Predictors of Dietary Heterocyclic Amine Intake in Three Prospective Cohorts[1]

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Abstract

Cooking meat creates heterocyclic amines (HCAs) through pyrolysis of amino acids and creatinine. Although recognized as mutagenic, the etiological role of HCA in human cancer is unclear, due to the lack of information on the effect of typical food cooking methods on HCA concentrations and on variation in HCA exposure in populations. We estimated overall daily dietary HCA intake and variation in intake between individuals, using recent data on HCA concentrations in various meats prepared by cooking methods, temperatures, and times common in United States in the 1990s. Random samples of 250 participants from each of three large prospective cohorts were mailed a questionnaire to assess frequency of consumption, cooking method, and typical outside appearance of pan-fried, broiled, and grilled or barbecued chicken, fish, hamburger, and steak; fried, microwaved, and broiled bacon; fried sausage; roast beef; and homemade gravy. The 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), and 2-amino-3,4,8-trimethylimidazo[4,5,*f*]quinoxaline (DiMeIQx) concentrations, measured in composite samples by solid-phase extraction and high-performance liquid chromatography, were assigned to each food, cooking method, and doneness level. The dietary reports showed ~30-fold relative variation in 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline intake, 20-fold for 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, and over 110-fold for 2-amino-3,4,8-trimethylimidazo[4,5,*f*]quinoxaline, when the 10th and 90th percentiles of HCA intake were compared (90th/10th percentile value). These reported variations in HCA exposure among participants in these three large cohorts indicates that estimation of HCA intake and determination of association with disease risk are feasible, if additional information on meat cooking methods is obtained.

Introduction

Several case-control and cohort studies have reported positive associations between higher consumption levels of total red meat (1, 2) and well-done, browned, or BBQ[3] meat (3–5) and risk of colon cancer. One mechanism by which consumption of meat, particularly well-cooked meat, may be associated with increased cancer risk is through exposure to mutagenic HCAs, which are created during cooking (5–9). HCAs are formed by pyrolysis of amino acids and creatinine; formation of these mutagens increases with higher surface temperature and longer duration of cooking that produce heavily browned meat surfaces (8, 10). In human diets, PhIP is the most abundant HCA, followed by MeIQx and DiMeIQx (11, 12).

Feeding experiments in rats, mice and monkeys have shown that high levels of HCAs induce tumors of the liver, lung, forestomach, small intestine, colon, and mammary gland, as well as leukemias and lymphomas (7, 11, 13–16). However, there is evidence of variation in the ability to metabolize different HCA between species, which raises questions about the extrapolation of these findings to humans (11). Furthermore, in most of the animal studies that quantified the concentrations of HCAs formed while meat is cooked, the meat was cooked at high temperatures in a laboratory setting. Most individuals in the United States are unlikely to consume high quantities of meats cooked in this manner in their usual diet. However, it is not known to what extent HCA exposures typically found in the United States diet may contribute to the etiology of human cancers.

Layton *et al.* (6) published estimates of the average daily intake of HCAs in the United States diet, based on a United States Department of Agriculture food consumption survey that used 3-day diet records from 3563 individuals (Continuing Survey of Food Intakes of Individuals) in 1989. These authors compiled a database of HCA concentrations measured in cooked foods from numerous sources and used the average of selected values for the HCA concentration for each food and cooking method combination in their analyses (6). Using these average HCA concentrations for each type of meat, the variation in HCA intake between individuals in this analysis re-

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[3] The abbreviations used are: BBQ, barbecued; HCA, heterocyclic amine; 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-trimethylimidazo-[4,5,*f*]quinoxaline; NHS, Nurses' Health Study; HPFS, Health Professionals Follow-up Study.

flected only the variation in average daily intake based on the 3-day dietary records, not the variation in cooking practices. For a 54-kg individual, the reported average intake of total PhIP was 899 ng/day, total MeIQx was 141 ng/day, and total DiMeIQx was 44 ng/day (6). Combining the daily HCA intake with indicators of carcinogenic potencies, Layton *et al.* (6) estimated that ~28,000 cancers per year in the United States could be a result of dietary exposure to HCA (6). Recognizing the limitations of their analyses, the authors indicated the need for studies to characterize the specific types of foods, the cooking methods used, and the degree of doneness, reflecting both duration of cooking and temperature. A Swedish study analyzed roasted and fried meats for HCA and estimated daily intake of MeIQx, DiMeIQx, and PhIP from responses to a mailed food frequency questionnaire from 544 elderly residents of Stockholm (12). In this Swedish population, HCA intake from meat gravy made from pan residue after frying meat contributed ~30% of total HCA intake (12).

Little is known regarding the variation in HCA intake between individuals in the United States. Variation in HCA intake reflects differences in the types of foods consumed, the quantity consumed, the method of cooking, and the degree of doneness (8, 12, 17, 18). We estimated overall daily HCA intake from the diet and variation in intake between individuals, using recent data on HCA concentrations in a variety of meats prepared with cooking methods, temperatures, and times common in the United States in the 1990s (19–23). In addition to providing descriptive data for HCA intake among participants in three United States cohort studies, which vary in age, sex, and geographic distribution, we sought to identify a minimum set of questions about meat cooking methods and degree of doneness that explains a large degree of the variation in HCA intake between participants in each of these cohorts.

Materials and Methods

Between October 1995 and December 1995, we mailed our pilot questionnaire on food cooking preparation to a total of 750 participants from three large United States prospective cohort studies (250 from each cohort).

Study Subjects. The NHS was established in 1976, when 121,701 married female registered nurses born between 1921 and 1945 and living in 11 states returned the mailed self-administered questionnaire about their past and current health status. Every 2 years since then, cohort members have received a follow-up questionnaire with questions about disease outcomes and health-related topics (smoking, hormone use, menopausal status, and so on). The 1980, 1984, 1986, 1990, and 1994 questionnaires also included a food frequency assessment. In the NHS, participants eligible for this study included those who never reported a prior history or diagnosis of cancer (except nonmelanoma skin cancer) on any of their biennial questionnaires (1976–1992), returned the baseline dietary questionnaire in 1980, and returned the 1994 questionnaire. From these 45,375 women, 250 were randomly selected to receive the questionnaire mailing.

The NHS II was established in 1989 and modeled on the experience and procedures developed for the NHS. The 116,680 registered female nurses born between 1946 and 1964, who responded to the baseline questionnaire and reported no prior history of cancer, defined the cohort. In 1991 and 1995, food frequency questions were included as part of the follow-up questionnaires. Participants who were eligible for this study never reported a prior history or diagnosis of cancer (except nonmelanoma skin cancer) on any of their biennial questionnaires (1989–1993), returned the baseline dietary questionnaire in 1991, and had returned the 1995 questionnaire by September 15, 1995. From these 61,932 women, 250 were randomly selected to receive the questionnaire mailing.

The HPFS, which began in 1986, is a prospective cohort study of men modeled on the experience and procedures developed in the NHS. This cohort consists of 51,529 male health professionals (dentists, pharmacists, osteopaths, optometrists, podiatrists, and veterinarians) who were born between 1911 and 1946. In this cohort, food frequency questionnaires were collected in 1986, 1990, and 1994. In the HPFS cohort, 250 eligible subjects were randomly selected from the 34,757 participants who had returned the 1994 questionnaire with dietary information and who had no reported history of cancer (except nonmelanoma skin cancer).

HCA Assessment. A four-page questionnaire with instructional cover letter and a postage-paid return envelope was mailed to the 250 randomly selected participants from each cohort. A second mailing was sent to nonrespondents. The questionnaire focused on specific cooking methods for eight food items for which measured levels of HCA were available in the database developed at the United States National Cancer Institute (20–23). Participants reported the frequency (ranging from never to 4+/week) of eating 4–6 oz of chicken, 3–5 oz fish, 1 patty of hamburger, 4–6 oz of steak, 4–6 oz of roast beef, 2–3 oz of homemade beef gravy, 2 slices of bacon, and 2 links or patties of sausage. Portion sizes were chosen to reflect typical quantities of consumption of cooked foods. Participants were also instructed to indicate the method by which each type of meat, chicken, and fish that they eat was usually cooked. Questions included the frequency of consumption and, to assess the degree of doneness, the description of the outside appearance for pan-fried, broiled, and grilled or BBQ chicken, fish, hamburger, and steak; fried, microwaved, and broiled bacon; fried sausage; roast beef; and homemade gravy. For each method of cooking chicken and fish, participants were also asked if it was usually cooked with the skin on and if they usually eat the skin.

The methods used to prepare the food and to measure the HCA content to create the database have been described in detail (20–23). In brief, multiple portions of each meat type, including chicken, fish, hamburger, steak, roast beef, homemade beef gravy, bacon, and sausage, were cooked under controlled and measured conditions. All pieces of the same type of food that had been cooked by the same method and to the same degree of doneness (based primarily on internal temperature and secondarily by appearance of surface browning) were ground together to form a composite sample. The levels of MeIQx, DiMeIQx, and PhIP were measured in each composite sample by solid-phase extraction and high-performance liquid chromatography, and the UV absorbance spectrum of each chromatograhic peak was compared with library spectra acquired from standard solutions for confirmation, following the methods of Gross and Gruter (24). Laboratory investigators were blinded to the cooking method and degree of doneness of each sample, as well as quality control samples that were interspersed throughout the investigation for a measure of reproducibility. In the quality control samples measured in the development of the database, the coefficients of variation for the measures from hamburger were 0.36 (with an average concentration of 7.2 ng/g in cooked hamburger) for MeIQx, 0.40 (1.7 ng/g) for DiMeIQx, and 0.24 (10.9 ng/g) for PhIP (20). The relative concentration of each compound in a given food item varies both with the degree of doneness and with the

Table 1 Characteristics of responders to the study questionnaire assessing HCA intake in each cohort

Factor	NHS (*n* = 226)	NHS II (*n* = 231)	HPFS (*n* = 216)
Mean age in June 1995 (yr)	62.1	40.9	62.5
Race[a] (% nonwhite)	3	3	1
Marital status[a]			
Married (%)	79	76	92
Divorced/separated (%)	5	7	6
Widowed (%)	10	1	3
Never married (%)		13	
Region of residency[a]			
Northeast (%)	47	31	23
Midwest (%)	21	33	26
South (%)	12	11	21
West (%)	20	26	30
Use of multivitamins[a] (%)	42	42	48
Smoking status[a]			
Never (%)	49	68	39
Past (%)	36	21	44
Current (%)	15	11	10
Work/employment status[a]			
Employed full or part-time (%)	59	87	71
Retired (%)	16		28
Homemaker (%)	20	9	
Body mass index[a] (mean kg/m^2)	25.7	25.3	26.0
Percentage calories from fat[b] (mean)	30.8	31.8	30.7

[a] Mean value or distribution based on most recent available response in each cohort. For example, the body mass index values are based on the 1992 questionnaire for NHS participants, the 1993 questionnaire for NHS II participants, and the 1994 questionnaire for HPFS. Percentages may not add to 100%, due to rounding and/or missing responses.
[b] Nutrient values were based on the most recent available food frequency data from 1990 for NHS, from 1991 for NHS II, and from 1990 for HPFS.

cooking method. For example, the MeIQx levels were greater than the PhIP levels for very well-done pan-fried hamburger, but the PhIP levels were greater than the MeIQx levels for very well-done grilled or BBQ hamburger (22).

Statistical Methods. The demographic characteristics of the respondents from each cohort were compared. For each cohort, information from the most recent available biennial questionnaires that asked about the demographic factor of interest was used. To determine the HCA intake for each participant in this study, the reported frequency of consumption in servings per day was multiplied by weight of the serving (in g) and the database value of MeIQx, DiMeIQx, and PhIP per g of the specific food for the given cooking method and degree of doneness reported by the participant. The values for each specific food were then summed over all of the cooking methods for that food to determine the overall MeIQx, DiMeIQx, and PhIP intake from each food. Next, the food-specific intakes were summed across all foods to calculate the overall MeIQx, DiMeIQx, and PhIP intakes. Stepwise linear regressions were conducted to ascertain the food and cooking method combinations that explained the variation in the estimated HCA intake separately for each cohort. All food and cooking method combinations were allowed to enter the models. Thus, the percentage of the variance in the modeled HCA explained by a food-cooking method combination depends on the other food-cooking method combinations already in the model. Although the results of the stepwise linear regressions differed some what depending on whether the variables were transformed on a natural log scale, the results are presented in the original scale for clarity.

Table 2 Mean (SD) reported daily food consumption (g/day) in each cohort

Food (g in one serving)	NHS	NHS II	HPFS
Chicken (141.75)	21.2 (21.4)	22.8 (20.7)	26.8 (25.8)
Fish (113.4)	8.1 (9.7)	6.3 (9.6)	14.6 (14.4)
Hamburger (113.4)	10.8 (10.5)	13.8 (14.0)	15.6 (15.5)
Steak (141.75)	7.6 (7.7)	7.9 (8.8)	11.6 (13.0)
Roast (141.75)	4.8 (5.0)	4.0 (4.7)	5.4 (7.6)
Beef gravy (75)	1.7 (2.7)	1.1 (1.9)	1.3 (2.8)
Bacon (13)	0.6 (1.2)	0.5 (0.8)	0.9 (1.6)
Sausage (26)	0.4 (0.6)	0.4 (0.8)	0.8 (2.5)

Results

A total of 226 participants from the NHS, 231 from NHS II, and 216 from HPFS responded to the mailed questionnaire (Table 1). The participants in the NHS and HPFS were similar in mean age (62.1 and 62.5 years, respectively), although the participants in the NHS II were younger (mean age = 40.9 years). Although all NHS participants were married at the time of identification in 1972, only 79% reported being married at the time of the 1992 questionnaire, whereas 92% of the HPFS participants reported being married in 1992. The participants in the HPFS represent a more uniform geographic distribution of the United States, whereas participants in the NHS were more likely to reside in the northeast United States, and participants in the NHS II were slightly more likely to reside in the Midwest. The participants from these three cohorts differed slightly in their reported use of multivitamins, smoking behavior, and employment status. Table 2 presents the mean (g/day) intake of chicken, fish, hamburger, steak, roast, beef gravy, bacon, and sausage for each cohort. The participants in the HPFS reported greater consumption for all foods except gravy. Slight differences were noted between reported intake among NHS and NHS II participants, with those in the NHS reporting greater intake of fish, roast, gravy, and bacon and those in NHS II reporting greater intake of chicken, hamburger, and steak.

Of the 18 food-cooking method combinations queried, the participants in the HPFS reported the most frequent consumption for 16 (Table 3). Participants in the NHS II were more likely to report frequent consumption of grilled or BBQ chicken, whereas participants in the NHS and HPFS were more likely to report frequent consumption of broiled chicken. Participants in the NHS II reported infrequent consumption of fish. Respondents from both NHS II (11.3%) and HPFS (10.4%) reported frequent consumption (≥1/week) of grilled/BBQ hamburger compared to those from the NHS (5.2%). HPFS (8.9%) and NHS II (6.5%) participants were more likely to report frequent consumption of grilled/BBQ steak than those from NHS (1.4%). Overall, a lower percentage of respondents from HPFS indicated consuming foods of which the usual appearance was well done, compared to those from NHS II, who reported the highest percentage consuming hamburger and steak, the usual appearance of which was well done, and NHS participants, who indicated a high percentage consumption of gravy made from well-done drippings, well-done bacon, and well-done sausage. Thus, although members of both the NHS and NHS II are female, differences in consumption patterns existed between the younger and older cohort. In addition, although the participants from both NHS and HPFS were similar in age, there were sex differences in consumption patterns.

In each of the three cohorts there was a wide within-cohort variation of HCA exposure. MeIQx intake ranged from 5 to 76

Table 3 Percentage in each cohort with high frequency of consumption and percentage in each cohort in high doneness category for each food-cooking method combination

Cohort, food/cooking method[a]	NHS		NHS II		HPFS	
	% consuming ≥1/week	% in high doneness category[b]	% consuming ≥1/week	% in high doneness category[b]	% consuming ≥1/week	% in high doneness category[b]
Pan-fried chicken	3.6	2.1	4.0	0.6	8.1	1.5
Broiled chicken	23.2	2.8	16.3	1.0	24.3	2.9
Grilled/BBQ chicken	12.2	7.7	22.0	10.0	19.0	8.0
Pan-fried fish	1.4	2.7	0.9	2.1	6.1	2.0
Broiled fish	10.5	0.0	4.6	2.2	18.4	1.6
Grilled/BBQ fish	1.4	5.0	1.4	5.0	8.5	4.9
Pan-fried hamburger	9.3	48.8	15.8	55.6	18.6	46.1
Broiled hamburger	6.1	43.8	5.4	48.9	8.3	44.1
Grilled/BBQ hamburger	5.2	54.0	11.3	58.1	10.4	53.4
Pan-fried steak	0.5	36.2	1.4	39.4	2.0	37.3
Broiled steak	2.4	39.3	1.9	40.7	4.9	36.6
Grilled/BBQ steak	1.4	42.6	6.5	46.1	8.9	43.4
Roast beef	4.7	25.0	4.2	28.3	7.5	19.6
Homemade beef gravy	3.2	36.7	1.8	32.0	3.2	18.5
Fried bacon	7.2	67.5	3.9	51.1	12.6	43.8
Microwaved bacon	3.1	59.5	1.9	56.3	5.2	39.0
Broiled bacon	0.5	67.6	0.0	40.9	0.5	23.9
Fried sausage	1.6	54.9	1.0	51.1	6.5	43.1

[a] The number reporting each food-cooking method combination varied slightly due to missing data.
[b] Those indicating blackened or charred chicken; blackened or charred fish; well-browned, blackened, or charred hamburger; well-browned, blackened, or charred steak; well-browned roast beef; gravy from well-browned beef; well-done/extra crispy bacon; and well-done sausage.

ng (10th to 90th percentile) for the NHS respondents, 4 to 77 ng for those from NHS II, and 3 to 100 ng for those from HPFS. The range of PhIP intake was from 39 to 584 ng (10th percentile to 90th percentile) for the NHS participants, 46 to 854 ng for NHS II, and 76 to 853 ng for HPFS. The range of DiMeIQx intake was from 0.1 to 9 ng (10th percentile to 90th percentile) for the respondents from NHS, 0.1 to 10 ng for NHS II, and 0 to 9 ng for HPFS.

The participants in the NHS consumed the lowest mean levels of MeIQx (33 ng/day), PhIP (286 ng/day), and DiMeIQx (3.5 ng/day), whereas participants in the HPFS consumed the highest mean levels of MeIQx (45 ng/day) and PhIP (458 ng/day) but not DiMeIQx (mean = 4.0 ng/day; Table 4). However, this pattern of the overall HCA levels being greater in the HPFS and lowest in the NHS was not necessarily true across all types of food. Although consumption of chicken had the greatest impact on overall PhIP and DiMeIQx, differences in consumption of chicken, fish, and steak between the three cohorts influenced the overall variation in PhIP intake. The highest intake of HCAs from beef gravy was in the NHS participants, reflecting the high reporting of use of well-done drippings to make homemade gravy. Similarly, participants in the NHS II reported the highest intake of MeIQx from hamburger, reflecting the high percentage reporting consumption of well-done hamburgers. Hamburger was the one food source that contributed the greatest levels to MeIQx intake in all three cohorts.

Table 5 summarizes results of the different food and cooking method combinations that explain the total intake of MeIQx, PhIP, and DiMeIQx in each cohort. A greater number of food and cooking combinations was needed in all cohorts to explain 90% of the variance in MeIQx and DiMeIQx than to explain PhIP. Across the three cohorts, between 83 and 88% of the variance in MeIQx intake, 95 and 98% of the variance in PhIP intake, and 81 and 96% of the DiMeIQx intake was explained by the top three cooked foods that contributed. Although different foods contributed to the intake of each measured HCA, chicken (grilled or BBQ chicken, broiled chicken, and pan-fried chicken) explained at least 95% of the variance in PhIP intake for each of the three cohorts.

Discussion

We estimated intake of HCA from meat (red meat, poultry, and fish), taking into account frequency of consumption, cooking method, degree of doneness, and HCA content for foods in a pilot study conducted in three prospective cohorts. Although frequency of consumption of meat as well as other foods has been ascertained previously by food frequency questionnaires in each of these cohorts, it was not possible to calculate HCA intake because questions on cooking method and degree of doneness had not been included. This study was designed to identify a set of questions that explains the greatest proportion of the variation in dietary HCA intake, with the aim of including a reduced set of questions on subsequent biennial questionnaires mailed to everyone in the three cohorts.

The addition of these questions for the assessment of HCA intake in the three prospective cohorts will allow for a number of issues to be addressed in the future. One question that was raised in the past was whether the cooking method of the meat and the degree of doneness have an independent effect on disease risk beyond the effects associated with high quantities of consumption of red meat (2). To date, none of the epidemiological studies that evaluated associations between fried foods and cancer risk directly assessed the HCA or mutagenic activity of the foods consumed (25). The association between total red meat intake and colon cancer previously reported in the HPFS was independent of the other dietary exposures but did not evaluate cooking methods and degree of doneness (2). Knekt *et al.* (26) reported associations between mean intake of fried meat and level of intake of other food groups and nutrients. Although other studies assessed questions on cooking methods or degree of doneness as part of larger dietary assessments,

Table 4 Mean (SD) HCA values (in ng/day) in each cohort overall and by type of food

HCA	Source[a]	NHS		NHS II		HPFS	
		Mean ng/day	SD	Mean ng/day	SD	Mean ng/day	SD
MeIQx	Overall	33.0	35.6	36.0	36.7	44.8	53.7
	Chicken	6.0	8.1	7.7	10.1	10.9	17.2
	Fish						
	Hamburger	9.9	16.4	15.4	24.6	15.0	25.6
	Steak	7.9	12.6	7.9	11.4	12.7	21.9
	Roast						
	Beef gravy	5.7	12.1	3.2	8.0	3.9	16.3
	Bacon	1.7	4.5	1.0	2.0	1.7	2.9
	Sausage	0.9	1.6	1.0	2.6	1.9	7.6
PhIP	Overall	285.5	426.5	346.2	524.9	457.9	735.2
	Chicken	204.0	370.0	288.8	514.9	313.8	653.7
	Fish	40.8	56.8	31.0	57.1	69.8	89.1
	Hamburger	1.2	12.9	1.1	7.8	5.8	52.2
	Steak	31.8	39.3	37.3	46.5	62.7	145.2
	Roast						
	Beef gravy	2.7	7.1	1.5	4.7	1.7	9.4
	Bacon	2.9	7.3	1.6	3.1	2.7	6.3
	Sausage	0.02	0.05	0.02	0.08	0.05	0.2
DiMeIQx	Overall	3.5	5.1	4.1	5.2	4.0	4.9
	Chicken	1.5	3.7	2.6	4.9	1.8	3.9
	Fish						
	Hamburger	0.5	0.8	0.7	0.9	0.9	1.5
	Steak	0.5	1.4	0.4	0.9	0.7	1.6
	Roast						
	Beef gravy	0.7	1.9	0.4	1.3	0.5	2.5
	Bacon	0.2	0.5	0.09	0.2	0.1	0.3
	Sausage						

[a] If an individual was missing information (not reported) for any of the component foods, they were not included in the overall MeIQx, PhIP, or DiMeIQx calculations, but they may be in the totals for individual foods if no information was missing for that food.

information of the associations between other dietary exposures and the sources of HCA intake has been minimal (3, 5).

Therefore, future studies analyzing the associations between HCA and cancer risk need to be able to control for the possible confounding of other dietary factors such as fat, protein, and fruit and vegetable intake.

The results of this study are highly dependent on the measured values of HCA in the database for each food, cooking method, and doneness combination. The database used in this analysis is currently the best available to determine the HCA levels in foods cooked in a usual manner in the United States, with all analyses being conducted under the same laboratory conditions (20–23). Many factors influence the determination of HCA intake. Variation occurs in reporting of food intake and in how doneness is defined, and there is also laboratory variation in the assessment of the HCA compounds. Furthermore, differences in food preparation may also impact the levels of HCA. Salmon *et al.* (27) reported that marinating chicken prior to grilling decreased the PhIP and increased the MeIQx formed during the cooking process. Because application of this database involves all of these potential sources of misclassification, there is more confidence in the ability to identify those with either low or high HCA levels than there is in the ability to accurately determine intake at intermediate levels. However, the relatively low levels of laboratory reproducibility in assessing HCA indicates the need to reevaluate this study if more precise measures of HCA levels in food or adequate human biomarkers of exposure become available.

In deciding which items to include in subsequent cohort-wide questionnaires to assess the influence of the cooking method and degree of doneness of meat on risk of disease, other information beyond the study results was considered. Due to the low frequency of consumption and the low values of HCA in the database associated with roast beef intake, this food did not contribute to the total HCA exposures assessed in this study among participants in any of the three prospective cohorts. However, previous epidemiological studies suggested eating well-done roast beef may be associated with increased colon cancer risk (3). In addition, in the database used in this study HCA were assessed for only one type of fish fillet. Other reports have indicated that different types of fish cooked at high temperature are a possible source of increased HCA exposure (28). Therefore, questions about the consumption of both roast beef and broiled fish were included. In the food frequency questionnaires administered to all three cohorts, participants were asked about the consumption of bacon. Among those that reported consuming bacon in this study of cooking methods and doneness levels, most indicated that they ate fried bacon, and there was little variation among bacon eaters in the level of doneness reported (only 1.5% indicated consuming lightly browned bacon). Therefore, HCA exposure from bacon can be estimated from the food frequency questionnaire without additional questions of cooking method or doneness.

On the basis of the results of this study conducted in the three prospective cohorts, participants' responses indicated that a mailed questionnaire could assess a wide variation in HCA exposure with relatively few questions. Although the same food items explained a great deal of the variation within each cohort, some distinctions remained between the three cohorts. Among the men in the HPFS, consumption of grilled/BBQ steak explained a high proportion of the variance in MeIQx intake, but this food item did not add substantially to the explanation of MeIQx levels in the women in NHS and NHS II. Therefore, the same reduced set of questions may not be appropriate to ascer-

Table 5 Predictors[a] of HCA intake by foods and cooking methods in each cohort

Cohort	NHS		NHS II		HPFS	
	Order[b]	% of variance explained[c]	Order[b]	% of variance explained[c]	Order[b]	% of variance explained[c]
Predictors of MeIQx						
Pan-fried hamburger	1	49.1	1	56.0	1	50.6
Pan-fried steak	2	28.6	6	2.2	5	3.2
Beef gravy	3	10.3	2	18.8	6	3.5
Grilled/BBQ hamburger	4	4.7	5	3.7	8	1.1
Grilled/BBQ steak	5	2.4	7	1.2	2	24.5
Pan-fried chicken	6	2.1	3	8.7	3	10.8
Broiled steak	7	1.3	8	0.8	9	1.1
Grilled/BBQ chicken	8	0.8	4	7.7	4	3.1
Sausage	11	0.2	9	0.4	7	1.7
Predictors of PhIP						
Grilled/BBQ chicken	1	78.5	1	93.8	1	85.8
Broiled chicken	2	16.2	2	2.7	4	3.8
Pan-fried chicken	3	3.1	3	1.6	2	5.0
Broiled fish	4	1.0	4	0.9	5	0.6
Grilled/BBQ steak	5	0.6	5	0.6	3	4.3
Predictors of DiMeIQx						
Pan-fried steak	1	43.8	3	2.2	4	6.3
Grilled/BBQ chicken	2	27.8	1	81.4	1	55.3
Beef gravy	3	19.2	2	12.5	5	5.5
Pan-fried chicken	4	3.8	6	0.7	3	10.0
Broiled chicken	5	2.2	10	0.01		
Pan-fried bacon	6	1.2	8	0.2	8	0.2
Pan-fried hamburger	7	0.9	5	1.4	6	4.8
Grilled/BBQ hamburger	8	0.8	4	1.4	7	1.7
Broiled steak	9	0.3	7	0.2	2	16.2

[a] Table includes all food-cooking method combinations across all three cohorts that explained ≥1% of the variance of each HCA in any of the cohorts.
[b] Order that each food/cooking method combination entered a linear stepwise regression model to predict intake of each HCA, given that the items with lower order value were already in the model.
[c] Additional percentage variance in predicting total intake of each HCA compound explained by each food-cooking method combination, given that all other factors with lower order value were already in the model.

tain HCA exposure in different populations. However, to allow for comparability across cohorts, a similar set of questions was selected.

The first biennial cohort-wide questionnaire to be developed after this study was in the HPFS. The questions that were added asked participants to indicate "During the past year, how often (never, <1/month, 1/month, 2–3/month, 1/week, 2–3/week, or 4+/week) did you eat the following: pan-fried chicken, broiled chicken, grilled/BBQ chicken, broiled fish, roast beef, pan-fried steak, grilled/BBQ steak, and homemade beef gravy?" In addition, for each food item, participants were asked to indicate the usual outside appearance (lightly browned, medium browned, well-browned, or blackened/charred). A separate question asked the participants when they "... eat chicken, how often it was cooked with the skin on?" and "How often do you eat the skin?" Consumption of pan-fried hamburger explained a high proportion of the variance in MeIQx among all of the cohorts. Although the consumption of grilled/BBQ steak explained over 24.5% of the variance of MeIQx in the HPFS, this food explained less than 2.5% of the variance in the NHS and NHS II cohorts. In the questions to be included in the biennial questionnaire to the NHS and NHS II participants, replacing the question on grilled/BBQ steak with questions about pan-fried hamburger would better predict the total levels of MeIQx in these cohorts.

Although the participants in each of these three cohorts are all members of various United States health care professions, their reported exposure to HCA is not uniform and reflects a wide range of exposure to HCA in their reported diet. The relative comparison of the 10th and 90th percentile (90th/10th percentile value) was ~30-fold for of MeIQx intake, 20-fold for PhIP intake, and >110-fold for DiMeIQx. Given these variations in HCA exposure among participants in the three cohorts, future epidemiological studies that assess the cooking methods and degree of doneness, in addition to the frequency of consumption of chicken, beef, fish, and pork, should be able to determine whether dietary exposures at these levels are related to disease risk.

The reduced number of questions that explained the majority of the variation in HCA exposure in these three cohorts may not necessarily serve the same function in other populations. This study noted differences in reported food preparation practices by sex and age. Furthermore, participants in the three cohorts are predominantly white and were selected based on their occupational training. Although the participants in the three cohorts represent a broad geographic representation of the United States, this smaller study was not able to address questions regarding geographic variation in food preparation practices. Similar to the study conducted in Sweden, the gravy made from pan drippings analyzed for this study contained high levels of HCA (12). However, due to less frequent consumption in the three United States cohorts studied, gravy did not contribute as much as in the Swedish study to overall HCA daily intake. Thus, in other populations, different questions may be necessary to measure the variation in dietary HCA exposure. In these three cohorts, the majority of the variation in HCA exposures could be explained by consumption of chicken and beef. Overall beef consumption has declined in the United

States, whereas chicken consumption has increased. Although the members of these cohorts also report more frequent consumption of chicken than red meat, their diet may not represent typical United States dietary patterns. Members of these three cohorts may be more health conscious with >40% of each cohort reporting the use of multivitamins and ≤15% reporting being a current smoker. Despite differences between members of these three cohorts and others living in the United States, the respondents in these cohorts were exposed to a wide range of dietary HCA. In conclusion, these data suggest that, instead of extensive questions to encompass all cooking methods and assess the degree of doneness of all relevant foods, a pilot study such as this may enable investigators to identify a reduced set of questions that explain most of the variance in HCA in the specific population of interest.

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