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Heterocyclic Amine Content in Beef Cooked by Different Methods to Varying Degrees of Doneness and Gravy made from Meat Drippings

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Abstract—Meats cooked at high temperatures sometimes contain heterocyclic amines (HCAs) that are known mutagens and animal carcinogens, but their carcinogenic potential in humans has not been established. To investigate the association between HCAs and cancer, sources of exposure to these compounds need to be determined. Beef is the most frequently consumed meat in the United States and for this study we determined HCA values in beef samples cooked in ways to represent US cooking practices, the results of which can be used in epidemiological studies to estimate HCA exposure from dietary questionnaires. We measured five HCAs [2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx) and 2-amino-1-methyl-6-phenylimidazo-[4,5-*b*]pyridine (PhIP)] in different types of cooked beef using solid-phase extraction and HPLC. Steak and hamburger patties were pan-fried, oven-broiled, and grilled/barbecued to four levels of doneness (rare, medium, well done or very well done), while beef roasts were oven cooked to three levels of doneness (rare, medium or well done). The measured values of the specific HCAs varied with the cut of beef, cooking method, and doneness level. In general, MeIQ× content increased with doneness under each cooking condition for steak and hamburger patties, up to 8.2 ng/g. PhIP was the predominant HCA produced in steak (1.9 to 30 ng/g), but was formed only in very well done fried or grilled hamburger. DiMeIQx was found in trace levels in pan-fried steaks only, while IQ and MeIQ were not detectable in any of the samples. Roast beef did not contain any of the HCAs, but the gravy made from the drippings from well done roasts had 2 ng/g of PhIP and 7 ng/g of MeIQx. Epidemiological studies need to consider the type of meat, cooking method and degree of doneness/surface browning in survey questions to adequately assess an individual's exposure to HCAs. *© 1998 Elsevier Science Ltd. All rights reserved*

Abbreviations: HCAs = heterocyclic amines; 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; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ = 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline.

Keywords: heterocyclic amines; beef; cooking method; MeIQx; PhIP; DiMeIQx.

INTRODUCTION

Heterocyclic amines (HCAs) are sometimes formed in meats cooked at high temperature (Adamson, 1990; Felton and Knize, 1990; Skog, 1993). HCAs are known to be potent mutagens and animal carcinogens (Ghoshal *et al.*, 1994; Ito *et al.*, 1991; Ohgaki *et al.*, 1986; Weisburger *et al.*, 1994); however, the carcinogenic potential in humans has not been established (Steineck *et al.*, 1993).

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Epidemiological studies of colon and breast cancer using crude surrogates for HCA exposure from meat (e.g. doneness, surface browning, frying, intake of gravy) have produced suggestive but somewhat inconsistent results (Gerhardsson *et al.*, 1991; Knekt *et al.*, 1994; Muscat and Wynder, 1994; Ronco *et al.*, 1996; Schiffman *et al.*, 1990; Schiffman and Felton, 1990; Steineck *et al.*, 1993). Currently used surrogates such as doneness of 'red meat' may be inadequate to assess an individual's exposure to HCAs since substantial heterogeneity of HCA levels is present in a variety of meats all considered as 'well done' (Sinha and Rothman, 1997). The resultant misclassification may result in decreased ability to observe a true association. Thus, to decrease misclassification and better assess true risk of HCAs in cancer aetiology there is a need to improve exposure assessment of HCAs. To this end we are developing a database of HCA concentrations in commonly consumed meat items cooked by various techniques and to various degrees of doneness.

There is an extensive literature on the presence of HCAs in meats cooked at high temperature, but in many of the reported studies the cooking was not well described, analysis methods varied and might not be comparable, or samples were cooked to maximize the production of HCAs, and not to be representative of the way meats are usually cooked by the general population in the US (Aeschbacher, 1991; Gross *et al.*, 1993; Johansson and Jagerstad, 1994; Layton *et al.*, 1995; Skog, 1993; Sugimura *et al.*, 1988). Thus, systematic measurement of HCA values in most commonly consumed meat types cooked by representative practices are needed to accurately assess HCA exposure in epidemiological studies in the US.

Beef is the most frequently consumed meat in the US (National Live Stock and Meat Board, 1994), and is likely to be one of the main contributors to HCA exposure. Exposure to HCAs varies depending on the cooking technique and the degree of doneness. In this study we report the HCA content of some of the most commonly consumed cuts of beef: hamburger patties, steak, roast beef, and gravy made from roast drippings cooked by different methods to varying degrees of doneness.

MATERIALS AND METHODS

Three types of beef products were purchased from a local supermarket: hamburger patties of freshly ground lean beef (15% fat, 1.5 to 2.0 cm thick × 10 cm diameter); steaks (top loin, New York strip, USDA choice steaks, 2.6 to 3.3 cm thick); and roasts (eye round roast, 4–6 lb). Hamburger patties and steaks were pan-fried, oven-broiled, or grilled/barbecued, while roasts were cooked in an oven.

The beef was cooked by nutritionists at the Human Nutrition Research Center, US Department of Agriculture, Beltsville, MD. For each cooking method, three hamburger patties, one steak and one roast were cooked in five, five and two different sessions, respectively. The cooked meat was finely ground in a Robot Coupe mixer (Jackson, MS, USA) to form a composite sample for the specific meat type, method of cooking and degree of doneness.

The hamburger patties and steaks were cooked to four levels of doneness: rare, medium, well done and very well done. The roasts were cooked to three levels of doneness: rare, medium and well done. The degree of doneness for the different types of beef products was primarily defined by internal temperature. We defined beef cooked to the internal temperature of 60°C as rare, 70°C as medium, 80°C as well done and 90°C as very well done. Internal temperature was taken using a tissue implantable thermocouple microprobe (type 1T-18, Physitemp Instruments, Inc., Clifton, NJ, USA) connected to a digital thermometer (model no. 08500-40, Cole-Parmer, Chicago, IL, USA). The interior colour was noted by visual inspection of meat. The level of surface browning was judged to be one of the following categories: not browned; moderately browned; well browned; and very well browned/charred (Plates 1 and 2).

We used the cooking methods most commonly used in the US: pan-fried, oven-broiled, grilled/barbecued, or roasted. Hamburger patties and steaks were pan-fried in a teflon-coated frying pan without added oil. The temperature on the pan surface was monitored with surface thermometers (PTC, Pacific Transducer Corp., Los Angeles, CA, USA). Oven-broiled hamburger patties and steaks were cooked in a commercial gas-range broiler with the meat placed 12 cm away from the heat source. The cooking surface temperature was monitored with a thermocouple probe near the surface of the broiling pan. Grilled/barbecued patties and steaks were prepared on a gas barbecue unit with ceramic briquettes (Sunbeam model 44M39 27 $\frac{1}{2}$ in. × 15 in., 44,000 BTU) sold for home use. The surface temperature was monitored with surface thermometers on the grill surface. Roast beef was cooked in a gas oven heated to 160°C (monitored by a thermocouple probe placed in the oven). Drippings were collected from two roasts and combined. Gravy was made by combining the drippings with flour and water.

Detailed information on the parameters used to classify doneness, such as internal temperature and surface browning, was recorded. Other information gathered to further define the cooking methods included: weight of meat before and after cooking to calculate the percent loss of weight with cooking; the total cooking times, and cooking surface and oven temperature.

The levels of [2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quin-

Table 1. Cooking parameters for steak prepared by three different methods to four degrees of doneness

Cooking method and degree of doneness	Number of cooking sessions[a]	Weight loss during cooking (%)	Cooking surface temp. (°C)	Internal temp. (°C)	Cooking time (min)	Surface browning/ charring
Pan-fried						
Rare	5	16	188	52	15	Slight
Medium	5	22	186	70	16	Moderate
Well done	5	30	189	84	26	Dark brown
Very well done	5	35	191	93	33	Blackened/charred
Oven-broiled						
Rare	5	21	181	50	10	Slight
Medium	5	33	179	68	15	Moderate
Well done	5	40	185	83	20	Dark brown
Very well done	5	44	178	92	24	Blackened/charred
Grilled/barbecued						
Rare	4	22	273[b]	67	20	Slight
Medium	3	26	260	69	16	Moderate
Well done	5	34	260	78	24	Dark brown
Very well done	5	47	249	89	41	Blackened/charred

[a]One steak cooked in each of five sessions except for rare and medium grilled/barbecued steaks samples, for which there were only four and three sessions, respectively. A session began when the whole cooking procedure was started anew with clean pans, etc.
[b]There was flashing of flames due to dripping fat on the hot briquettes of all the grilled/barbecued samples. The temperature of the grilling surface was recorded throughout cooking as well as the maximum temperature (500°C) reached with flashing of flames.

oline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]-quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)] were measured in duplicate samples by solid-phase extraction and analysed by HPLC by the method of Gross and Gruter (1992) and has been described in detail by Knize *et al*. (1995). HCA recoveries for each sample were determined from the average of duplicate samples spiked with all five compounds. The identities of peaks at the retention time of known HCA were confirmed by photodiode-array UV spectra in all cases. The investigators measuring HCA content were blinded to the type of meat, cooking method and degree of doneness. Quality control samples with relatively low and high content of HCAs were interspersed throughout the analysis to check on measurement reproducibility. These samples were made from hamburger cooked at low temperature (containing low levels of HCAs) or hamburger patties cooked at high temperature (containing high levels of HCAs). The average concentrations of MeIQx, PhIP and DiMeIQx found in the high temperature samples ($n = 13$) were: 7.2 (coefficient of variation: 0.36), 10.9 (0.24) and 1.7 (0.40) ng/g, respectively. IQ and MeIQ were not present in the high-temperature quality control samples and none of the five HCAs was found in the low-temperature quality control samples. The limit of detection was approximately 0.2 ng/gram of cooked meat for all the five compounds.

RESULTS

The cooking conditions for the various cuts of beef are described in Tables 1–3. The percent of loss (water and fat) from cooking the meat varied with the cooking method. The greatest percent loss was found in grilled/barbecued samples and the least in pan-fried samples. As expected, degree of

Table 2. Cooking parameters for hamburger patties prepared by three different methods to four degrees of doneness

Cooking method and degree of doneness	Number of cooking sessions[a]	Weight loss during cooking (%)	Cooking surface temp. (°C)	Internal temp. (°C)	Cooking time (min)	Surface browning/ charring
Pan-fried						
Rare	15	17	180	59	6	None
Medium	15	28	180	69	10	Moderate
Well done	15	36	189	81	15	Dark brown
Very well done	15	40	191	90	20	Blackened/charred
Oven-broiled						
Rare	15	22	183	56	6	None
Medium	15	30	186	68	8	Moderate
Well done	15	40	175	83	10	Dark brown
Very well done	15	48	181	98	12	Blackened/charred
Grilled/barbecued						
Rare	15	19	185[b]	56	9	None
Medium	15	26	213	69	12	Moderate
Well done	15	40	216	84	24	Dark brown
Very well done	15	56	240	90	38	Blackened/charred

[a]Three patties cooked in each of five sessions. A session began when the whole cooking procedure was started anew with clean pans, etc.
[b]There was flashing of flames due to dripping fat on the hot briquettes of all the grilled/barbecued samples. The temperature of the grilling surface was recorded throughout cooking as well as the maximum temperature (500°C) reached with flashing of flames.

Table 3. Cooking parameters for roast cooked to three degrees of doneness

Degree of doneness	Number of cooking sessions[a]	Weight loss during cooking (%)	Oven temp. (°C)	Internal temp. (°C)	Cooking time (min)	External brownness
Roast beef						
Rare	2	14	160	53	96	None
Medium	2	24	160	64	120	Moderate
Well done	2	34	160	86	182	Moderate

[a]One roast cooked in each of two sessions.

doneness increased with cooking time for each method of cooking meat. Less cooking time was required for oven-broiling than for pan-frying or grilling/barbecuing to achieve the same degree of doneness. The degree of doneness was primarily based on the internal meat temperature and somewhat on the surface browning.

MeIQx and PhIP were detected in most of the cooked beef samples in substantial quantities (Figs 1 and 2 and Table 4). DiMeIQx was found at trace or undetectable levels and IQ and MeIQ were not detected in any of the samples. Steak (Fig. 1) usually contained the highest amounts of MeIQx and PhIP. The maximum levels of MeIQx were found in very well done pan-fried steak samples. Oven-broiled and grilled/barbecued samples generally contained lower levels of MeIQx for all degrees of doneness than pan-fried samples. PhIP was found in almost all of the steak samples with the highest values in very well done pan-fried and grilled/barbecued samples. DiMeIQx was not detected in any of the steak samples (data not shown) except pan-fried very well done steak (1 ng/g of cooked meat).

The MeIQx content of pan-fried hamburger samples increased with greater degrees of doneness (Fig. 2). Similar levels of MeIQx were detected in pan-fried and grilled/barbecued hamburger patties, while MeIQx was detected only in very well done oven-broiled samples. Of the two hamburger patty samples containing appreciable levels of PhIP, very well done grilled/barbecued samples had much higher levels than very well done pan-fried samples. DiMeIQx was not detected in any of the hamburger patty samples (data not shown).

The roast beef did not contain detectable levels of any of the HCAs measured even when cooked to a well done state (Table 4) consistent with the finding that there was no mutagenic activities in these samples (data not shown). However, gravy made from drippings from well done roast beef contained substantial amounts of MeIQx (7 ng/g), as well as 1 ng/g of DiMeIQx, 4 ng/g of PhIP, and mutagenic activity of 814 TA98 rev/g. The gravy made with drippings from rare and medium roast beef contained only 1 ng/g of MeIQx and non-detectable levels of DiMeIQx and PhIP with mutagenic activity of 158 and 73 TA98 rev/g, respectively.

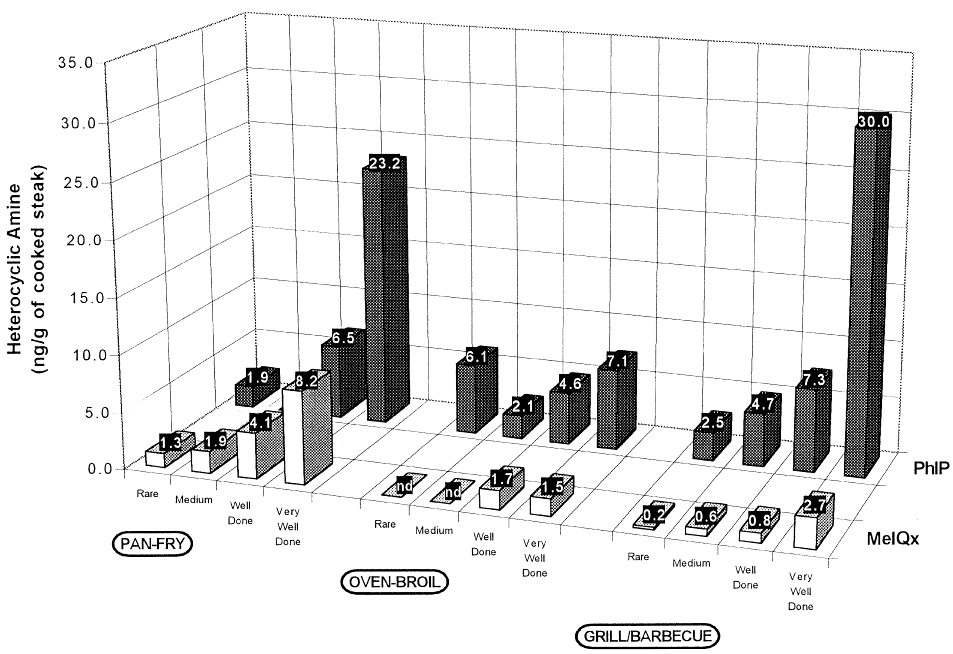

Fig. 1. MeIQx and PhIP content of steaks cooked by three methods to four degrees of doneness.

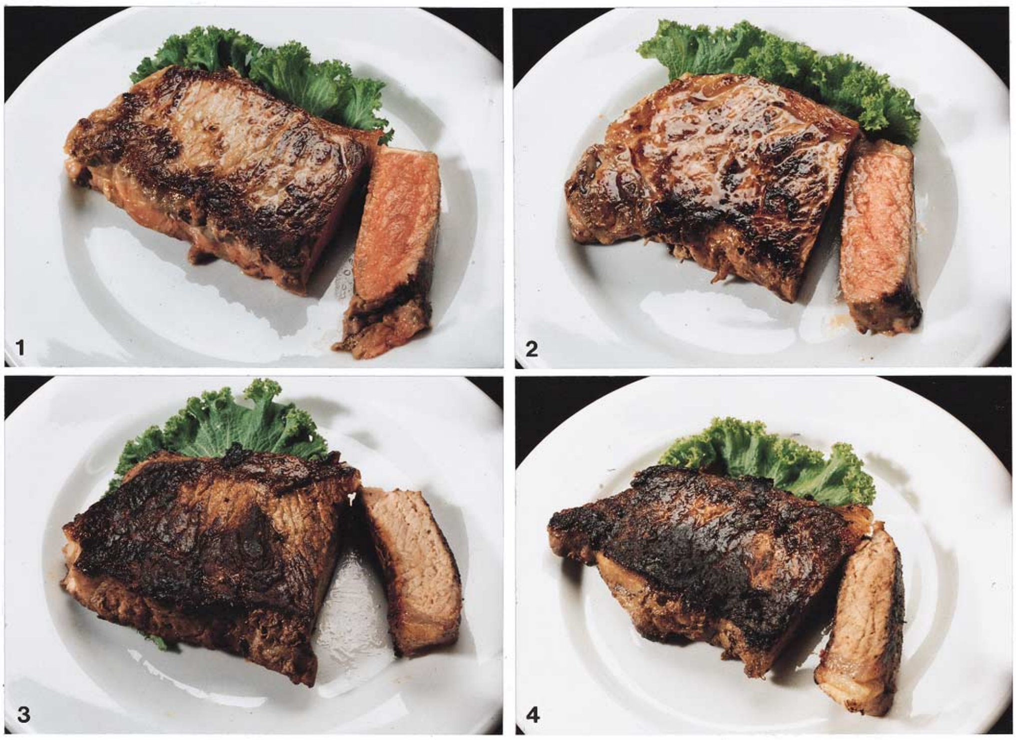

Plate 1. External and internal appearance of beef steaks pan-fried to four levels of doneness: 1—rare; 2—medium; 3—well done; 4—very well done.

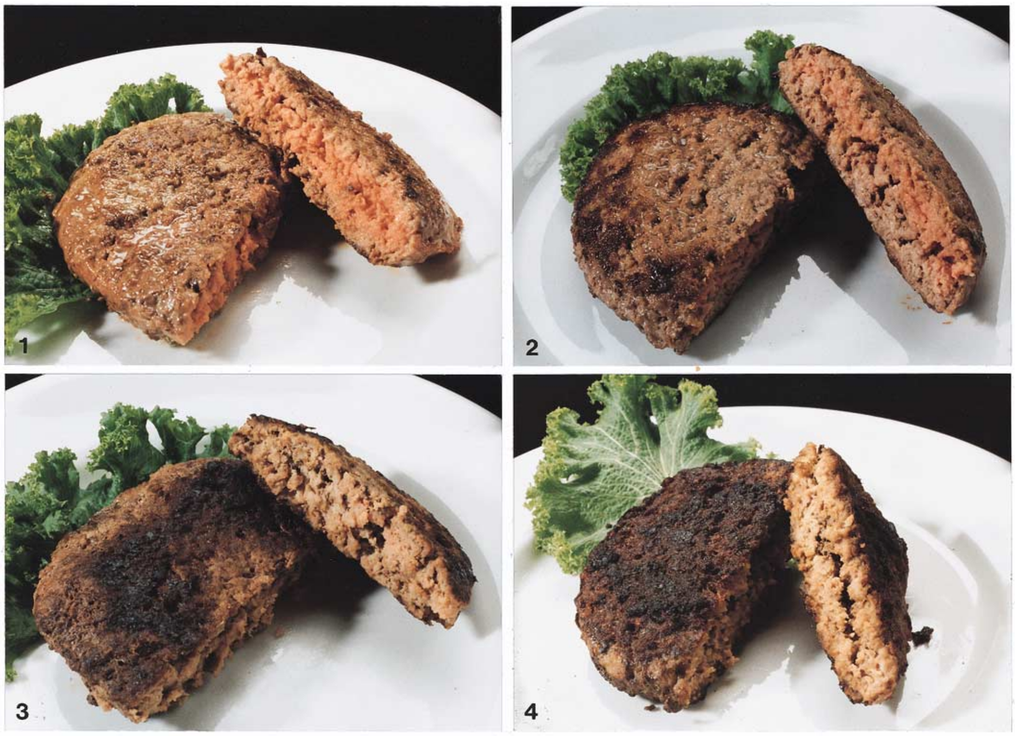

Plate 2. External and internal appearance of hamburger patties pan-fried to four levels of doneness: 1—rare; 2—medium; 3—well done; 4—very well done.

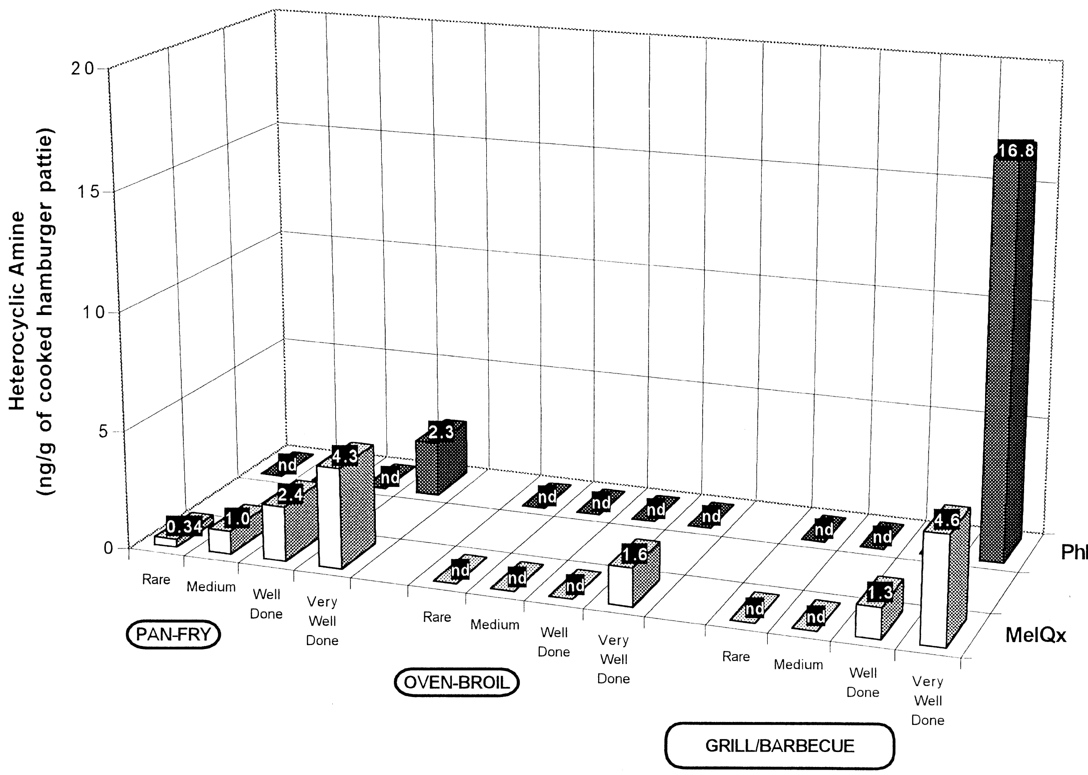

Fig. 2. MeIQx and PhIP content of hamburger patties cooked by three methods to four degrees of doneness.

DISCUSSION

Beef steaks, hamburger patties, roast beef and gravy cooked by commonly used methods to varying degrees of doneness is reported here. The HCA content of steaks and hamburger patties increased by doneness level, but the individual HCAs measured were not produced to the same extent by each cooking method and doneness level. Furthermore, HCAs were not detected in roast beef, but gravy made from the drippings of all roast beef samples contained MeIQx, and gravy from well-done roast beef also contained PhIP and DiMeIQx. This confirms the reported finding of MeIQx and other HCAs in meat pan residues from which gravies are made (Johansson and Jagerstad, 1994; Skog *et al.*, 1995).

The contribution of gravy to the total HCA intake can be substantial. For example, a portion of approximately a quarter-cup of gravy would contain 398 ng of MeIQx, 62 ng of DiMeIQx, and 230 ng of PhIP. Interestingly, one would have to consume approximately 200 g of pan-fried well done hamburger or 400 g of well done grilled/barbecued steak to have a similar intake of MeIQx. Further, higher intake of gravy has been associated with greater risk of colon cancer (Gerhardsson *et al.*, 1991).

Epidemiological studies to date have not specifically estimated HCA exposure, but have used surro-

Table 4. MeIQx, DiMeIQx, and PhIP content of roast beef cooked to three degrees of doneness and gravy made from the roast beef drippings

	MeIQx (ng/g cooked meat or gravy made from drippings)		DiMeIQx (ng/g cooked meat or gravy made from drippings)		PhIP (ng/g cooked meat or gravy made from drippings)	
Degree of doneness	Roast	Gravy[a]	Roast	Gravy	Roast	Gravy
Roast beef						
Rare	nd	1.1	nd	nd	nd	nd
Medium	nd	1.0	nd	nd	nd	nd
Well done	nd	7.1	nd	1.1	nd	4.1

nd = not detectable

[a]Gravy was made using the crusty drippings after skimming off all the fat except one-quarter cup (56 g). In a saucepan, one-quarter cup of flour, one and three-quarter cups of water, and one-quarter cup of fat with all the drippings were mixed. This mixture was cooked until thick and bubbly. Makes two cups (448 g).

gates such as cooking methods and doneness levels of various kinds of meats. The results have been mixed whereby some studies find that the amount of well done or well browned red meat, fried or barbecued meats and gravy made from drippings increases the risk of colorectal cancer (Gerhardsson *et al*., 1991; Lang *et al*., 1994; Skog *et al*., 1995; Steineck *et al*., 1993). In contrast, other studies find no association between colorectal cancers and degree of doneness of meat or with various methods of cooking meats (Muscat and Wynder, 1994; Steineck *et al*., 1993). The conflicting results in the literature may be due to the variability in HCA formation, thereby leading to misclassification of exposure.

The results presented here and previously reported work for chicken and meat products from fast-food restaurants (Knize *et al*., 1995; Sinha *et al*., 1995) indicate that questions only on red meat consumption and general questions of cooking and doneness are likely to misclassify HCA exposure; for the same level of doneness, steaks, hamburger patties and roast beef have different levels of HCAs. The way that each of these meat items is cooked also affects HCA production: oven-broiled very well done steaks and grilled/barbecued steaks differ vastly in HCA content. Even within the same type of meat and doneness level, place of cooking (e.g. fast-food restaurants, non-fast-food restaurant, and home), or cooking method (pan-fry, oven-broil and grill/barbecue) can play a major role in determining HCA content (Sinha and Rothman, 1997).

Degree of doneness, which is often closely related to surface browning and total cooking time, is a key issue for HCA production in cooked meat. MeIQx and PhIP content increased in hamburgers and steak with greater degrees of doneness mainly in pan-fried and grilled/barbecued meat. Increases in both of these compounds were seen in well-done steak samples with a further substantial increase in the very well done steak. For hamburgers, trends were similar, but in general the levels of the various HCAs within cooking categories were lower. The difference between steaks and hamburger patties may be due to the shorter time needed for hamburger patties to reach the same internal temperature as steaks. Thus, even with high-temperature cooking methods, the meat must be cooked for a longer time to achieve a well done or very well done state allowing for increased production of these compounds.

The three high-temperature cooking methods (pan-frying, oven-broiling and grilling/barbecuing) produce varying levels of the different HCAs. Pan-frying and grilling/barbecuing appear to produce more MeIQx and PhIP. Oven-broiling produces very little of either of these compounds. The reason may be the shorter length of time needed for the meat to reach the same internal temperature or some other cooking-related factor. The parameters, which appear to be important in forming HCAs, are direct contact of the meat with a hot flat surface such as in pan-frying or very high-temperature such as in grilling/barbecuing. In practical terms, when estimating HCA exposure through questionnaires, combining cooking methods such as pan-frying and oven-broiling in one question may not be the best approach when inquiring about meat preparation.

The HCA values generated here regarding type of meat, cooking method, and doneness matrix are being used to quantitate the exposure of individuals to these compounds using a meat cooking module within a food frequency questionnaire. The frequency of consumption of a particular meat, the way it is cooked and the level of doneness is obtained in the food frequency questionnaire module. The values from this database are used within each cell (type of meat, cooking method, doneness level) and added for all the meat items in the questionnaire to obtain an individual's exposure to each of the HCAs. The meat cooking module is being validated using multiple food diaries with detailed information on meat cooking.

When all types of meats cooked by different methods and to various degrees of doneness are combined into one database, we observe that specific HCAs, such as MeIQx and PhIP, do not correlate well with each other. Moreover, since the mutagenic, and to a lesser extent, carcinogenic potencies of these compounds also differ (Layton *et al*., 1995), there is some uncertainty about how best to combine these data for use in epidemiological studies. Risk of specific disease outcomes is best analysed by various methods, since a strictly reductionistic approach of evaluating only HCAs may not be appropriate. This is especially true with red meat itself, which is a risk factor for certain cancers (Giovannucci *et al*., 1993 and 1994; Willett *et al*., 1990). Thus, data from food frequency questionnaire responses should be analysed by type of meat and by cooking practice as well as each of the HCAs separately and/or by a sum of individual compounds weighted for some measure of mutagenic activity or carcinogenic potency.

In summary, of all the meats consumed in the US, consumption of beef is by far the highest (National Live Stock and Meat Board, 1994). Therefore, it is important to estimate the exposure to HCAs from cooked beef in epidemiological studies investigating the role of HCAs to cancer aetiology. In this report we provide a database of HCA values for different cuts of beef, cooked by various methods to varying degrees of doneness, which may be useful in estimating HCA exposure from dietary questionnaires. However, to accurately classify HCA exposure, details on type and cut of meat, method of cooking and degree of doneness need to be obtained in a food frequency questionnaire as MeIQx, DiMeIQx, and PhIP levels vary greatly with these three parameters.

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