Dietary Intake of Heterocyclic Amines and Cancers of the Esophagus and Gastric Cardia

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Abstract
The results of two epidemiological studies suggest that high intake of heterocyclic amines, which are formed on the surface of meats cooked at high temperatures, might be associated with increased risk of esophageal or cardia cancers. Our aim was to further investigate heterocyclic amine intake and risk of these cancers. We examined data from a nationwide, population-based, case-control study of risk factors for adenocarcinoma of the esophagus and gastric cardia and squamous cell carcinoma of the esophagus in Sweden, with 185, 258, and 165 cases, respectively, and 815 controls. Heterocyclic amine intake was estimated based on the frequency of consumption and degree of surface browning of commonly fried meats, and the consumption of pan juices. Statistically nonsignificant 50–70% higher risks of esophageal squamous cell carcinoma were observed among individuals in the highest quartile levels of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline, and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine relative to those in the lowest. Dose-risk trends were evident. Subjects reporting high intake of all three heterocyclic amines had an odds ratio of 2.4 (95% confidence interval, 1.2–4.8) relative to those with low intake of all three substances. In contrast, no association was found with risk of adenocarcinoma of the esophagus or gastric cardia. Heterocyclic amine intake might be associated with an increase in risk of squamous cell carcinoma of the esophagus. Given the dearth of epidemiological data regarding these cancers and the lack of established biological mechanisms, confirmatory data are needed.

Introduction
To date, no risk factor has offered a satisfactory explanation for the rapid increase in the incidence rates of esophageal adenocarcinoma observed in Western populations (1), and the quest for relevant factors continues. Heterocyclic amines are carcinogenic substances formed through pyrolysis of amino acids and creatine or creatinine when meats are cooked at high temperature, particularly by pan-frying (2). Heterocyclic amine values increase with cooking temperature, with the type and shape of the cooked piece of meat, with the degree of browning on the surface, and with the cooking method (3, 4). Although the relationship between heterocyclic amine intake and human cancer is unclear, there is ample evidence from in vitro and animal experiments that heterocyclic amines can damage DNA and cause tumors of the colon, breast, and prostate (5–7). In humans, some epidemiological studies (2), but not all (8), have suggested that heterocyclic amine intake is associated with risk of colon cancer. Because colon-like metaplasia (Barrett’s esophagus) precedes the development of esophageal adenocarcinomas (9), heterocyclic amines might also play a role in the etiology of this cancer.

The three most abundant heterocyclic amines found in cooked (especially pan-fried) meat are MeIQx, DiMeIQx, and PhIP (4, 5). These compounds have been judged by the IARC (5) to be probable human carcinogens. One population-based case-control study of esophageal and gastric cardia adenocarcinomas evaluated the doneness level of cooked meat as a surrogate of heterocyclic amine exposure (10). Compared with individuals who preferred beef cooked rare/medium rare, those who preferred medium or medium/well-done beef had a 2.5-fold higher risk of gastric adenocarcinoma, and those who preferred eating beef well done had a >3-fold higher risk. In that study, consumption of gravy made with pan juices from cooked meat, which can contain high levels of PhIP and MeIQx (11), was associated positively with risk of esophageal adenocarcinoma.

Intake of heterocyclic amines might also be positively associated with risk of squamous cell carcinoma of the esophagus. Cigarette smoking is a strong risk factor for esophageal squamous cell carcinoma (12), and heterocyclic amines have been shown to induce mutations identical to those induced by CSC (13). A hospital-based case-control study in Uruguay (14) found a positive association between heterocyclic amine intake and squamous cell carcinomas of the upper aerodigestive tract, about half of which were esophageal. Given the positive associations found in the two previous epidemiological studies (10, 14), we examined the association between heterocyclic amine

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3 The abbreviations used are: MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; MeIQ, 2-amino-3,4-dimethylimidazo[4,5-f]quinoline; ICV, cytochrome P450; NAT, N-acetyltransferase; PAH, polycyclic aromatic hydrocarbon; OR, odds ratio; CI, confidence interval; BMI, body mass index; CSC, cigarette smoke condensate.
intake and risk of adenocarcinomas of the esophagus and gastric cardia and esophageal squamous cell carcinoma.

Materials and Methods

Study Population and Design. We performed a nationwide, population-based, case-control study of causes of esophageal and cardia cancers in Sweden. The study base comprised all native Swedes below the age of 80 years, living in Sweden from 1995 through 1997. All new patients with adenocarcinoma of the esophagus or gastric cardia diagnosed in the study base and half of the patients with esophageal squamous cell carcinoma (born on even dates) were eligible as cases. We included 185 patients with esophageal adenocarcinoma (87% of all eligible), 258 patients (84%) with gastric cardia adenocarcinoma, 165 patients (73%) with esophageal squamous cell carcinoma, and 815 (73%) control subjects. The latter were selected randomly from age and gender strata in the study base to mimic the age and gender distribution among cases (frequency matching). Special study protocols from endoscopy, surgery, and histopathology served as a basis for classification of tumor site. Specimens from all tumors were reviewed by the study pathologist, Dr. Anders Lindgren. Criteria for cardia cancer were adenocarcinomas occurring within 2 cm proximal and 3 cm distal to the gastroesophageal junction, defined as the place of origin of the longitudinal gastric folds. However, if Barrett’s esophagus was histologically verified adjacent to the tumor, it was classified as esophageal. Nonparticipation among cases was attributed mainly to poor clinical condition or death shortly after diagnosis. Nonparticipation among controls was most often due to unwillingness.

Dietary Assessment. Using previously evaluated food frequency questions in computer-aided face-to-face interviews with all study subjects by trained personnel from Statistics Sweden, we asked about the consumption of 63 food items 20 years prior to interview (15). The food frequency questionnaire was constructed in collaboration with representatives of the Swedish National Food Administration, who maintain databases on dietary habits in Sweden. Questions were asked about the usual frequency of consumption of each item in terms of number of times per day, week, month, or year. Average daily intake for every food item was created for each subject by multiplying the frequency of consumption of specific food items by standard, item-specific portion sizes, based on National Food Administration handbook (16). Daily total energy and nutrient intakes were calculated using the Swedish food composition database (16). Energy adjustment of nutrients was performed using the residual methodology recommended by Willett and Stampfer (17). Quantification of Heterocyclic Amine Intake. Frying is the most common way of cooking meat in Sweden (3, 18). The amounts of heterocyclic amines (MeIQx, Di MeIQx, and PhIP) associated with the various fried meat surfaces were estimated through experimental cooking and subsequent laboratory analysis (3). For six of the most frequently fried meats in Sweden (bacon, minute-steak, sausage, pork chops, meatballs, and hamburgers), questions were asked about the usual number of the specified items consumed during an average meal as well as the frequency of consumption (as described above). Four pictures were used for each item to assess the usual degree of surface browning (each picture corresponding to meat fried at 150°C, 175°C, 200°C, and 225°C, respectively; Ref. 3). Questions were also asked about the usual frequency of consumption of barbecued meat and of using pan juices (after frying) in sauces and gravies. Based on an estimated 30 g of gravy per serving, we calculated additional heterocyclic amine intake using experimental values obtained for pan residues (3).

Statistical Analyses. Age- and gender-adjusted and multivariate-adjusted OR and 95% CIs were calculated from unconditional logistic regression models (19). In the full multivariate models, we included factors that were related to risk in our data as well those that are suspected risk factors based on the results of previous studies. Multivariate models included age (as a continuous variable), gender, total energy intake (as a continuous variable), BMI (in quartiles of kg/m²), gastro-esophageal reflux symptoms at least once per week (yes, no), cigarette smoking status (never, past, and current), education (years of formal education in three categories), alcohol consumption (in quartiles of grams per day), dietary fiber intake (quartiles), and measures of antioxidant intake (vitamin C, β-carotene, and α-tocopherol, in quartiles). For tests of trend in risk across successive levels of categorical variables, median values of each category were fitted in the risk models as successive integers (20).

Results

Baseline characteristics of the study subjects are shown in Table 1. The median age was 67 years for the combined cases and 68 years for the controls. The patients were comprised mostly of males (82%) than females. Subjects with esophageal adenocarcinoma had the highest median BMI and had a higher prevalence of symptomatic gastro-esophageal reflux than other subjects. Patients with esophageal squamous cell carcinoma were more likely than those with adenocarcinomas or controls to be smokers or drinkers. Intake of vitamin C, dietary fiber, and multivitamin supplements was highest in the control group, as was the percentage of individuals with post-secondary education. The Pearson correlation between MeIQx and DiMeIQx was 0.98; between MeIQx and PhIP, the correlation coefficient was 0.90, and between DiMeIQx and PhIP, it was 0.90. Intake of these heterocyclic amines was not strongly correlated with the consumption of alcohol or tobacco. Individuals who reported having high levels of education had somewhat lower intakes of heterocyclic amines than those with lower levels of education. Correlations between meat variables and intake of specific heterocyclic amines ranged from 0.08 to 0.27.

Dietary intake of heterocyclic amines was positively associated with risk of esophageal squamous cell carcinoma in both age- and sex-adjusted and multivariate-adjusted models (Table 2). A 50–70% increased risk was observed for individuals in the highest quartile compared with those in the lowest quartile of MeIQx, DiMeIQx, and PhIP intake; positive, monotonic associations were observed, but trend tests were statistically significant (P < 0.05) only for DiMeIQx. However, the high correlation among the individual heterocyclic amines makes it difficult to distinguish their independent effects. Individuals with the highest intake of all three heterocyclic amines, representing the group with the highest total exposure, were more than twice as likely to be diagnosed with esophageal squamous cell carcinoma as those with the lowest intakes. No association was observed between heterocyclic amine intake and esophageal adenocarcinoma or gastric cardia adenocarcinoma. The results were similar in models that adjusted for age and sex only (data not shown) and were essentially unaltered by statistical adjustment for total meat consumption. There was a suggestion of increased risk of esophageal squamous cell carcinoma among individuals who consumed pan-fried red meats (pan-fried red or processed meat, but not pan-fried pork)
or pan juices twice per week or more (data not shown); there was no association with total meat, red meat, white meat, or fish, when cooking method was not considered. If anything, consumption of barbecued meat was associated inversely with esophageal cancer risk. A formal test of interaction between intake of heterocyclic amines and smoking status in relation to esophageal squamous cell carcinoma risk was not statistically significant ($P = 0.51$).

**Discussion**

In this nationwide case-control study, there was a positive association between intake of heterocyclic amines and risk of esophageal squamous cell carcinoma. The findings were also suggestive of an increased risk with high intake of pan-fried red or processed meats, but the associations were less clear than those with the specific heterocyclic amines. In contrast, there was no association between intake of heterocyclic amines and risk of adenocarcinoma of the esophagus or the gastric cardia.

The strengths of our study include its population-based design. We identified practically all newly diagnosed cases of the three tumors in the study base and rigorously classified each according to histological type and tumor site. A random sample from the entire study base acting as control subjects reduced the possibility of selection problems existing in the fact that some potential cases did not survive long enough to participate in our study, and some potential controls declined to participate. However, given the relatively high participation rates in the present study, important differences in heterocyclic amine intake according to participation do not seem likely. Moreover, the number of cases in our study was limited, especially when examining associations between intake of heterocyclic amines and risk of the specific tumors by strata of other variables.

The findings of the present study are consistent with those of a hospital-based case-control study in Uruguay (14), which found positive association between squamous cell carcinoma of the upper aerodigestive tract and heterocyclic amine intake, especially PhIP. In that study, assessment of heterocyclic amine intake was based only on the frequency of consumption and usual doneness preference of cooked meat, increasing the likelihood of measurement error (4). Nonetheless, as with our study, the investigators observed stronger associations with the specific heterocyclic amines than with any of the meat variables (14, 21). Although the associations among the heterocyclic amines and the various squamous cell carcinomas were all positive, they were somewhat stronger for laryngeal tumors than for tumors of the oral cavity or esophagus. However, the number of cases was small ($n = 140$ upper aerodigestive tract cancers), especially when the specific tumor sites were examined separately.

In addition to MeIQx, DiMeIQx, and PhIP, other substances are formed on the surface of meat during the cooking process. Included among these are other heterocyclic amines, such as 2-amino-3-methylimidazo[4,5-f]quinoline and MelIQ. 2-Amino-3-methylimidazo[4,5-f]quinoline and MelIQ, which are considered a possible and a probable human carcinogen, respectively, by the IARC (5), are found positive association betweens squamous cell carcinoma of the esophagus (14), which was no association between intake of heterocyclic amines and smoking status in relation to esophageal squamous cell carcinoma risk, was limited, especially when examining associations between intake of heterocyclic amines and risk of the specific tumors by strata of other variables.

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**Table 1** Baseline characteristics of the study subjects

<table>
<thead>
<tr>
<th></th>
<th>Adenocarcinoma of esophagus</th>
<th>Adenocarcinoma of gastric cardia</th>
<th>Squamous cell carcinoma of esophagus</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>185</td>
<td>258</td>
<td>165</td>
<td>815</td>
</tr>
<tr>
<td>Medians</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Age (yrs)</td>
<td>69</td>
<td>66</td>
<td>67</td>
<td>68</td>
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<tr>
<td>BMI (kg/m$^2$)</td>
<td>25.4</td>
<td>24.7</td>
<td>23.6</td>
<td>23.7</td>
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<tr>
<td>Alcohol (g/day)</td>
<td>5.2</td>
<td>6.2</td>
<td>6.2</td>
<td>6.4</td>
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<tr>
<td>Total energy (kcal/day)</td>
<td>2315</td>
<td>2308</td>
<td>2293</td>
<td>2231</td>
</tr>
<tr>
<td>Total dietary fiber (g/day)</td>
<td>13.2</td>
<td>12.9</td>
<td>13.4</td>
<td>15.5</td>
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<tr>
<td>Vitamin C (mg/day)</td>
<td>47.8</td>
<td>49.1</td>
<td>47.3</td>
<td>51.3</td>
</tr>
<tr>
<td>α-Tocopherol (mg/day)</td>
<td>6.2</td>
<td>5.4</td>
<td>6.0</td>
<td>5.5</td>
</tr>
<tr>
<td>β-Carotene (mg/day)</td>
<td>1.3</td>
<td>1.5</td>
<td>1.3</td>
<td>1.8</td>
</tr>
<tr>
<td>No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>162 (87.6)</td>
<td>219 (84.9)</td>
<td>118 (71.5)</td>
<td>675 (82.8)</td>
</tr>
<tr>
<td>Reflux symptoms present</td>
<td>111 (60.0)</td>
<td>74 (28.7)</td>
<td>25 (15.2)</td>
<td>136 (16.7)</td>
</tr>
<tr>
<td>Smoking (2 years before interview)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>56 (30.1)</td>
<td>43 (16.7)</td>
<td>22 (13.3)</td>
<td>323 (39.6)</td>
</tr>
<tr>
<td>Former</td>
<td>86 (46.5)</td>
<td>124 (48.1)</td>
<td>44 (26.7)</td>
<td>313 (38.4)</td>
</tr>
<tr>
<td>Current</td>
<td>43 (23.2)</td>
<td>91 (35.3)</td>
<td>99 (60.0)</td>
<td>179 (22.0)</td>
</tr>
<tr>
<td>Supplement user for at least 3 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivitamins</td>
<td>27 (14.6)</td>
<td>40 (15.5)</td>
<td>29 (17.6)</td>
<td>155 (19.0)</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>3 (1.6)</td>
<td>6 (2.3)</td>
<td>2 (1.2)</td>
<td>11 (1.3)</td>
</tr>
<tr>
<td>Alpha-tocopherol</td>
<td>1 (0.5)</td>
<td>1 (0.4)</td>
<td>1 (0.6)</td>
<td>8 (1.0)</td>
</tr>
<tr>
<td>Post-secondary education</td>
<td>46 (24.9)</td>
<td>91 (35.3)</td>
<td>48 (29.1)</td>
<td>318 (39.0)</td>
</tr>
</tbody>
</table>
ally found on the surface of heavily burnt meat, has been shown to be mutagenic in the colon (22), and it may be associated positively with risk of squamous cell carcinomas of the esophagus (14). These substances, which were unmeasured in our study, may have confounded the associations with MeIQx, PhIP, and may have contributed to misclassification of total heterocyclic amine exposure.

The biological mechanisms that might underlie an association between intake of heterocyclic amines and risk of esophageal squamous cell carcinoma are unclear. Heterocyclic amines are activated to carcinogenic intermediates by N-oxidation, which is catalyzed principally by CYP1A2 in the liver and by CYP1A1 and CYP1B1 in extrapulmonary tissues (23). The latter two isozymes are expressed in the esophagus of healthy humans (24), in Barrett's esophagus (25), and in tumorous and non-tumorous esophageal mucosa of esophageal cancer patients (26–28). In humans, the extrapulmonary CYPs may be more important than previously thought in the activation of dietary heterocyclic amines (29–31). NAT2 and NAT1, enzymes that O-acetylate heterocyclic amines and facilitate DNA adduct formation (23), are also found in human esophageal tissue (32). Hence, the presence of these NATs might lead to further activation of heterocyclic amines that were N-hydroxylated at the esophagus. However, whether esophageal epithelial cells carry out any of the activation steps of heterocyclic amines to DNA-reactive species is unknown.

There may be reasons to suspect heterocyclic amine intake in the etiology of squamous cell carcinoma of the esophagus and not adenocarcinoma. For example, cigarette smoking is a strong risk factor for esophageal squamous cell carcinoma, whereas the association between smoking and esophageal adenocarcinoma is less clear (12). In *Salmonella*, CSC was shown to induce a frameshift mutation spectrum identical to that induced by the heterocyclic amine 2-amino-6-methyl(dipyrido[1,2-a:3’,2’,6’-d]imidazole (13). As noted previously (33), this finding is consistent with bioassay-directed fractionation studies showing that aromatic amines account for most of the frameshift specificity of CSC in *Salmonella* (34). In contrast, the base substitution mutation spectrum induced by CSC was similar to that induced by non-heterocyclic aromatic amines and benzo(a)pyrene (13), which is consistent with a role for the PAH component of cigarette smoke in tumorigenesis at target organ sites (33). PAHs (and not heterocyclic amines) have been associated with esophageal squamous cell carcinoma in rodent models (35). Nonetheless, it is important to view the latter findings in light of the uncertain relationship between esophageal tumorigenesis in rodents versus humans. Indeed, differences between the esophageal cells of rodents and humans have been observed in vitro with respect to their sensitivity to the toxic effects of carcinogens found in tobacco smoke, including PAHs and N-nitrosamines (36–38).

The results of the present study suggest that dietary intake of heterocyclic amines may increase the risk of esophageal squamous cell carcinoma, but not that of esophageal or gastric cardia adenocarcinoma. These results further suggest that the increase in the incidence rates of the latter tumors observed in Western populations is not due to dietary changes related to heterocyclic amines. The biological mechanisms underlying the heterocyclic amine-cancer pathway are unclear, although DNA-adduct formation appears to be important (2). Given the dearth of data regarding the role of heterocyclic amines in the etiology of squamous cell carcinoma of the esophagus, it is important to view the latter findings in light of the uncertain relationship between esophageal tumorigenesis in rodents versus humans. Indeed, differences between the esophageal cells of rodents and humans have been observed in vitro with respect to their sensitivity to the toxic effects of carcinogens found in tobacco smoke, including PAHs and N-nitrosamines (36–38).
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References

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