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Short Communication

Intake of heterocyclic aromatic amines from meat in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Heidelberg cohort

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It was the aim of the present study to estimate the intake of heterocyclic aromatic amines (HCA) from meat, which have been associated with cancer risk in several epidemiological studies, of 21 462 subjects who participated in the European Prospective Investigation into Cancer and Nutrition (EPIC) in Heidelberg. This was accomplished by using a detailed dietary questionnaire that assessed meat consumption, cooking methods, and degree of browning of the respective food items. Median total HCA intake from meat was 31 ng/d (mean 69 ng/d), which was lower than results observed in previous studies. 2-Amino-1-methyl-6-phenylimidazo[4,5b]pyridine was the most common HCA in this cohort (median 17; mean 48 ng/d). The present study offers the opportunity of a detailed examination of the associations between meat cooking as well as HCA intake from meat and cancer risk in a prospective way.

Heterocyclic aromatic amines: Meat: Cancer risk: European Prospective Investigation into Cancer and Nutrition

Meat and fish are usually cooked before being consumed. Cooking methods that induce high temperatures and a direct exposure to a hot surface, such as grilling or frying, or to direct flame, for example, barbecuing, are discussed in relation to carcinogenesis. Results from several epidemiological studies show associations between meat prepared at high temperatures and the risk of several types of cancer[1–5]. One of the reasons for the increased risk is thought to be the heat-dependent formation of heterocyclic aromatic amines (HCA). When meat is cooked at temperatures over 130°C, for example, when frying, barbecuing or grilling, these compounds are formed from amino acids, creatinine and sugar[6]. The amount of HCA production depends mainly on cooking method, temperature and the type of meat, with amounts ranging in most studies from 1 to 80 ng/g meat for 2-amino-1-methyl-6-phenylimidazo[4,5b]pyridine (PhIP), the most abundant HCA in the human diet, followed by 2-amino-3,8-dimethyl-3H-imidazo[4,5f]quinoxaline (MeIQx), with usual amounts up to 6 ng/g meat and 2-amino-3,4,8-trimethyl-3H-imidazo[4,5f]quinoxaline (DiMeIQx), with usually up to 1 ng/g meat[7]. Recent epidemiological studies have shown associations between the estimated intake of HCA from diet and the risk of colorectal[1,8], breast[4] and prostate[9] cancer, although other studies did not observe positive associations[10,11].

We examined the distribution of HCA intake from meat in a German cohort of middle-aged men and women. Previous studies reported a wide range of the daily HCA intake from as low as 77 ng/d in a Swedish study[10] to more than 1 μg/d in a US study[12]. The intake of HCA in a pilot project has been described previously[13]. This new analysis was conducted in the entire European Prospective Investigation into Cancer and Nutrition (EPIC)-Heidelberg cohort.

Material and methods

EPIC is a prospective cohort study conducted in ten countries that started in the early 1990s[14]. In Heidelberg (Germany), 25 544 subjects, aged 35–65 years (women) and 40–65 years (men), were recruited for participation between 1994 and 1998. During recruitment, information on diet, lifestyle and health have been collected. All subjects are being contacted in approximately 2-year intervals to collect information on chronic disease status as well as diet and lifestyle[15]. During the second follow-up (2001–3), 25 049 participants have been contacted. Of those, 86 % completed a 158-item FFQ that assessed food consumption during that past 12 months, which had previously been used in the baseline assessment (1994–8) (for details, see Bohlscheid-Thomas *et al.*[16]). This FFQ included questions on meat consumption and preparation. Participants were asked how often they consumed sixteen types of meat (beef roast, including goulash, roulade; beef steak, filet or tenderloin; pork roast, including goulash; pork

Abbreviations: DiMeIQx, 2-amino-3,4,8-trimethyl-3H-imidazo[4,5f]quinoxaline; EPIC, European Prospective Investigation into Cancer and Nutrition; HCA, heterocyclic aromatic amine; MeIQx, 2-amino-3,8-dimethyl-3H-imidazo[4,5f]quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5b]pyridine.

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steak, schnitzel, cutlet, filet or tenderloin; hamburgers or meatballs; frying sausage; Wieners; bacon; liverloaf; fried chicken, turkey breast, turkey goulash) and which cooking methods they prefer for each type of meat (steaming or boiling, pan-frying, breading and frying, frying or broiling, grilling or barbecuing). Additionally, with the help of four pictures, subjects stated which degree of browning they favoured (lightly browned, moderately browned, strongly browned, extremely browned)[17]. If a subject indicated to vary between two or more cooking methods per food items these cooking methods were weighted equally. The same degree of browning was assumed for each cooking method used for a specific type of meat.

Total HCA concentration and concentration of the most abundant HCA PhIP, MeIQx and DiMeIQx were estimated using published data of their content in different types of meat[18–21]. HCA intake from steaming or boiling or from breading and frying was considered to be zero[7]. In addition to meat cooking, participants were asked about the use of meat drippings to prepare gravy. The intake of HCA from gravy was calculated by multiplying the amount of gravy with the HCA concentration in gravy of the corresponding meat item. This was automatically added to a specific meat item's HCA intake. By combining information on degree of browning, cooking method and the amount of meat intake, the mean daily dietary intake of HCA from meat was estimated. We also calculated HCA intake per MJ to take into account differences in energy intake that might contribute to differences in HCA intake. Because HCA intake was not normally distributed we computed medians and interquartile ranges of HCA intake and used the Wilcoxon test and Kruskal–Wallis test to compare the intake of different subgroups of our participants. All tests were two-sided; P values $<0{\cdot}05$ were considered to be statistically significant. EPIC-Heidelberg has been approved by the ethical committee of the Heidelberg University Medical School.

Results

The median intake of total HCA from meat was 30·6 ng/d (mean 69·4 ng/d), with PhIP contributing most to total HCA intake from meat (median 16·8 ng/d; mean 47·6 ng/d) (Table 1). Intake was highest from roast beef, followed by chicken or turkey, hamburgers or meatballs, and beef steak. Statistically significant differences in HCA intake were observed by sex, age, education, BMI and smoking status. Men had a higher HCA intake than women and smokers a higher intake than non-smokers (Table 2). Intake decreased with age and subjects with a higher educational level had a lower HCA intake than those with a lower educational level. Intake also differed by BMI, with more obese participants having a higher HCA intake from meat (Table 2). These differences were similar for HCA intake per MJ.

Table 1. Intake of total heterocyclic aromatic amines (HCA), 2-amino-1-methyl-6-phenylimidazo[4,5b]pyridine (PhIP), 2-amino-3,8-dimethyl-3H-imidazo[4,5f]quinoxaline (MeIQx) and 2-amino-3,4,8-trimethyl-3H-imidazo[4,5f]quinoxaline (DiMeIQx) as well as total HCA by meat type in European Prospective Investigation into Cancer and Nutrition (EPIC)-Heidelberg (*n* 21 462)

(Medians and interquartile ranges)

	Intake (ng/d)	
	Median	Interquartile range
Total HCA from meat	30·6	13·0–71·3
Intake of single HCA		
PhIP	16·8	6·2–43·2
MeIQx	9·5	3·9–21·2
DiMeIQx	1·7	0·6–3·9
Total HCA intake by meat type		
Beef roast, including goulash, roulade	4·8	0–17·5
Fried chicken, turkey breast, turkey goulash	3·6	0·5–12·9
Hamburgers, meatballs	1·3	0·4–3·8
Beef steak, filet or tenderloin	1·1	0–5·9
Pork steak, schnitzel, cutlet, filet or tenderloin	0·8	0–3·2
Pork roast, including goulash	0·2	0–1·7
Frying sausage	0	0–1·7
Bacon, pork belly	0	0
Wieners	0	0
Liverloaf	0	0

Discussion

This is the first large European cohort study that attempts to assess the intake of HCA from meat using a detailed questionnaire on food intake and food preparation methods. We have previously estimated the intake of HCA in a smaller group of EPIC-Heidelberg participants[13], which revealed a higher intake of total HCA (median 103 ng/d) compared with the present investigation (median 30·6 ng/d). In a Swedish study that used a similar approach to assess HCA intake, median total HCA intake was 77 ng/d, which also included HCA intake from fish[10]. Similar amounts were calculated in a large Japanese study[22] and slightly lower levels in a Singapore study (mean intake 49·95 ng/d)[23]. In US studies, estimated HCA intake is generally higher (mean PhIP intake 78·1 ng/d; mean MeIQx intake 21·9 ng/d) than in European studies[24]. This might be explained by larger portions of meat consumed in the USA than in Germany, but also by differences in cooking method preferences, for example, a preference for HCA-forming methods such as grilling in US cohorts and for non-HCA-forming methods such as boiling in Germany. Also, meat is usually consumed at a higher degree of browning in US cohorts[12] than in our cohort, leading to a higher intake of HCA. In addition, different consumption habits contribute to the observed differences. 'Roast beef, roulade and goulash' contributed most to the intake of total HCA in our cohort; however, the contribution of roast beef to HCA intake was negligible in three US cohorts[25]. The difference in HCA intake between the pilot study[13] and the present evaluation can, at least in part, be explained by some changes in questionnaire design. We added the possibility to mark the preparation method 'boiling' that does not contribute to HCA intake. Second, in contrast to the pilot study, we did not consider fish in the present study because HCA intake from fish varies widely depending on the type of fish and its preparation[20,26]. Third, HCA intake differed between subgroups mainly due to higher meat consumption, for example, in men, but also due to the preference of cooking methods or degrees of browning. Men tended to consume meat darker than

Table 2. Total heterocyclic aromatic amines (HCA) intake by sex, age, education, smoking status and body mass index in European Prospective Investigation into Cancer and Nutrition (EPIC)-Heidelberg (*n* 21 462)

(Medians and interquartile ranges)

		Total HCA intake from meat (ng/d)		Total HCA intake from meat (ng/d per MJ)	
	n	Median	Interquartile range	Median	Interquartile range
Sex					
Men	9864	40·7	17·6–92·3	4·7	2·1–10·3
Women	11 598	24·0	10·1–54·2	3·5	1·5–7·7
P		<0·0001*		<0·0001*	
Age group (years)†					
50–59	5770	37·4	14·9–87·9	4·8	2·0–11·1
60–69	7569	32·0	13·5–73·9	4·1	1·8–9·1
70 +	8123	26·2	11·5–57·9	3·5	1·6–7·3
P		<0·0001‡		<0·0001‡	
BMI (kg/m^2)					
< 18·5	195	14·0	3·2–38·8	2·0	0·6–5·3
18·5–24·9	9224	24·8	10·2–57·3	3·3	1·4–7·4
25·0–29·9	8691	34·2	15·1–77·8	4·4	2·0–9·3
30 +	3320	43·2	18·4–98·0	5·3	2·4–11·8
P		<0·0001‡		<0·0001‡	
Education					
Primary school or none	5933	35·1	15·4–81·1	4·6	2·0–10·0
Technical or professional school	7346	30·1	13·1–68·1	4·1	1·8–8·8
Secondary school	1546	28·1	10·5–67·1	3·8	1·5–8·7
University degree	6633	27·9	11·6–65·8	3·5	1·5–7·9
P		<0·0001‡		<0·0001‡	
Smoking status					
Never	8672	26·3	11·3–60·2	3·6	1·6–7·7
Former	8691	31·5	13·3–73·0	4·1	1·8–9·0
Current	4099	39·5	16·6–91·3	5·0	2·2–11·0
P		<0·0001‡		<0·0001‡	

* Wilcoxon test.
† Age at second follow-up (2001–3).
‡ Kruskal–Wallis test.

women and younger participants tended to prepare meat more often by frying or grilling than older participants (data not shown).

In conclusion, we estimated an HCA intake from meat that was lower than observed in previous studies in Europe or the USA. Statistically significant differences were seen for age, sex, BMI, education and smoking status. EPIC-Heidelberg offers the opportunity to examine the association of meat consumption and HCA intake from meat with cancer risk in a prospective way. Further, future studies can take into account potential confounders, especially genetic variation in metabolic pathways of HCA as well as secondary plant products such as phenolic acids that are well known to have an impact on HCA metabolism and cancer risk.

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References

1. Nowell S, Coles B, Sinha R, MacLeod S, Luke Ratnasinghe D, Stotts C, Kadlubar FF, Ambrosone CB & Lang NP (2002) Analysis of total meat intake and exposure to individual heterocyclic amines in a case-control study of colorectal cancer: contribution of metabolic variation to risk. *Mutat Res* **506–507**, 175–185.
2. Zheng W, Gustafson DR, Sinha R, Cerhan JR, Moore D, Hong CP, Anderson KE, Kushi LH, Sellers TA & Folsom AR (1998) Well-done meat intake and the risk of breast cancer. *J Natl Cancer Inst* **90**, 1724–1729.
3. Sinha R, Kulldorff M, Chow WH, Denobile J & Rothman N (2001) Dietary intake of heterocyclic amines, meat-derived mutagenic activity, and risk of colorectal adenomas. *Cancer Epidemiol Biomarkers Prev* **10**, 559–562.
4. Sinha R, Gustafson DR, Kulldorff M, Wen WQ, Cerhan JR & Zheng W (2000) 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, a carcinogen in high-temperature-cooked meat, and breast cancer risk. *J Natl Cancer Inst* **92**, 1352–1354.
5. Sinha R, Kulldorff M, Swanson CA, Curtin J, Brownson RC & Alavanja MC (2000) Dietary heterocyclic amines and the risk of lung cancer among Missouri women. *Cancer Res* **60**, 3753–3756.
6. Sugimura T (1997) Overview of carcinogenic heterocyclic amines. *Mutat Res* **376**, 211–219.
7. Skog KI, Johansson MA & Jagerstad MI (1998) Carcinogenic heterocyclic amines in model systems and cooked foods: a review on formation, occurrence and intake. *Food Chem Toxicol* **36**, 879–896.

8. Butler LM, Sinha R, Millikan RC, Martin CF, Newman B, Gammon MD, Ammerman AS & Sandler RS (2003) Heterocyclic amines, meat intake, and association with colon cancer in a population-based study. *Am J Epidemiol* **157**, 434–445.
9. Cross AJ, Peters U, Kirsh VA, Andriole GL, Reding D, Hayes RB & Sinha R (2005) A prospective study of meat and meat mutagens and prostate cancer risk. *Cancer Res* **65**, 11779–11784.
10. Augustsson K, Skog K, Jagerstad M, Dickman PW & Steineck G (1999) Dietary heterocyclic amines and cancer of the colon, rectum, bladder, and kidney: a population-based study. *Lancet* **353**, 703–707.
11. Delfino RJ, Sinha R, Smith C, *et al.* (2000) Breast cancer, heterocyclic aromatic amines from meat and *N*-acetyltransferase 2 genotype. *Carcinogenesis* **21**, 607–615.
12. Keating GA & Bogen KT (2004) Estimates of heterocyclic amine intake in the US population. *J Chromatogr B Analyt Technol Biomed Life Sci* **802**, 127–133.
13. Rohrmann S & Becker N (2001) Die Aufnahme heterozyklischer aromatischer Amine in Deutschland - Ergebnisse eine Pilotstudie aus EPIC Heidelberg (The intake of heterocyclic aromatic amines in Germany – results from an EPIC-Heidelberg pilot study). *Ernährungs-Umschau* **48**, 447–450.
14. Riboli E, Hunt KJ, Slimani N, *et al.* (2002) European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr* **5**, 1113–1124.
15. Bergmann MM, Bussas U & Boeing H (1999) Follow-up procedures in EPIC-Germany - data quality aspects. European Prospective Investigation into Cancer and Nutrition. *Ann Nutr Metab* **43**, 225–234.
16. Bohlscheid-Thomas S, Hoting I, Boeing H & Wahrendorf J (1997) 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* **26**, Suppl. 1, S59–S70.
17. Augustsson K, Skog K, Jagerstad M & Steineck G (1997) Assessment of the human exposure to heterocyclic amines. *Carcinogenesis* **18**, 1931–1935.
18. Sinha R, Rothman N, Salmon CP, Knize MG, Brown ED, Swanson CA, Rhodes D, Rossi S, Felton JS & Levander OA (1998) Heterocyclic amine content in beef cooked by different methods to varying degrees of doneness and gravy made from meat drippings. *Food Chem Toxicol* **36**, 279–287.
19. Sinha R, Rothman N, Brown ED, Salmon CP, Knize MG, Swanson CA, Rossi SC, Mark SD, Levander OA & Felton JS (1995) 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* **55**, 4516–4519.
20. Skog K, Augustsson K, Steineck G, Stenberg M & Jagerstad M (1997) Polar and non-polar heterocyclic amines in cooked fish and meat products and their corresponding pan residues. *Food Chem Toxicol* **35**, 555–565.
21. Skog K, Steineck G, Augustsson K & Jagerstad M (1995) Effect of cooking temperature on the formation of heterocyclic amines in fried meat products and pan residues. *Carcinogenesis* **16**, 861–867.
22. Kobayashi M, Hanaoka T, Nishioka S, Kataoka H & Tsugane S (2002) Estimation of dietary HCA intakes in a large-scale population-based prospective study in Japan. *Mutat Res* **506–507**, 233–241.
23. Wong KY, Su J, Knize MG, Koh WP & Seow A (2005) Dietary exposure to heterocyclic amines in a Chinese population. *Nutr Cancer* **52**, 147–155.
24. Cantwell M, Mittl B, Curtin J, Carroll R, Potischman N, Caporaso N & Sinha R (2004) Relative validity of a food frequency questionnaire with a meat-cooking and heterocyclic amine module. *Cancer Epidemiol Biomarkers Prev* **13**, 293–298.
25. Byrne C, Sinha R, Platz EA, Giovannucci E, Colditz GA, Hunter DJ, Speizer FE & Willett WC (1998) Predictors of dietary heterocyclic amine intake in three prospective cohorts. *Cancer Epidemiol Biomarkers Prev* **7**, 523–529.
26. Gross GA & Gruter A (1992) Quantitation of mutagenic/carcinogenic heterocyclic aromatic amines in food products. *J Chromatogr* **592**, 271–278.

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