)	—	
Missing information [n (%)]	127 (24.6)	—	
Person-years (y)	5.4 ± 2.4[4]	7.8 ± 1.7	
Age (y)	55.1 ± 6.2	52.9 ± 7.6	<0.0001
HCA intake (ng/d)			
PhIP	44.8 ± 62.1	41.0 ± 117.5	0.25
MeIQx	21.5 ± 35.0	16.8 ± 29.7	0.004
DiMeIQx	3.8 ± 6.0	3.0 ± 4.5	0.003
BMI (kg/m^2)	26.6 ± 3.8	26.2 ± 4.0	0.02
Energy intake (kcal/d)	2003 ± 676	1935 ± 654	0.03
Vegetable intake (g/d)	116.5 ± 55.9	119.0 ± 58.9	0.35
Fruit intake (g/d)	114.1 ± 87.5	121.0 ± 86.2	0.09
Fiber intake (g/d)	20.1 ± 6.8	20.4 ± 7.3	0.39
Total flavonoid intake (mg/d)	97.6 ± 81.6	100.3 ± 90.7	0.48
Total folic acid intake (μg/d)	204.5 ± 58.0	205.8 ± 62.5	0.65
Red and processed meat intake (g/d)	91.9 ± 58.6	81.5 ± 60.7	0.0002
Ethanol intake (g/d)	23.3 ± 26.2	17.2 ± 21.8	<0.0001
Vigorous physical activity (%)[5]			
None	34.3	36.2	
<2 h/wk	38.6	37.8	
≥ 2 h/wk	26.2	24.9	0.85
Regular NSAID use (%)	9.5	9.3	0.90
Smoking status (%)			
Never	35.9	43.6	
Former	45.4	39.6	
Current	18.8	16.9	0.004
Education (%)			
None or primary school	30.6	29.8	
Technical or professional school	32.6	35.4	
Secondary school	5.4	5.4	
University degree	31.4	29.5	0.75
Family history of colon cancer (%)	17.8	11.6	0.0003

[1] PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; DiMeIQx, 2-amino-3,4,8-dimethylimidazo[4,5-*f*]quinoxaline; NSAID, nonsteroidal antiinflammatory drug; HCA, heterocyclic aromatic amine.
[2] t Test for continuous variables or chi-square test for categorical variables.
[3] Proximal colon includes *International Classification of Diseases* (ICD) for Oncology codes C180, C181, C182, C183, C184, C1841, C1842, C1843, and C185; distal colon includes ICD codes C186 and C187; and rectum includes ICD codes C199, C209, C2091, C2092, and C2093.
[4] Mean ± SD (all such values).
[5] Missing information on physical activity for 121 participants.

with subjects without adenomas, subjects with adenomas were statistically significantly older, had a higher body mass index, and had significantly higher intakes of MeIQx, DiMeIQx, energy, red and processed meat, and ethanol. Also, subjects with adenomas were more likely to be smokers at baseline and more often reported a family history of colorectal cancer. Spearman correlation coefficients between intakes of different HCAs were as follows: 0.74 for PhIP/MeIQx, 0.62 for PhIP/DiMeIQx, and 0.82 for MeIQx/ DiMeIQx.

In the crude model, the risk of colorectal adenomas increased with higher intakes of PhIP, MeIQx, and DiMeIQx (**Table 2**). After potential confounders were taken into account, the RRs

TABLE 2
Association between heterocyclic aromatic amine intake, by quartile (Q), and relative risk (RR) of colorectal adenomas in the European Prospective Investigation into Cancer and Nutrition (EPIC)–Heidelberg Study[1]

		RR[2] (95% CI)		
	No. of cases	Unadjusted	Adjusted[3]	RR (95% CI) Adjusted[4]
PhIP (ng/d)				
Q1 (<6.5)	100	1.00	1.00	1.00
Q2 (≥6.5 and <16.8)	106	1.10 (0.83, 1.45)	1.05 (0.79, 1.39)	0.98 (0.73, 1.31)
Q3 (≥16.8 and <41.4)	143	1.38 (1.07, 1.80)	1.29 (0.99, 1.69)	1.19 (0.90, 1.59)
Q4 (≥41.4)	197	1.54 (1.19, 1.99)	1.47 (1.13, 1.93)	1.39 (1.04, 1.86)
P for trend		0.0002	0.002	0.01
MeIQx (ng/d)				
Q1 (<3.8)	102	1.00	1.00	1.00
Q2 (≥3.8 and <9.3)	123	1.21 (0.92, 1.57)	1.14 (0.87, 1.50)	1.05 (0.79, 1.39)
Q3 (≥9.3 and <19.9)	134	1.24 (0.95, 1.61)	1.17 (0.89, 1.53)	1.04 (0.78, 1.38)
Q4 (≥19.9)	157	1.41 (1.09, 1.83)	1.27 (0.97, 1.68)	1.16 (0.86, 1.57)
P for trend		0.01	0.10	0.36
DiMeIQx (ng/d)				
Q1 (<0.5)	117	1.00	1.00	1.00
Q2 (≥0.5 and <1.5)	121	1.05 (0.81, 1.36)	0.99 (0.77, 1.29)	0.93 (0.71, 1.21)
Q3 (≥1.5 and <3.8)	118	0.99 (0.76, 1.28)	0.93 (0.72, 1.21)	0.86 (0.65, 1.12)
Q4 (≥3.8)	160	1.29 (1.01, 1.64)	1.18 (0.92, 1.53)	1.09 (0.83, 1.42)
P for trend		0.06	0.25	0.57

[1] PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; DiMeIQx, 2-amino-3,4,8-dimethylimidazo[4,5-*f*]quinoxaline.
[2] Cox regression models were used for these evaluations.
[3] Adjusted for energy intake without energy from alcohol (quartiles), ethanol intake (quartiles), milk and milk product consumption (quartiles), fiber consumption (quartiles), BMI (in kg/m^2; <25, 25–30, or >30), family history of colorectal cancer (bivariate), physical activity (none, <2 h/wk, or ≥2 h/wk), intake of nonsteroidal antiinflammatory drugs, smoking (never, former, or current), pack-years of smoking (≤5, >5–10, >10–15, or >15), education (none or primary, technical or professional school, secondary school, or university degree), age (1-y categories), and sex.
[4] Additionally adjusted for red and processed meat intake.

were attenuated but remained statistically significant for PhIP intake. Subjects with the highest intake of PhIP had a 47% (95% CI: 13, 93%) higher risk of developing adenomas than did those with the lowest intake. The RRs for high intakes of MeIQx and DiMeIQx also remained elevated, yet were no longer statistically significant. After the consumption of red and processed meat was included in the model, only the association between PhIP and colorectal adenoma risk remained significantly increased (RR: 1.39; 95% CI: 1.04, 1.86). Also, after mutual adjustment (adjustment of HCAs for one another) in the multivariate model, the association of PhIP with adenoma risk remained statistically significant (RR: 1.50; 95% CI: 1.07, 2.11; quartile 4 compared with quartile 1), but no association existed for MeIQx or DiMeIQx (data not shown). Exclusion of the first 2 y of follow-up resulted in slightly stronger associations of PhIP ($RR_{adjusted}$: 1.59; 95% CI: 1.19, 2.12; quartile 4 compared with quartile 1), MeIQx (RR: 1.35; 95% CI: 1.01, 1.81), and DiMeIQx intake (RR: 1.29; 95% CI: 0.99, 1.69) with adenoma risk (complete data not shown).

Because the formation of HCAs in meat depends on meat type, preparation methods, and degree of browning, we also analyzed the relation of these factors with adenoma risk. The consumption of red and processed meat was associated with an increased adenoma risk, although the association was only borderline statistically significant (*P* for trend = 0.07; **Table 3**); no association was seen for white meat consumption. Also, the amount of meat consumed strongly or extremely browned was statistically significantly associated with an increased risk of colorectal adenomas (RR: 1.36; 95% CI: 1.05, 1.76; quartile 4 compared with quartile 1), but there was no consistent association for the amount of fried, broiled, or grilled/barbecued meat (Table 3).

Subanalyses by adenoma location were conducted for proximal and distal colon and rectum (Table 3). PhIP intake was associated with a significantly increased risk of adenoma in the colon (multivariate RR: 1.56; 95% CI: 1.12, 2.19; quartile 4 compared with quartile 1). This effect seemed to be based on the association seen in the distal colon: participants with the highest PhIP intake had a 74% (95% CI: 13%, 167%) higher risk than did those with the lowest intake. After red and processed meat was included in the model, this association remained with borderline significance (Table 3). Total red and processed meat consumption was related to an increased risk of adenomas in the colon (*P* for trend = 0.03).

PhIP intake was associated with a statistically significantly higher risk of small adenomas in quartiles 3 (RR: 1.58; 95% CI: 1.04, 2.40) and 4 (RR: 1.73; 95% CI: 1.13, 2.63); this association became slightly stronger after adjustment for red and processed meat intake (Table 3). No statistically significant associations for PhIP intake were seen by morphology (data not shown). In contrast with this result, the association between total red and processed meat intake and meat consumed strongly/extremely browned were stronger in large than in small adenomas, whereas a high consumption of fried, broiled, or grilled/barbecued

TABLE 3
Association between relative risk (RR) of colorectal adenoma and consumption, by quartile (Q), of red and processed meat, white meat, meat prepared by hazardous preparation methods, meat strongly or extremely browned, and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine intake in the European Prospective Investigation into Cancer and Nutrition (EPIC)–Heidelberg Study

	Red and processed meat consumption[1]		White meat consumption		Total meat prepared by hazardous cooking methods[1]		Total meat consumed strongly or extremely browned		PhIP intake		
	n	Adjusted[2] RR[3] (95% CI)	*n*	Adjusted[2] RR (95% CI)	*n*	Adjusted[2] RR (95% CI)	*n*	Adjusted[2] RR (95% CI)	*n*	Adjusted[2] RR (95% CI)	Adjusted[4] RR (95% CI)
Adenomas at all sites											
Q1	83	1	127	1	103	1	112	1	100	1	1
Q2	119	1.30 (0.97, 1.75)	128	0.98 (0.76, 1.25)	107	0.95 (0.72, 1.26)	121	1.13 (0.87, 1.47)	106	1.05 (0.79, 1.39)	0.98 (0.73, 1.31)
Q3	161	1.62 (1.20, 2.18)	123	0.89 (0.69, 1.15)	148	1.28 (0.98, 1.67)	115	1.03 (0.79, 1.34)	143	1.29 (0.99, 1.69)	1.19 (0.90, 1.59)
Q4	153	1.33 (0.95, 1.85)	138	1.01 (0.78, 1.30)	158	1.18 (0.89, 1.57)	168	1.36 (1.05, 1.76)	167	1.47 (1.13, 1.93)	1.39 (1.04, 1.86)
P for trend		0.07		0.87		0.08		0.04		0.001	0.01
Adenomas in the colon											
Q1	51	1	79	1	63	1	77	1	63	1	1
Q2	73	1.29 (0.89, 1.86)	83	0.98 (0.71, 1.34)	69	0.97 (0.68, 1.37)	74	1.01 (0.73, 1.39)	61	0.94 (0.66, 1.35)	0.88 (0.60, 1.27)
Q3	99	1.63 (1.12, 2.37)	82	0.92 (0.67, 1.26)	93	1.29 (0.92, 1.81)	72	0.94 (0.68, 1.30)	95	1.38 (0.99, 2.0)	1.26 (0.88, 1.80)
Q4	106	1.53 (1.01, 2.30)	85	0.96 (0.70, 1.31)	104	1.24 (0.87, 1.78)	106	1.24 (0.90, 1.71)	110	1.56 (1.12, 2.19)	1.43 (1.0, 2.05)
P for trend		0.03		0.70		0.10		0.25		0.001	0.01
Adenomas in the proximal colon											
Q1	21	1	38	1	24	1	38	1	25	1	1
Q2	35	1.51 (0.86, 2.65)	32	0.78 (0.48, 1.27)	35	1.17 (0.68, 2.01)	30	0.82 (0.51, 1.34)	32	1.20 (0.70, 2.07)	1.10 (0.63, 1.93)
Q3	40	1.58 (0.88, 2.82)	36	0.84 (0.52, 1.34)	41	1.38 (0.81, 2.35)	37	0.96 (0.60, 1.53)	49	1.73 (1.04, 2.90)	1.57 (0.92, 2.69)
Q4	50	1.63 (0.87, 3.05)	40	0.90 (0.57, 1.44)	46	1.30 (0.75, 2.26)	41	0.88 (0.54, 1.43)	40	1.44 (0.83, 2.47)	1.29 (0.72, 2.30)
P for trend		0.17		0.75		0.33		0.75		0.11	0.25
Adenomas in the distal colon											
Q1	30	1	41	1	39	1	39	1	38	1	1
Q2	38	1.19 (0.72, 1.95)	51	1.15 (0.75, 1.76)	34	0.84 (0.53, 1.36)	44	1.18 (0.76, 1.82)	29	0.76 (047, 1.25)	0.71 (0.43, 1.18)
Q3	59	1.71 (1.04, 2.79)	46	1.01 (0.65, 1.56)	52	1.26 (0.81, 1.96)	35	0.92 (0.58, 1.48)	46	1.18 (0.75, 1.85)	1.07 (0.66, 1.72)
Q4	56	1.50 (0.87, 2.59)	45	1.02 (0.66, 1.58)	58	1.24 (0.78, 1.99)	65	1.62 (1.05, 2.49)	70	1.74 (1.13, 2.67)	1.59 (1.00, 2.54)
P for trend		0.09		0.90		0.17		0.07		0.002	0.01
Adenomas in the rectum											
Q1	23	1	32	1	30	1	24	1	30	1	1
Q2	36	1.59 (0.91, 2.79)	26	0.87 (0.51, 1.48)	24	0.76 (0.430, 1.34)	29	1.27 (0.72, 2.22)	23	0.78 (0.44, 1.36)	0.72 (0.41, 1.28)
Q3	31	1.30 (0.70, 2.40)	24	0.78 (0.45, 1.36)	31	0.97 (0.56, 1.68)	24	1.03 (0.57, 1.86)	29	0.94 (0.54, 1.62)	0.91 (0.51, 1.62)
Q4	25	0.85 (0.42, 1.74)	33	1.13 (0.67, 1.89)	30	0.83 (0.46, 1.50)	38	1.61 (0.92, 2.82)	33	1.08 (0.62, 1.86)	1.12 (0.63, 2.00)
P for trend		0.51		0.75		0.73		0.16		0.64	0.51
Size of adenoma, small adenomas[5]											
Q1	37	1	49	1	40	1	48	1	39	1	1
Q2	54	1.28 (0.83, 1.98)	57	1.16 (0.79, 1.72)	45	1.10 (0.71, 1.71)	61	1.29 (0.88, 1.89)	46	1.21 (0.78, 1.87)	1.17 (0.74, 1.83)
Q3	78	1.67 (1.08, 2.59)	50	0.97 (0.65, 1.47)	71	1.74 (1.15, 2.63)	46	1.00 (0.66, 1.52)	66	1.58 (1.04, 2.40)	1.54 (1.00, 2.39)
Q4	55	0.97 (0.58, 1.62)	68	1.36 (0.93, 1.99)	68	1.52 (0.97, 2.38)	69	1.36 (0.91, 2.03)	73	1.73 (1.13, 2.63)	1.81 (1.16, 2.83)
P for trend		0.90		0.21		0.02		0.29		0.01	0.003
Size of adenoma, large adenomas[5]											
Q1	23	1	48	1	36	1	30	1	34	1	1
Q2	35	1.39 (0.81, 2.40)	39	0.73 (0.47, 1.13)	35	0.82 (0.51, 1.33)	37	1.36 (0.84, 2.21)	33	0.88 (0.54, 1.44)	0.78 (0.47, 1.29)
Q3	48	1.82 (1.05, 3.16)	39	0.69 (0.44, 1.06)	39	0.85 (0.52, 1.38)	38	1.22 (0.75, 1.99)	43	1.06 (0.66, 1.70)	0.89 (0.54, 1.46)
Q4	59	1.98 (1.09, 3.58)	39	0.72 (0.46, 1.12)	55	1.00 (0.62, 1.63)	60	1.78 (1.11, 2.83)	55	1.31 (0.82, 2.08)	1.07 (0.65, 1.76)
P for trend		0.02		0.14		0.86		0.03		0.16	0.53

[1] Sum of meat consumed by broiling, frying, and grilling/barbequing.
[2] Adjusted for energy intake without energy from alcohol (quartiles), ethanol intake (quartiles), milk and milk product consumption (quartiles), fiber consumption (quartiles), BMI (in kg/m^2; <25, 25–30, or >30), family history of colorectal cancer (bivariate), physical activity (none, <2 h/wk, or ≥2 h/wk), intake of nonsteroidal antiinflammatory drugs, smoking (never, former, or current), pack-years of smoking (≤5, >5–10, >10–15, or >15 y), education (none or primary, technical or professional school, secondary school, or university degree), age (1-y categories), and sex.
[3] Cox regression models were used for these analyses.
[4] Additionally adjusted for red and processed meat intake.
[5] Results for 127 adenomas without size information are not shown.

meat was more strongly related to the risk of small adenomas (Table 3).

Following our hypothesis that flavonoid intake may modulate HCA metabolism in humans, we analyzed the interaction between PhIP and flavonoid intake (total flavonoids and flavonoid subgroups) on adenoma risk. The *P* values for interaction were not statistically significant for any of these evaluations, but were strongest for flavonols (*P* for interaction = 0.16). In subjects with a flavonol intake below the median intake in the cohort, the RR progressively increased with higher PhIP intake (RR: 1.76; 95% CI: 1.17, 2.65; *P* for trend = 0.01; quartile 4 compared with quartile 1). However, no statistically significant associations were observed for subjects with a high flavonol intake (RR: 1.24; 95% CI: 0.85, 1.80; *P* for trend = 0.14; quartile 4 compared with quartile 1; complete data not shown).

DISCUSSION

In this prospective cohort study, we observed an increased risk of colorectal adenomas in subjects with a high intake of PhIP—the most abundant HCA in the human diet. After potential confounders were taken into account, subjects with a high intake of PhIP had a 46% higher risk of developing colorectal adenomas than did those with a low intake. This result is supported by positive associations between the consumption of strongly/extremely browned meat and the consumption of meat prepared by methods that are related to a high formation of HCAs.

Only one other cohort study analyzed the association between DiMeIQx, MeIQx, and PhIP intake and adenoma risk, but only MeIQx intake showed a positive association with distal colon adenoma (10). Our results are in line with those of a US case-control study (8) that reported positive associations of DiMeIQx, MeIQx, and PhIP intake with the risk of colorectal adenomas. The largest case-control study conducted thus far, with almost 3700 left-sided adenomas, reported an increased adenoma risk with high intakes of MeIQx and PhIP (7). Another recent US case-control study (6) observed positive associations between MeIQx and DiMeIQx intake, but not PhIP intake, and recurrence of multiple adenomas. Two additional US case-control studies observed no statistically significant associations of PhIP, MeIQx, or DiMeIQx intake with the risk of colorectal adenomas (3, 9).

In our study, comparison of the highest with the lowest intake remained statistically significant for PhIP after adjustment for multiple confounders, even after total red and processed meat consumption was added to the model. This suggests that the association between PhIP intake and adenoma risk cannot be completely explained by other, potentially hazardous components in red or processed meat, but is in fact due to the effect of PhIP. Our observation of a positive association between the consumption of strongly/extremely browned meat and colorectal adenoma risk supports this conclusion because the degree of browning is a major determinant of HCA formation.

Our results showed that the adenoma risk in the distal colon increased with increasing PhIP intake, which confirmed previous observations (7, 10). Also, the lack of association between PhIP intake and rectal adenoma risk has been reported by other researchers (7, 28). It has been hypothesized that these findings may be caused by different local bowel milieu (29), water content of feces (29), and the different flora in the gut sections (30, 7).

The positive association between small adenomas and PhIP intake is in line with results from the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, in which the intakes of MeIQx and PhIP were related to an increased risk of nonadvanced but not advanced distal adenomas (7). No association for any HCA was seen in small or large adenomas in the Tennessee Polyp Study, although an increased risk of large adenomas in subjects with a high consumption of total meat or red meat cooked well-done or very well-done was noted (3). This last observation, however, is in line with our observations for total red and processed meat consumption and high intake of darkly browned meat.

We examined whether the intake of flavonoids modified the association between HCA intake and adenoma risk. As hypothesized, a statistically significant association of PhIP intake with adenoma risk was observed in subjects with a low flavonol intake, whereas no significant association was seen in those with a high flavonol intake. However, the P value for interaction was not statistically significant ($P = 0.16$). Several cross-links with dietary flavonoids have been described in experimental studies with respect to the suggested interactive effects with phase I and phase II enzymes on the risk of HCA-associated cancers (31). Larger studies with greater power will be needed to determine a possible effect modification by polyphenolic compounds.

Strengths of this study included its prospective design, completion of follow-up by a large number of subjects, and medically confirmed diagnoses of adenoma. Limitations of this study were the possible misclassification of HCA intake, even though the 2 major determinants (ie, degree of browning and preparation method) were included in the photo-based questionnaire. Furthermore, as in other studies (3, 6–10), only recent consumption habits were documented, perhaps neglecting behavior at early stages of adenoma development. The estimated HCA intake was lower than in a previous study in Europe (32) and much lower than in a US study (33). This difference was partly explained by variations in meat consumption habits (24). Furthermore, we decided to include only participants with a negative colonoscopy in our control group. This was done previously (10) and is justified by the fact that adenomas are frequently asymptomatic and, thus, participants are not aware of adenoma growth. However, including only participants with a colonoscopy might have altered the outcomes, because this subgroup might have been in need of a colonoscopy and, thus, might not have been completely healthy. On the other hand, this subgroup might have been more health-conscious and, thus, more likely to participate in the colorectal screening program.

In the Health Professionals Follow-Up Study, only the association of HCA intake with distal adenomas was examined; therefore, it was not clear whether a participant had had a colonoscopy or a sigmoidoscopy only (10). This may have explained in part why we observed a stronger effect in the distal but not in the proximal colon. However, in a US case-control study, the association between HCA intake and risk of adenomas was not altered after cases in the distal colon were excluded (8).

In this first European cohort study of the association between HCA intake and the risk of adenoma, the data showed a significantly elevated risk between the estimated HCA intake from meat and colorectal adenomas. This effect was strongest and most robust for PhIP. We also observed a higher adenoma risk with increasing consumption of meat prepared by cooking methods that lead to a high formation of HCAs as well as consumption of strongly or extremely browned meat, which further supported the findings for HCA and adenoma risk. Our results indicate that HCA (particularly PhIP) intake is an independent risk factor for the development of colorectal adenomas. Thus, recommendations concerning meat intake must not miss the aspects of preparation methods and doneness/degree of browning—both of which predict HCA formation and eventually dietary HCA intake.

The authors' responsibilities were as follows—SR: statistical analysis and data interpretation; SH: data interpretation and draft of the manuscript; and JL: study concept and design, data acquisition, critical revision of the manuscript, and funding. None of the authors had any personal or financial conflicts of interest or competing interests.

REFERENCES

1. Morson BC. Evolution of cancer of the colon and rectum. Cancer 1974; 34(suppl):845–9.
2. Austin GL, Adair LS, Galanko JA, Martin CF, Satia JA, Sandler RS. A diet high in fruits and low in meats reduces the risk of colorectal adenomas. J Nutr 2007;137:999–1004.
3. Shin A, Shrubsole MJ, Ness RM, et al. Meat and meat-mutagen intake, doneness preference and the risk of colorectal polyps: the Tennessee colorectal polyp study. Int J Cancer 2007;121:136–42.
4. Adamson RH, Snyderwine EG, Thorgeirsson UP, et al. Metabolic processing and carcinogenicity of heterocyclic amines in nonhuman primates. Princess Takamatsu Symp 1990;21:289–301.
5. Shirai T, Tamano S, Sano M, Masui T, Hasegawa R, Ito N. Carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in rats: dose-response studies. Princess Takamatsu Symp 1995;23:232–9.
6. Martinez ME, Jacobs ET, Ashbeck EL, et al. Meat intake, preparation methods, mutagens and colorectal adenoma recurrence. Carcinogenesis 2007;28:2019–27.
7. Sinha R, Peters U, Cross AJ, et al. Meat, meat cooking methods and preservation, and risk for colorectal adenoma. Cancer Res 2005;65: 8034–41.
8. Sinha R, Kulldorff M, Chow WH, Denobile J, Rothman N. Dietary intake of heterocyclic amines, meat-derived mutagenic activity, and risk of colorectal adenomas. Cancer Epidemiol Biomarkers Prev 2001;10: 559–62.
9. Gunter MJ, Probst-Hensch NM, Cortessis VK, Kulldorff M, Haile RW, Sinha R. Meat intake, cooking-related mutagens and risk of colorectal adenoma in a sigmoidoscopy-based case-control study. Carcinogenesis 2005;26:637–42.
10. Wu K, Giovannucci E, Byrne C, et al. Meat mutagens and risk of distal colon adenoma in a cohort of U.S men. Cancer Epidemiol Biomarkers Prev 2006;15:1120–5.
11. Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA. Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr 2001;74:418–25.
12. Uhl M, Ecker S, Kassie F, et al. Effect of chrysin, a flavonoid compound, on the mutagenic activity of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and benzo(a)pyrene (B(a)P) in bacterial and human hepatoma (HepG2) cells. Arch Toxicol 2003;77:477–84.
13. Hammons GJ, Fletcher JV, Stepps KR, et al. Effects of chemoprotective agents on the metabolic activation of the carcinogenic arylamines PhIP and 4-aminobiphenyl in human and rat liver microsomes. Nutr Cancer 1999;33:46–52.
14. Kukongviriyapan V, Phromsopha N, Tassaneeyakul W, et al. Inhibitory effects of polyphenolic compounds on human arylamine N-acetyltransferase 1 and 2. Xenobiotica 2006;36:15–28.
15. Riboli E. The European Prospective Investigation into Cancer and Nutrition (EPIC): plans and progress. J Nutr 2001;131:170S–5S.
16. Bohlscheid-Thomas S, Hoting I, Boeing H, Wahrendorf J. Reproducibility and relative validity of food group intake in a food frequency questionnaire developed for the German part of the EPIC project. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997;26(suppl 1):S59–70.
17. Rohrmann S, Becker N. Development of a short questionnaire to assess the dietary intake of heterocyclic aromatic amines. Public Health Nutr 2002;5:699–705.
18. Sinha R, Knize MG, Salmon CP, et al. Heterocyclic amine content of pork products cooked by different methods and to varying degrees of doneness. Food Chem Toxicol 1998;36:289–97.
19. Sinha R, Rothman N, Brown ED, et al. High concentrations of the carcinogen 2-amino-1-methyl-6-phenylimidazo- [4,5-*b*]pyridine (PhIP) occur in chicken but are dependent on the cooking method. Cancer Res 1995;55:4516–9.
20. Sinha R, Rothman N, Salmon CP, et al. Heterocyclic amine content in beef cooked by different methods to varying degrees of doneness and gravy made from meat drippings. Food Chem Toxicol 1998;36:279–87.
21. Skog K, Augustsson K, Steineck G, Stenberg M, Jagerstad M. Polar and non-polar heterocyclic amines in cooked fish and meat products and their corresponding pan residues. Food Chem Toxicol 1997;35:555–65.
22. Skog K, Solyakov A, Arvidsson P, Jagerstad M. Analysis of nonpolar heterocyclic amines in cooked foods and meat extracts using gas chromatography-mass spectrometry. J Chromatogr A 1998;803:227–33.
23. Skog K, Steineck G, Augustsson K, Jagerstad M. Effect of cooking temperature on the formation of heterocyclic amines in fried meat products and pan residues. Carcinogenesis 1995;16:861–7.
24. Rohrmann S, Zoller D, Hermann S, Linseisen J. Intake of heterocyclic aromatic amines from meat in the European Prospective Investigation into Cancer and Nutrition (EPIC)–Heidelberg cohort. Br J Nutr 2007;98: 1112–5.
25. Linseisen J, Radtke J, Wolfram G. [Flavonoid intake of adults in a Bavarian subgroup of the national food consumption survey.] Z Ernahrungswiss 1997;36:403–12 (in German).
26. Radtke J, Linseisen J, Wolfram G. [Phenolic acid intake of adults in a Bavarian subgroup of the national food consumption survey.] Z Ernahrungswiss 1998;37:190–7 (in German).
27. Radtke J, Linseisen J, Wolfram G. Fasting plasma concentrations of selected flavonoids as markers of their ordinary dietary intake. Eur J Nutr 2002;41:203–9.
28. Wei SC, Su YN, Tsai-Wu JJ, et al. Genetic analysis of the APC gene in Taiwanese familial adenomatous polyposis. J Biomed Sci 2004;11: 260–5.
29. Iacopetta B. Are there two sides to colorectal cancer? Int J Cancer 2002; 101:403–8.
30. Kassie F, Rabot S, Kundi M, Chabicovsky M, Qin HM, Knasmuller S. Intestinal microflora plays a crucial role in the genotoxicity of the cooked food mutagen 2-amino-3-methylimidazo [4,5-*f*]quinoline. Carcinogenesis 2001;22:1721–5.
31. Hodek P, Trefil P, Stiborova M. Flavonoids-potent and versatile biologically active compounds interacting with cytochromes P450. Chem Biol Interact 2002;139:1–21.
32. Augustsson K, Skog K, Jagerstad M, Dickman PW, Steineck G. Dietary heterocyclic amines and cancer of the colon, rectum, bladder, and kidney: a population-based study. Lancet 1999;353:703–7.
33. Cantwell M, Mittl B, Curtin J, et al. Relative validity of a food frequency questionnaire with a meat-cooking and heterocyclic amine module. Cancer Epidemiol Biomarkers Prev 2004;13:293–8.