

## 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<sup>1–5</sup>. 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<sup>6</sup>. 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<sup>7</sup>. Recent epidemiological studies have shown associations between the estimated intake of HCA from diet and the risk of colorectal<sup>1,8</sup>, breast<sup>4</sup> and prostate<sup>9</sup> cancer, although other studies did not observe positive associations<sup>10,11</sup>.

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<sup>10</sup> to more than 1 µg/d in a US study<sup>12</sup>. The intake of HCA in a pilot project has been described previously<sup>13</sup>. 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<sup>14</sup>. 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<sup>15</sup>. 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.*<sup>16</sup>). 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)<sup>17</sup>. 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<sup>18–21</sup>. HCA intake from steaming or boiling or from breading and frying was considered to be zero<sup>7</sup>. 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 <0.05 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.

## 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<sup>13</sup>, which revealed a

**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

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<sup>10</sup>. Similar amounts were calculated in a large Japanese study<sup>22</sup> and slightly lower levels in a Singapore study (mean intake 49.95 ng/d)<sup>23</sup>. 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<sup>24</sup>. 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<sup>12</sup> 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<sup>25</sup>. The difference in HCA intake between the pilot study<sup>13</sup> 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<sup>20,26</sup>. 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)

	<i>n</i>	Total HCA intake from meat (ng/d)		Total HCA intake from meat (ng/d per MJ)	
		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
<i>P</i>		<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
<i>P</i>		<0.0001‡		<0.0001‡	
BMI (kg/m <sup>2</sup> )					
< 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
<i>P</i>		<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
<i>P</i>		<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
<i>P</i>		<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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