Dietary heterocyclic amines and cancer of the colon, rectum, bladder, and kidney: a population-based study

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Summary

Background Heterocyclic amines formed in cooked meat and fish are carcinogenic in animal models and form DNA adducts in human beings. We undertook a study to assess whether these substances are related to the risks of cancer in the large bowel and urinary tract.

Methods In a population-based case-control study, cases were identified from the Swedish cancer registry. Controls were randomly selected from the population register. Information on intake of various foods and nutrients was assessed by questionnaire, with photographs of foods cooked at various temperatures. We measured the content of heterocyclic amines in foods cooked under these conditions.

Findings Information was retrieved from 553 controls, 352 cases of colon cancer, 249 cases of rectal cancer, 273 cases of bladder cancer, and 138 cases of kidney cancer. The response rate was 80% for controls and 70% for cases. The estimated daily median intake of heterocyclic amines was 77 ng for controls, and 66 ng, 63 ng, 96 ng, and 84 ng for cases with cancer of the colon, rectum, bladder, and kidney, respectively. The relative risk for the intake of heterocyclic amines (highest *vs* lowest quintile) was 0·6 (95% CI 0·4–1·0) for colon cancer, 0·7 (0·4–1·1) for rectal cancer, 1·2 (0·7–2·1) for bladder cancer, and 1·0 (0·5–1·9) for kidney cancer. Seven cases, but no controls, had an estimated daily intake of heterocyclic amines above 1900 ng.

Interpretation Intake of heterocyclic amines, within the usual dietary range in this study population, is unlikely to increase the incidence of cancer in the colon, rectum, bladder, or kidney. For daily intakes above 1900 ng, our data are consistent with human carcinogenicity, but the precision was extremely low.

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Introduction

Two decades of laboratory research have highlighted a possible relation between dietary heterocyclic amines and cancer in human beings. Heterocyclic amines are formed by amino acids, creatine or creatinine, and sugar during cooking,[1–3] and were initially found in the charred parts of meat and fish.[4,5] Long-term animal studies show a multisite carcinogenic effect,[6] and DNA-adduct formation in human tissues has been documented.[7,8] A review by the International Agency for Research on Cancer suggests that 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) is a probable human carcinogen, and the following are possible human carcinogens: 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). Hence, it is reasonable to investigate, with epidemiological methods, whether heterocyclic amines are a cause of some diet-related cancers in human beings.[6]

In the western diet, fried meat is the main source of exposure to heterocyclic amines. The amount of meat eaten probably influences the risk of human cancer,[9–16] but the absence of information on cooking methods in available studies makes interpretation of the cancer risk associated with heterocyclic amines difficult. The challenge for epidemiological research in this area is to disentangle the effect of these compounds from the effect of meat in itself, or other potentially carcinogenic constituents in meat. We have developed a method, based on several preparatory studies,[17–20] to study the effect of intake of heterocyclic amines separately from the intake of meat and fish. The method was used in a population-based case-control study designed to investigate whether heterocyclic amines are related to the risk of cancer in the large bowel and urinary tract.

Methods

Source population and data collection

The source population was people born in Sweden between 1918 and 1942, who had a permanent address in Stockholm at least one month between Nov 1, 1992, and Dec 31, 1994. Information about the cases was retrieved from the population-based cancer registry in Stockholm. Notification to the registry is mandatory by law for attending physicians and pathologists. The following sites were included, according to the codes of the International Classification of Diseases, Injuries and Causes of Death (ICD) ninth revision:[21] colon (ICD 153), rectum (154), bladder (188), and kidney (189, except renal pelvis, ie, 189·1 and 189·3). Controls were randomly selected from a population register during the observation period and frequency matched according to age (5-year bands) and sex distribution among the patients with cancer of the colon. Information was collected by a postal questionnaire, and completed by a telephone interview.

Cooking procedures and analyses

In total, 22 dishes with corresponding pan residues were cooked and analysed—15 fried meat dishes, three roasted meat dishes, two fried fish dishes, fried eggs, and black pudding.

	Controls	Colon cancer	Rectal cancer	Bladder cancer	Kidney cancer
Derivation of study population					
Number of controls and cases identified	692	521	354	391	186
Number participating	553 (80%)	352 (68%)	249 (70%)	273 (70%)	138 (74%)
Reasons for non-participation (%)					
Died	1	9	8	6	13
Illness	3	6	9	9	4
Refused	12	9	7	8	6
Other reasons	4	5	3	4	2
Questionnaire lost	0	4	3	3	1
Characteristics of participants					
Men	283 (51%)	199 (57%)	154 (62%)	205 (75%)	74 (54%)
Women	270 (49%)	153 (43%)	95 (38%)	68 (25%)	64 (46%)
Mean (range) age (years)	67 (51–76)	67 (51–77)	67 (51–77)	68 (52–77)	66 (51–76)
Mean (SE) body-mass index (kg/m²)	24·6 (0·2)	24·9 (0·2)	24·9 (0·2)	24·9 (0·2)	25·6 (0·5)
Current smokers	135 (24%)	79 (22%)	55 (22%)	134 (49%)	37 (27%)
Median (IQR) daily intake (ng) of heterocyclic amines*	77 (164)	66 (133)	63 (122)	96 (185)	84 (152)
Mean (SE) food intake (g daily)					
Meat and fish	148 (3·9)	156 (5·0)	153 (5·5)	156 (5·1)	162 (9·4)
Fruit and vegetables	277 (8·3)	251 (8·4)	249 (11·2)	233 (11·0)	260 (15·0)
Dietary fibre	20 (0·3)	20 (0·4)	20 (0·5)	19 (0·4)	20 (0·7)
Saturated fat	35 (0·7)	39 (0·9)	38 (1·0)	40 (1·2)	37 (1·4)
Monosaturated fat	30 (0·6)	33 (0·8)	32 (0·8)	33 (0·9)	32 (1·2)
Polyunsaturated fat	12 (0·2)	13 (0·4)	12 (0·3)	13 (0·3)	12 (0·4)
Mean (SE) daily intake of nutrients					
Energy (kcal)†	2058 (27·2)	2206 (39·8)	2162 (42·6)	2225 (48·3)	2139 (61·1)
Fat (% of energy)	36 (1·0)	36 (1·2)	36 (1·5)	36 (1·4)	36 (2·0)
Carbohydrate (% of energy)	44 (1·1)	44 (1·3)	44 (1·6)	43 (1·5)	44 (2·1)
Protein (% of energy)	16 (0·6)	16 (0·8)	16 (0·9)	16 (0·8)	16 (1·1)
Alcohol (% of energy)	4 (0·2)	4 (0·2)	4 (0·2)	5 (0·3)	4 (0·3)

Data are mean (SE), number (%) of individuals, or median (IQR).

*Sum of all five heterocyclic amines studied. Median values with interquartile range. †MJ=kcal×0·0042.

Table 1: **Characteristics of study participants and reasons for non-participation**

Dishes were fried in a standard way,[19,20] with equipment previously described by Övervik and colleagues,[22] at 150°C, 175°C, 200°C, and 225°C, and roasting was done in a normal household oven at 150°C and 200°C. We have reported elsewhere the chemical analyses and the obtained concentrations of IQ, MeIQ, MeIQx, DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline, and PhIP in the cooked food, as well as findings on quality assurance such as: accuracy, recovery, detection level, linearity, and variation between duplicate measurements with this method.[19,20] Mutagenic activity was assayed by use of the Ames' test on *Salmonella typhimurium* strain TA98.[17,23]

Dietary assessment method

The dietary assessment method has been described in detail before.[18] In short, diet was assessed by an extensive semiquantitative food-frequency questionnaire including 188 food items (27 fried meat dishes, 16 roasted meat dishes, 11 boiled meat dishes, four grilled meat dishes, four gravies and sauces, seven fish dishes, fried eggs, omelettes, and black pudding). The inclusion of dishes was based on previous population-based studies on food intake in an elderly population in Stockholm.[17] All questions referred to eating habits 5 years previously.

Data used to assess the intake of heterocyclic amines were the type of meat or fish ingested, frequency of consumption, portion size, cooking methods, degree of surface browning, and concentration of heterocyclic amines.[18] Colour photographs showed six dishes, each fried at four different temperatures, giving varying degrees of surface browning. Each photograph corresponds to known frying temperature and amount of heterocyclic amines. The classification of surface browning for all other fried dishes was also linked to these photographs, but was not considered for roasted items. The intake of heterocyclic amines from pan residue and gravy was calculated by use of a method that gave the concentration in one individually assessed standard serving.[18]

Databases and statistical analysis

Information from the questionnaire on food consumption was linked to our database with information on concentrations of heterocyclic amines in cooked food and gravy,[19,20] and to the National Food Administration's food-composition database. 45 (3%) people were excluded from the analysis owing to missing responses for more than 10% of the food items. If less than 10% of the information about intake frequencies was missing, we used a median value based on all the people in the study.

Odds ratio was modelled with logistic regression, to estimate the incidence rate ratio (relative risk) between exposed and unexposed individuals in the source population. The main exposure variables of interest (heterocyclic amines, meat, and fish) were categorised into quintiles, based on the distribution among controls. Quintile 1, the group with the lowest intake, was used as the reference category. Over 75% of both cases and controls had zero intake of MeIQ, so we could not group the intake into quintiles. The comparison used was the group with an intake in the highest quintile and those with zero intake. The logistic-regression model included age (as a continuous variable) and sex; for bladder and kidney cancer smoking status (categorised as current, former, or never) was also included. In addition, we formed a multivariate nutrient density model,[24] in which the main exposure variable of interest was divided by the total energy intake to obtain the nutrient density before categorisation into quintiles. The natural logarithm of total energy intake was also included in the multivariate nutrient-density models. The models were fitted by means of the LOGISTIC and GENMOD procedures of SAS statistical software (version 6.12). Model goodness-of-fit was assessed by means of the Hosmer-Lemeshow goodness-of-fit test.

Results

Table 1 shows the number of participants and descriptive statistics. The estimated daily median intake of the five heterocyclic amines included in the study was less than 100 ng for both controls and cases. The highest estimated daily intake among the controls was 1816 ng. Seven cases reported a higher intake than this, of which four were colon cancer cases (daily intakes 2251 ng, 2482 ng, 5247 ng, and 5659 mg), two were bladder cancer cases (1905 ng and 3357 ng), and one a kidney cancer case (6761 ng).

Cancer site	Relative risk (95% CI)*			
	Quintile 2	Quintile 3	Quintile 4	Quintile 5
Colon	1·1 (0·7–1·7)	0·8 (0·5–1·3)	0·7 (0·5–1·1)	0·6 (0·4–1·0)
Rectum	1·3 (0·8–2·0)	0·8 (0·5–1·4)	0·7 (0·4–1·1)	0·7 (0·4–1·1)
Bladder	1·5 (0·9–2·6)	1·5 (0·8–2·6)	1·4 (0·8–2·4)	1·2 (0·7–2·1)
Kidney	1·3 (0·7–2·3)	1·2 (0·6–2·2)	1·1 (0·7–2·2)	1·0 (0·5–1·9)

*Adjusted for age, sex, and energy (and smoking status for bladder and kidney cancer); quintile 1=reference category.

Table 2: **Relative risk of cancer by quintile of total heterocyclic amine intake**

For colon and rectal cancer, the risks were lower for the highest estimated intake category than for the lowest intake category of heterocyclic amines (table 2). The relative risks for proximal and distal colon analysed separately were 0·7 and 0·6, respectively. Slightly increased risks of bladder cancer were found in all intake categories compared with the lowest, whereas the relative risks of kidney cancer were typically close to unity. Accounting of energy by the multivariant nutrient-density models or adjustment for other potential confounding factors (age, sex, smoking, fat, protein, dietary fibre, vegetables, fruits, physical activity) had little or no effect on the relative risks for the estimated intake of heterocyclic amines for all of the cancer sites. With the effect of heterocyclic amines measured as mutagenic activity, the relative risks were similar to those for the total estimated intake of the heterocyclic amines.

The relative risks for each of the heterocyclic amines separately were similar (table 3). For colon and rectal cancer, decreased risks were found when the highest estimated intake category was compared with the lowest category for MeIQx, DiMeIQx, and PhIP. IQ gave risks close to unity for all quintiles and MeIQ was associated with increased risks. Small increased risks of bladder cancer were found in almost all exposure categories of MeIQx, DiMeIQx, and PhIP, but not for IQ and MeIQ. Relative risks of kidney cancer were close to unity for different intakes of all five heterocyclic amines studied.

Cancer site	Relative risk (95% CI)*			
	Quintile 2	Quintile 3	Quintile 4	Quintile 5
Colon				
IQ	1·1 (0·7–1·7)	0·8 (0·5–1·3)	1·4 (0·9–2·1)	1·1 (0·7–1·6)
MeIQ	..	..	..	1·3 (0·9–1·8)
MeIQx	1·1 (0·7–1·6)	0·9 (0·6–1·3)	0·7 (0·4–1·1)	0·6 (0·4–1·0)
DiMeIQx	1·0 (0·7–1·5)	0·8 (0·5–1·2)	0·8 (0·5–1·2)	0·6 (0·4–0·9)
PhIP	1·0 (0·6–1·5)	0·7 (0·4–1·1)	0·8 (0·5–1·3)	0·6 (0·4–0·9)
Rectum				
IQ	0·9 (0·5–1·4)	0·8 (0·5–1·3)	1·4 (0·9–2·2)	0·8 (0·5–1·3)
MeIQ	..	..	..	1·5 (1·0–2·1)
MeIQx	1·3 (0·8–2·0)	0·9 (0·6–1·5)	0·6 (0·4–1·0)	0·7 (0·4–1·2)
DiMeIQx	1·2 (0·7–1·9)	1·0 (0·6–1·6)	0·7 (0·4–1·1)	0·6 (0·4–1·1)
PhIP	1·5 (0·9–2·4)	0·8 (0·5–1·3)	1·0 (0·6–1·6)	0·6 (0·4–1·1)
Bladder				
IQ	0·9 (0·5–1·5)	1·1 (0·7–1·9)	1·0 (0·6–1·8)	1·1 (0·7–1·9)
MeIQ	..	..	..	1·0 (0·7–1·5)
MeIQx	1·4 (0·8–2·4)	1·4 (0·8–2·5)	1·1 (0·6–1·9)	1·1 (0·6–1·9)
DiMeIQx	1·2 (0·7–2·0)	1·0 (0·6–1·8)	1·5 (0·9–2·6)	1·0 (0·6–1·7)
PhIP	1·5 (0·9–2·7)	1·2 (0·7–2·1)	1·4 (0·8–2·5)	1·2 (0·7–2·1)
Kidney				
IQ	1·0 (0·5–1·8)	0·6 (0·3–1·2)	1·3 (0·7–2·3)	0·9 (0·5–1·6)
MeIQ	..	..	..	1·1 (0·7–1·8)
MeIQx	1·3 (0·7–2·5)	1·7 (0·9–3·2)	1·0 (0·5–2·0)	0·9 (0·5–1·9)
DiMeIQx	1·1 (0·6–2·0)	1·1 (0·6–2·0)	1·0 (0·5–2·0)	1·1 (0·6–2·0)
PhIP	1·1 (0·6–2·1)	1·0 (0·6–1·9)	1·1 (0·6–2·0)	0·9 (0·5–1·7)

*Adjusted for age, sex, and energy (and smoking status for bladder and kidney cancer); quintile 1=reference category, except for MeIQ, for which quintiles 1–4=reference.

Table 3: **Relative risk of cancer by quintile of estimated intake of the five heterocyclic amines studied**

Cancer site	Relative risk (95% CI)*			
	Quintile 2	Quintile 3	Quintile 4	Quintile 5
Colon	1·4 (0·9–2·2)	1·4 (0·9–2·1)	1·7 (1·1–2·6)	0·9 (0·5–1·4)
Rectum	1·4 (0·9–2·3)	1·4 (0·8–2·3)	1·4 (0·9–2·4)	1·0 (0·6–1·6)
Bladder	1·6 (1·0–2·8)	1·7 (1·0–2·9)	1·9 (1·1–3·2)	0·8 (0·5–1·5)
Kidney	0·9 (0·5–1·7)	1·3 (0·7–2·4)	1·4 (0·7–2·5)	1·0 (0·5–1·9)

*Adjusted for age, sex, and energy (and smoking status for bladder and kidney cancer); quintile 1=reference category.

Table 4: **Relative risk of cancer by quintile of total meat and fish intake**

Intake of meat and fish together, irrespective of the cooking method, was associated with an increased risk of colon and bladder cancer (table 4). The increased relative risks, however, were mostly seen in quintiles 2–4, and not in quintile 5.

We found that the total energy intake was associated with an increased risk of colon cancer, whereas intakes of fat, carbohydrate, protein, and dietary fibre were not related to risk of cancer of the colon, rectum, bladder, or kidney after adjustment for energy intake. Current smoking was strongly associated with cancer of the bladder (relative risk 5·0 [95% CI 3·3–7·6]) and less strongly with kidney cancer (1·3 [0·8–2·1]), and not at all with colon (1·0 [0·7–1·4]) or rectal cancer (0·9 [0·6–1·4]).

Discussion

This population-based case-control study, designed to study the cancer risk associated with heterocyclic amines, showed that heterocyclic amines in the doses typically ingested by the Stockholm population are not a major cause of the cancers we investigated. In comparisons of the group with an estimated intakes of heterocyclic amines in the highest quintile with the group that had estimated intakes in the lowest quintile, only slightly increased risks were found for bladder cancer, no difference in risk for kidney cancer, and a slightly decreased risk for colorectal cancer. Our findings are consistent with an increased cancer risk when the intake of heterocyclic amines is high (above 1900 ng daily) but because few people had such high exposures the precision is extremely low at these intakes.

There have been suggestions that the previously reported positive association between meat intake and risk of colorectal cancer[10,11,13–16] may be explained by intake of heterocyclic amines. In this study we were able in some part, if not entirely, to disentangle the effect of meat and fish from the effect of heterocyclic amines. The observed decreased relative risk of colon cancer with increasing intake of heterocyclic amines was not explained by the intake of meat and fish, which primarily increased the risk. The intake of heterocyclic amines depends on, in addition to the intake of meat and fish, the type of meat consumed, proportion size, cooking methods, cooking temperature, the extent to which pan residue and gravy is ingested, and the concentration of heterocyclic amines in dishes cooked in the normal way, in households and restaurants. Our findings suggest that meat and fish may contain carcinogenic substances other than heterocyclic amines, that are of importance for human carcinogenicity. Candidates include polycyclic aromatic hydrocarbons, nitrosamines, and less polar heterocyclic amines.

A possible limitation of this study is bias from errors in the estimated intake of heterocyclic amines. Our method for assessing heterocyclic amines is based on the experience of three previous case-control investigations[9,12,14] and four preparatory studies.[17–20] Among controls, the mean daily intake of meat, fish, and major macronutrients, as well as the proportion of energy from fat, protein, carbohydrates, and alcohol, was in agreement with other reports based on Swedish eating habits.[25] The exposure measurements were used to rank individuals according to their estimated intake of heterocyclic amines at the appropriate time before the observation period. An error in the estimated absolute intake may, or may not, misclassify a person in this regard. In a separate analysis, we found that when the intake of heterocyclic amines was estimated from 15 meat and fish dishes in the questionnaire instead of 39, only a small degree of misclassification was introduced, and its effect on stimulated relative risks was small. For classification of an intake not associated with an increased risk, errors in assessment of absolute intake would, however, always give erroneous threshold boundaries. Measurement of DNA adducts, excretion of heterocyclic amines in urine, or the collection of double portions of food for chemical analysis, provide additional methodological difficulties, but these methods are candidates for validation studies. Adjustments for potential confounding factors had no major effect on the relative risks, and the nutrient-density model did not change the findings.

The dietary assessment of heterocyclic amines was based on data from chemical analyses of 152 samples of fried and roasted dishes with corresponding gravies. All dishes were cooked according to Swedish recipes so as to resemble the food cooked in households and restaurants. Our quality-assurance variables for the chemical analyses of heterocyclic amines were generally good.[19,20] Furthermore, our laboratory is participating in a collaborative study to calibrate the measurements of heterocyclic amines (EU MAT I-CT 930042, 1994–1997).

The main contributors to the surface browning during frying and roasting are the so-called non-enzymatic browning reactions (Maillard reactions).[26] These reactions take place mainly between reducing sugars and amino groups and need not always correlate with the concentration of heterocyclic amines, although an increase in the concentration of heterocyclic amines with increasing cooking temperatures and time[19,20] is typically observed, especially on whole meat. The relation between cooking conditions (temperature and time), degree of browning, and concentration of heterocyclic amines judged from coloured photos of six typical dishes has limitations. A previous Swedish study found that the colour of meat, measured as the absorbance, was correlated with the frying temperature and amount of mutagenic activity in pure meat when the frying duration was fixed.[27]

Heterocyclic amines have been shown to be carcinogenic in animals.[6] This carcinogenic effect is induced by high doses, such as 10–400 mg/kg bodyweight. The lack of a carcinogenic effect of heterocyclic amines in our study may be due to the much lower intake in the study population (median 1 ng/kg bodyweight). However the highest noted estimated intakes of heterocyclic amines (>1900 ng) were found among people with cancer, and these individuals were not the same as those reporting the highest intakes of meat and fish.

Assessment of human risks from animal data involves extrapolation from high to low doses. The impact of low exposure is unclear, and both a threshold dose and a linear dose-response relation have been proposed.[28] The metabolism of heterocyclic amines can vary between human beings and other species,[29] and the effect of heterocyclic amines may be strongly modified by genetic factors, such as acetylation status,[30] that vary between different populations.

Contributors

Katarina Augustsson drafted the paper and was responsible for the data collection, design of the questionnaire, and the cooking sessions. Kerstin Skog and Marharetha Jägerstad did the chemical analyses for heterocyclic amines. Paul Dickman did the statistical analyses. Gunnar Steineck was the principal investigator and designed the study. All investigators contributed to the writing of the paper.

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