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Comparison of heterocyclic amine levels in home-cooked meats with exposure indicators (United States)

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Abstract

Objective: To compare indicators of heterocyclic amine (HCA) exposure with HCA concentrations in home-cooked meat samples.
Methods: Pan-fried hamburger and steak samples were obtained from individuals stating a preference for medium, well done and very well done meat. Concentrations of DiMeIQx, IFP, MeIQx and PhIP were determined by HPLC.
Results: HCA concentrations at the three doneness levels were not significantly different using the participants' self-reported doneness preference to categorize samples. Using doneness levels determined at the time the meat was cooked and photograph analysis to categorize samples, HCA concentrations increased with doneness level and significant differences were observed between the very well done and lower doneness levels. When assigned to doneness levels by photograph analysis, mean concentrations (ng/g cooked meat) of DiMeIQx, IFP, MeIQx, and PhIP were 0.18, 0.16, 0.65 and 0.47 in well done hamburger and 0.61, 0.74, 1.88 and 2.04 in very well done hamburger. In steak, mean concentrations were 0.24, 0.10, 0.79 and 0.59 in well done steak and 0.45, 0.14, 1.87 and 0.62 in very well done steak.
Conclusions: HCA levels in home-cooked meat samples were significantly different when samples were visually classified for doneness, but not when self-reported doneness preference was used to classify doneness.

Introduction

The identification of heterocyclic amines (HCAs) in cooked meats and fish has led to numerous investigations of the potential health effects from their consumption in the diet. A key challenge to examining relationships between HCAs and disease is the accurate estimation of HCA dietary intake. Studies have shown that HCA concentrations in cooked meats are a function of cooking method, time, and temperature [1–5]. Therefore, information on dietary practices, such as the way meats are cooked and individual preference for the level of meat doneness, can be expected to improve HCA exposure assessment. However, recent epidemiologic studies estimating HCA intake by combining information on meat doneness preference, cooking method and meat consumption have led to mixed findings. Significant relationships between several cancers and frequent intake of well done or well browned meat have been found in some of these studies [6–11], whereas others using similar dietary information have found no relationships [12–14]. These contradictory findings indicate that a more thorough examination of dietary practices and preferences is needed to understand the relationship between HCA intake and cancer.

A major uncertainty in HCA exposure estimation is the designation of HCA concentrations in the cooked meats. The concentration data on HCAs collected to date have been obtained from meats cooked by researchers under conditions considered representative of

how these meats are prepared by the public. Virtually no data on HCA concentrations in meats actually cooked in residential settings are available, so the validity of using the data from controlled cooking studies to represent HCA levels in meals prepared by the public is unknown. A second uncertainty pertains to the use of self-reported doneness preference to categorize HCA exposure. Subjective definitions of doneness level among the population could lead to the misclassification of individuals into exposure categories and distort analyses of HCA intake and disease.

To address these uncertainties, we conducted a study of HCA concentrations in meats prepared in residential settings. Pan-fried meat samples were obtained from a study population whose predetermined cooking practices and dietary preferences were obtained from a food-frequency questionnaire. This information, as well as information obtained at the time the meats were cooked, and visual inspection of photographs of the meat samples, were used to categorize samples into levels of doneness. Our objectives were to evaluate the different indicators for characterizing HCA exposure (self-reported doneness preference, self-assessment of meat doneness and photograph assessment of meat doneness) and to compare HCA concentrations in meats prepared in residences with concentrations in meats prepared in standardized cooking trials. We sought to identify methods for improving estimation of exposure to HCAs.

Materials and methods

Pan frying was chosen as the cooking method for the study. HCA concentrations in meats pan-fried to different doneness levels have been determined in several standardized cooking studies [1, 3, 4] and provided a data set for comparison with the results of our study. Hamburger and beef steak (cut not specified) were the meat types selected, as these are the most frequently consumed pan-fried meats in the study population [15]. Sample sizes for the meat groups and doneness levels were chosen to detect significant differences between doneness levels based on expected variability in measurement reproducibility. A previous standardized cooking study found an average coefficient of variation for HCA concentrations of 33% in pan-fried hamburger [16]. The present study was designed to obtain single samples from 60 households and dual sample, from 10 households. In households providing two samples, the husband and wife each provided a separate pan-fried hamburger sample.

Participants were part of the Agricultural Health Study (AHS) cohort, a prospective health study of pesticide applicators and their families being conducted in Iowa and North Carolina [15]. As part of a larger questionnaire, AHS registrants answered questions about their dietary habits and cooking practices related to meat consumption. Registrants provided information on how frequently they ate hamburger and steak over the past 12 months, how they usually cooked steak and hamburger (choices: pan-fried, baked, grilled, oven-broiled, other, don't eat), and how they usually ate beef steak and hamburger (don't eat, rare, medium rare, medium, medium well, well done, very well done, don't know). This information, as well as the registrant's AHS identifier number, gender and county of residence, and the complementary questionnaire answers and personal information of the registrant's spouse, were obtained on AHS registrants in ten Iowa counties in electronic form. AHS registrants indicating that they consumed pan-fried hamburger or beef steak once or more per week, and ate hamburger either medium, well done, or very well done or steak either well done or very well done, were eligible for the study. A total of 1386 AHS registrants met the study criteria, 22% of the AHS registrants in the ten-county area.

Enrollment and management of the study were conducted by the AHS Field Station at the University of Iowa, Iowa City. The study area was limited to a ten-county area around Iowa City to facilitate acquisition of the samples. The name, phone number and mailing address of the eligible AHS registrants maintained at the Field Station were used to contact potential participants. Participants were selected from a randomly sorted list of eligible AHS registrants and sent an introductory letter briefly explaining the purpose and requirements of the study, and notifying them that they would be contacted by telephone. Registrants contacted by telephone were asked to confirm information on their AHS survey about their pan-frying preferences by answering the question, "Do you usually pan-fry [hamburger/steak] that you eat until it is pan-fried [medium-done/well-done/very well-done]?" with the information in brackets reflecting the registrant's AHS-recorded answers. If they confirmed the information, registrants were asked to participate in the study. In ten households (designated as spouse pairs), both the husband and wife were enrolled. To enroll spouse pairs, one eligible member of the household was interviewed first and asked to provide a sample without informing him/her that the spouse would be interviewed for the study as well. After the participant completed the protocol, his/her spouse was interviewed and asked to provide another sample. An equal number of men and women were enrolled in the study but not within each doneness category.

Participants were sent a sampling kit within a week of the telephone interview. The sampling kit consisted of two zip-lock plastic bags, instructions on how to obtain the sample of the meat, a questionnaire about how the meat was prepared, a disposable Kodak flash camera, and a paper ruler. The instructions contained an introduction asking the participant to prepare the pan-fried hamburger or steak the way he/she usually did without any reference to the participant's AHS-recorded doneness preference. The instructions were presented in text and graphic form to lead the participant through the procedures for cooking, photographing and storing the cooked meat. The questionnaire asked the participant to describe the meat (leanness, thickness, boneless), how the meat was prepared and cooked, to what level of doneness the meat was cooked, whether the participant cooked the meat himself or herself, and how frequently the participant consumed the meat.

Meat samples were initially stored frozen, then shipped on dry ice to Lawrence Livermore National Laboratory where they were stored frozen until analysis. Samples were analyzed for four HCAs, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx), 2-amino-1,6-dimethylfuro[3,2-*e*]imidazo[4,5-*b*]pyridine (IFP), amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), as previously described [17]. These analyses were performed by a Millennium 2010 HPLC system with a diode-array detector (Waters Corp., Milford, MA) and a Shimadzu fluorescence detector using the HPLC column under conditions reported previously [16]. IFP quantification was performed using the extinction coefficient corresponding to PhIP since a synthetic standard is not available. Extraction recoveries were determined by spiking one sample per day with a mixture of DiMeIQx, IFP, MeIQx, and PhIP. Average percent recoveries were 67, 69, 49, and 50 for DiMeIQx, IFP, MeIQx, and PhIP, respectively. Amounts of HCAs reported were corrected for incomplete recoveries.

Quality control samples analyzed repeatedly over the course of these analyses yielded coefficients of variation of 31, 17, 15, and 26% (steak, n = 6) and 49, 33, 18 and 28% (hamburger, n = 5) for DiMeIQx, IFP, MeIQx, and PhIP, respectively. These quality control results were within the range expected for part-per-billion analysis [18]. To account for nondetected HCAs in a sample, when an analyte was not detected, a concentration of one-half the value between 0 and the lowest value detected for the analyte was assigned to the sample. These values were 0.005, 0.04, 0.015, and 0.02 ng/g for DiMeIQx, IFP, MeIQx, and PhIP, respectively. The number of samples assigned these values were 19 (27%), 40 (57%), 8 (11%), and 21 (30%) for DiMeIQx, IFP, MeIQx, and PhIP, respectively.

HCA concentrations for the different doneness levels were compared within each meat type using three classifications of the samples. A sample was assigned to a doneness level based on the participant's doneness level preference recorded in the AHS survey (''Survey''), the doneness level recorded by the participant on the sample questionnaire (''Questionnaire''), and the photographs of the sample (''Photograph''). The possible responses and the corresponding doneness levels to which samples were assigned are shown in Table 1. To assign a sample to a doneness level by photograph analysis, six people (four women and two men, ages 35–58) were separately shown four photographs of each sample taken by the participant (uncooked meat, during cooking, after the meat was cooked and the meat sliced to show the interior) and asked whether or not they saw redness in the interior of the sample and if the sample was not browned, browned, or blackened. ''Not browned'' was selected to represent a discernible change in meat color to differentiate it from ''browned'' meat. To average the visual ratings for each sample, each potential combination of answers was converted to a numeric value (1, 2 or 3) and the average of the six values rounded to the nearest whole number. The averaged values were converted to doneness levels as follows: 1 = Medium, 2 = Well done, and 3 = Very well done (Table 1).

Table 1. Methods for classifying sample doneness level

Method	Question asked of participant:	Possible responses and doneness level[a]
AHS Survey	When you eat_____, how do you usually eat it?	Medium (M) Well done (WD) Very well done (VWD)
Sample questionnaire	How was the meat cooked?	Rare, Medium rare, Medium (M); Medium well, Well done (WD); Very well done (VWD)
Photograph analysis	Is the meat surface: Not browned, browned or blackened?	Not browned and red; not browned and not red; Browned and red (M)
	Is the interior of the meat: red or not red	Browned and not red; Blackened and red (WD); Blackened and not red (VWD)

[a] Letters in parentheses refer to doneness level to which a sample was assigned based on the preceding response (M = Medium, WD = Well done, VWD = Very well done).

Results

We targeted a total of 80 samples for our study, and of the 142 people called to solicit their participation, 23 were unable to participate, 49 declined to participate, and 70 agreed, giving us an enrollment of 59% for the study ($70 \div (70 + 49) \times 100$, Table 2). AHS registrants claiming a preference for very well done pan-fried meats were limited in the study cohort, and enrollment of the desired number of participants in this doneness category was not achieved. Compliance with the study was excellent and only minimal follow-up with the participants was required for return of the samples. In some cases, the participants did not provide the entire series of photographs requested in the instructions; however, a photograph of the cooked sample was obtained from every participant. One participant provided a sample of pan-fried ground beef rather than a hamburger patty, and this sample was excluded from the study.

Thirty-two of the study participants (46%) indicated a doneness level for their cooked meat that was different from their usual doneness preference recorded in the AHS survey. Almost half of these reclassifications were only one category different from the AHS survey (*e.g.* from medium to medium well), so to have sufficient sample sizes in the three doneness categories of interest, we grouped the sample questionnaire responses into three doneness categories in the following manner: rare/medium rare/medium = medium, medium well/well done = well done, and very well done = very well done. When classified this way, 33% of the study participants indicated on the sample questionnaire a doneness level that was different from their usual doneness preference recorded in the AHS survey (Table 3). Of the 23 participants who reclassified their meat samples to a different doneness level on the sample questionnaire, 11 had stated a preference of very well done on the AHS survey. Of the 31 meat samples reclassified by photograph analysis, 13 were from participants stating a preference of medium on the AHS survey. The average variation of the doneness levels obtained from the six individuals was 15 ± 10% (mean variation ± 1 s.d. of the variation obtained from the six visual ratings of each of the 69 samples; see Methods).

Before comparing HCA concentrations between doneness levels in a sample classification, significant differences between the individual and spouse pair hamburgers within doneness levels were evaluated. Significant differences in all four HCA concentrations were observed between the well done individual and spouse pair hamburgers categorized by the questionnaire and between the medium and well done individual and spouse pair hamburgers categorized by the survey (data not shown). No significant differences were detected between the individual and spouse pair hamburgers categorized by the photographs at any doneness level. Individual and spouse pair hamburger data in each sample classification were pooled for comparison of HCA concentrations at the different doneness levels.

HCA concentrations in the meats were not normally distributed under any sample classification, so pairwise comparisons between doneness levels were conducted with the nonparametric Mann–Whitney *U*-test. No significant differences in HCA concentrations were

Table 3. Distribution of doneness level reclassifications

Classification method response comparison	Doneness reclassification		
	Reduced doneness level	No change in doneness level	Increased doneness level
Survey *vs.* questionnaire	12	47	11
Questionnaire *vs.* photograph	6	38	25

Table 2. Study design and enrollment

Sample type	Sample size	Eligible registrants[a]	Telephone response					Samples received
			Call	No	NM[b]	Other[c]	Yes	
Hamburger[d]	40	1127	73	30	7	3	33	33
Spouse pairs	20	88	27	2	2	3	20	20
Steak	20	171	42	17	7	1	17	17
Totals	80	1386	142	49	16	7	70	70

[a] Registrants fulfilling study criteria. The total number of AHS registrants in the 10-county study area was 6259.
[b] Survey non-match. The telephone respondent did not confirm either cooking method or doneness preference as stated in their AHS survey.
[c] Unable to participate because of illness (3), death (4) or inability to locate subject (1).
[d] Includes single samples from individual households (33) and dual samples from spouse pair households (20). Spouse pairs are the households in which the two residents stated doneness preferences of medium and well done.

observed between any doneness levels of either the pooled hamburger or steak samples categorized by the AHS survey (data not shown). Well done and very well done hamburger concentrations of the four HCAs were significantly different when the samples were categorized by questionnaire and photograph analysis (Figure 1). No hamburger samples were rated as medium when doneness was assigned by photograph analysis.

No significant differences were detected between the doneness levels of the four HCAs when the steak samples were categorized by either the questionnaire or photograph rating (Figure 2). When assigned to doneness level by the questionnaire, HCA concentrations were highest in well done steak whereas when samples were assigned to doneness level by photograph analysis, HCA concentrations were highest in very well done steak.

When categorized by the AHS survey, significant differences in the concentrations of DiMeIQx, IFP, and MeIQx were observed between the medium and well done spouse pair hamburgers (Figure 3). When categorized by questionnaire and photograph analysis there were no significant differences between doneness levels for any of the HCA concentrations in the spouse pair hamburgers. No hamburger spouse pair samples were rated as medium when doneness was assigned by photograph analysis.

Sample variability was considerable with the coefficient of variation (CV, standard deviation/mean) exceeding 100% at most doneness levels for all HCAs (data not shown). The CVs for the pooled hamburger and steak data, averaged across all doneness levels and sample classifications, were 131% and 110%, respectively. Sample reclassification by either method had little effect on this variability. CVs obtained from the quality control samples were similar to previous values obtained in our laboratory for repeated determinations of MeIQx and PhIP in a pan-fried hamburger sample (36% and 24%, respectively [16]).

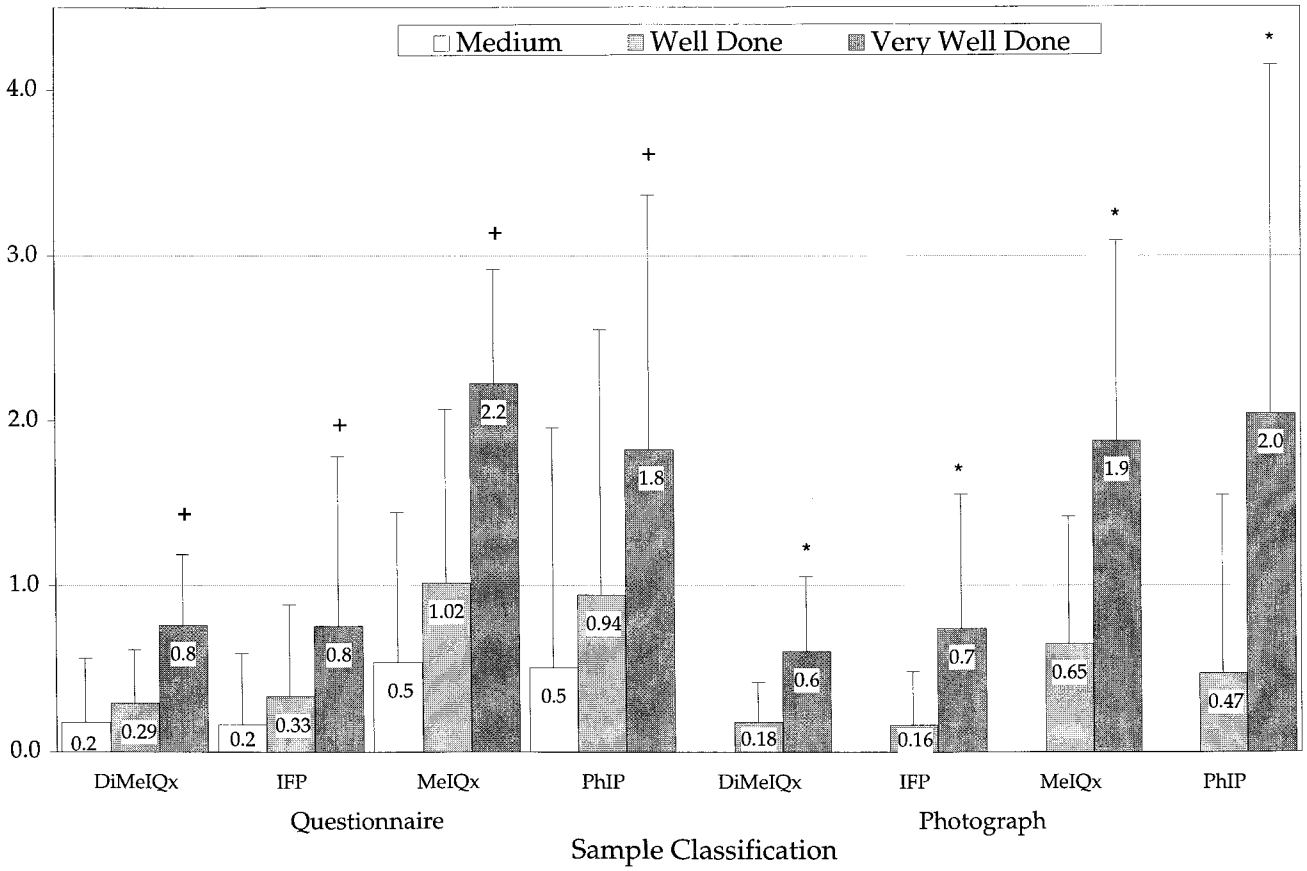

Fig. 1. HCA concentrations (ng/g cooked meat, ±1 standard deviation) in pan-fried hamburgers when samples were categorized by questionnaire and by photograph analysis. Symbol denotes significant differences between corresponding doneness level and all lower levels (+ = $p \leq 0.05$, * = $p \leq 0.005$). Numbers inside bars are the numeric values for the HCA concentrations. Questionnaire-categorized sample sizes for the medium, well done and very well done groups were 13, 36 and 3, respectively. Photograph-categorized sample sizes for the groups were 1, 37 and 14, respectively.

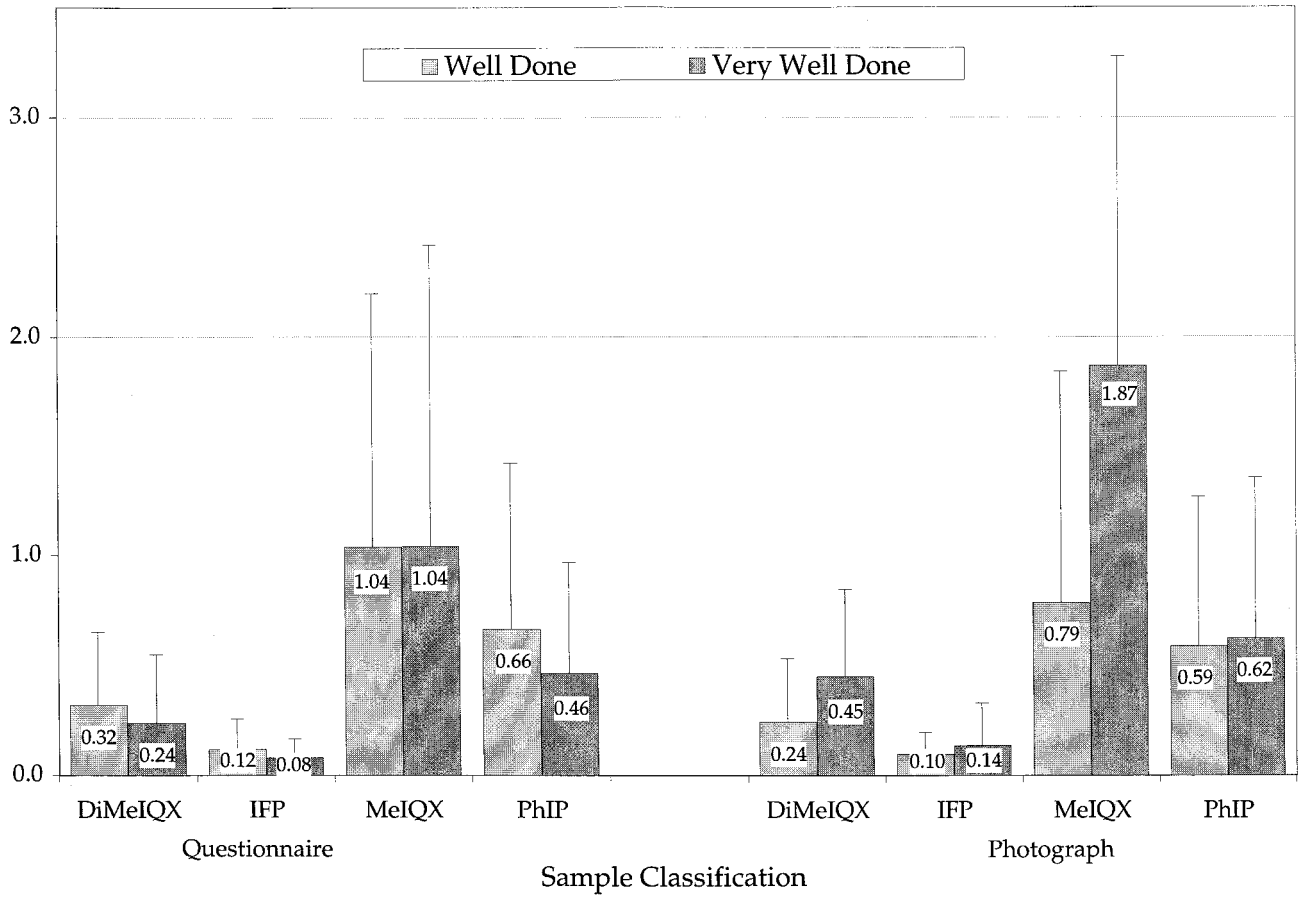

Fig. 2. HCA concentrations (ng/g cooked meat, ± 1 standard deviation) in pan-fried steak when samples were categorized by questionnaire or by photograph analysis. Numbers inside bars are the numeric values for the HCA concentrations. Questionnaire-categorized sample sizes for the well done and very well done groups were 11 and 6, respectively. Photograph-categorized sample sizes for the groups were 13 and 4, respectively.

Discussion

Classification of the samples by photograph analysis produced results most consistent with the relationships between doneness level and HCA concentrations observed in standardized cooking trials with hamburger and steak [1, 3, 4]. In these cooking trials, HCA levels rose with the level of doneness and exhibited the sharpest increase when meats were cooked very well done. When the samples in this study were classified by photograph analysis, HCA levels increased consistently with doneness level and the concentration differences between doneness levels were greatest. Using the AHS survey to categorize the meat samples produced consistent trends between HCA concentrations and doneness level only in the spouse pair hamburgers. Variability in doneness preference is the likely cause for this poor agreement between the AHS survey and HCA concentrations, as 46% of the participants reported a doneness level on the sample questionnaire that was different from their AHS-recorded preference. Reasons why the AHS survey was a good exposure predictor for the spouse pairs are unclear. The spouse pairs may represent a subset of meat consumers with a more consistent and distinguishable doneness preference so that meats from the same meal within the household are prepared differently. Households with such disparate doneness preferences were rare in our study area, comprising less than 2% of the households in the study area. Also, we cannot rule out a study effect on the spouse pairs. Spouse pairs were enrolled sequentially, with the second member being enrolled only after the first member completed the protocol. However, this study design did not prevent the second member from being blind to the contrast we were observing, and may have influenced how that person prepared his/her meat sample.

Using information obtained from either the questionnaire or photographs to classify a sample's doneness

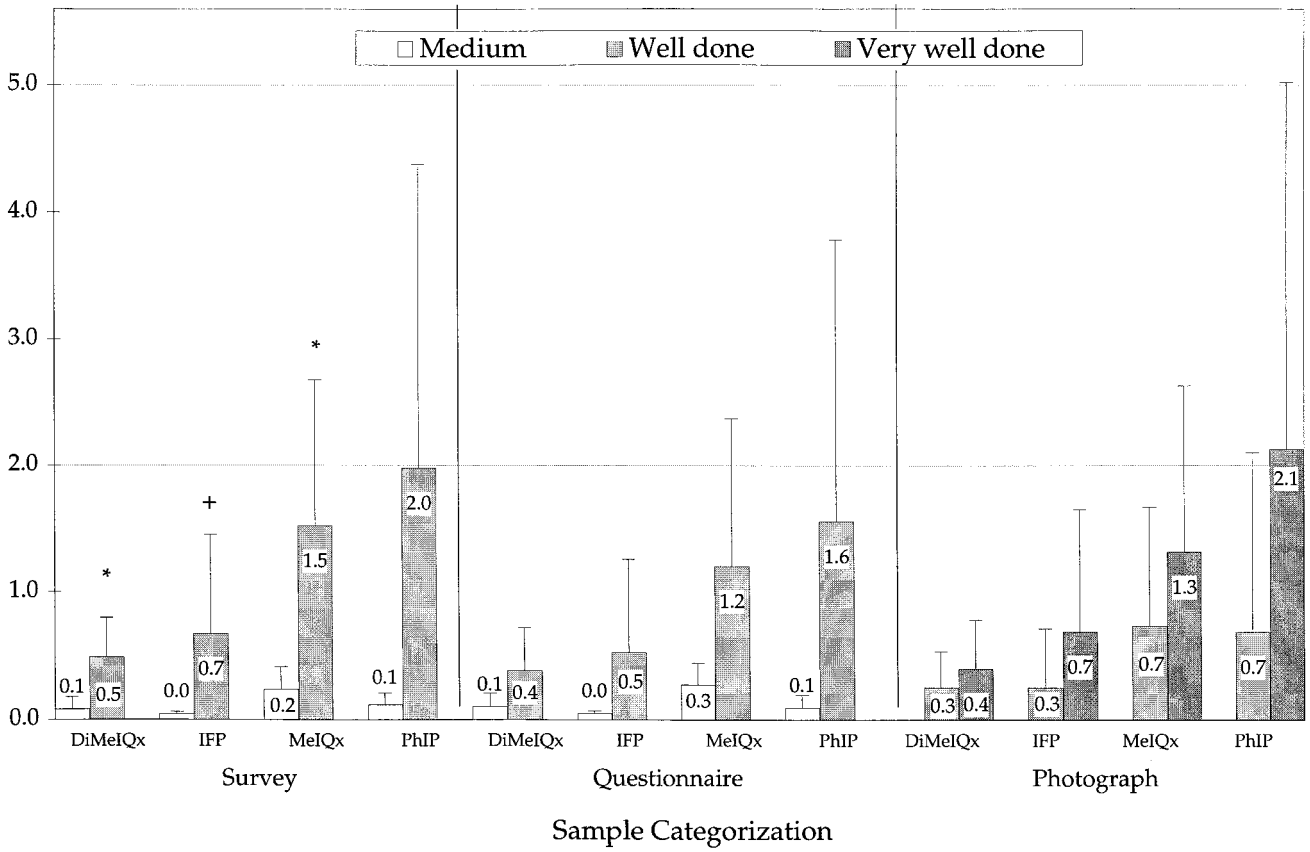

Fig. 3. HCA concentrations (ng/g cooked meat, ±1 standard deviation) in pan-fried spouse pair hamburgers when categorized by AHS survey, questionnaire or by photograph analysis. Symbol denotes significant differences between corresponding doneness level and all lower levels (+ = $p \leq 0.05$, * = $p \leq 0.005$). Numbers inside bars are the numeric values for the HCA concentrations. Survey-categorized sample sizes for the medium and well-done groups were 10 and 10, respectively. Questionnaire-categorized sample sizes for the medium and well-done groups were 7 and 13, respectively. Photograph-categorized sample sizes for the well done and very well done groups were 15 and 5, respectively.

level produced trends in HCA meat concentrations consistent with our expectation that concentrations would increase with the level of doneness. Trends were best with the photograph analysis (based on the lower *p*-values obtained for the doneness comparisons under this sample classification) which suggest that visual descriptors (interior and surface colors of the cooked meats) or depictions of cooked meats (photographs of meats cooked to different doneness levels) are better predictors of HCA concentration in cooked meats than the traditional terms used to describe meat doneness (medium, well done and very well done). Doneness level terminology may introduce subjective error that could result in exposure misclassification. In this study, participants were given no definitions of the doneness levels, so interindividual variability in assessing doneness would be expected to influence sample classification. By developing explicit criteria for doneness, and averaging the assessments of six people, subjective error was reduced with photograph analysis, and a better classification of samples into more representative doneness levels was achieved. Epidemiologic studies using photographs to characterize HCA exposure [9, 11, 14] have found relationships between high HCA intake and cancer. Therefore, in observational epidemiologic studies, more graphic descriptors of cooked meat or visual depiction of cooked meats may serve as better HCA exposure indicators than doneness assessment.

HCA concentrations in the cooked hamburger and steak samples in this study were lower than the concentrations reported for these meats in standardized cooking trials (Table 4). The levels observed in this study are most comparable to those of Skog *et al.* [3], and indicate that there are substantial differences in HCA concentrations in meats prepared in residential settings in Iowa compared with the standardized conditions used to date. The variability in HCA concentrations in our study was also substantially higher than

Table 4. HCA concentrations (ng/g cooked meat) in pan-fried meats from standardized cooking trials and this study

Study	Meat	Conditions (°C/min)	HCA concentration range (ng/g)		
			DiMeIQx	MeIQx	PhIP
Knize *et al.*, 1994 [1]	Hamburger	150–230/4–20	0–1.0	0–7.3	0–32.0
Skog *et al.*, 1995 [3]	Hamburger	150–225/5–7	0–0.8	0–2.2	0.01–1.1
Skog *et al.*, 1995 [3]	Steak	150–225/5–7	0.02–0.6	0.02–1.6	0.06–1.8
Sinha *et al.*, 1998 [4]	Hamburger	180–191/6–20	–	0.34–4.3	0–2.3
Sinha *et al.*, 1998 [4]	Steak	180–191/15–33	–	1.3–8.2	1.9–23.2
Norrish *et al.*, 1999 [19]	Hamburger	200/–	0.03–0.29	0.29–1.12	0–3.96
Norrish *et al.*, 1999 [19]	Steak	200/–	0.06–0.80	0–3.80	0.29–7.33
This study[a]	Hamburger	–	0.2–0.8	0.5–2.2	0.5–1.8
This study[a]	Steak	–	0.3–0.2	1.0–1.0	0.7–0.5

[a] Concentration ranges taken from sample distribution based on questionnaire-assigned doneness level. IFP concentrations in pan-fried hamburger and steak in this study were 0–0.80 and 0, respectively.

observed in the standardized cooking trials (30–40%). Our findings suggest that there are other factors that influence the formation of HCAs in residentially cooked meats not accounted for in the standardized cooking trials. Information obtained from the sample questionnaire and photographs indicate that a wide variety of meat cuts (of steak), meat preparations (thawing and seasoning), and cooking utensils were used by the participants. These factors may explain the differences in HCA concentrations observed between the home-cooked and standardized-cooked meats.

There were several elements to our study that limit its use in HCA exposure assessment. The small number of samples and lack of replicate sampling resulted in large variability in HCA concentrations. Our participants were drawn from a small regional area and a relatively homogeneous population. Therefore, extrapolation of HCA concentration data from this study to cooked meats in the larger US population is problematic. We are currently conducting a larger study with the same population examining HCA concentrations in grilled chicken, steak, hamburger and pork, with replicate sampling in a subset of homes. The use of the AHS survey to evaluate doneness level of a single sample can also be questioned as the survey was designed to assess diet over 12 months and was administered several years before the participants were enrolled in this study. Information provided at the time the meat was prepared, or obtained from visual inspection of the meat, as was done with the other two sample categorizations, would be expected to be better predictors of HCA concentration. To properly evaluate AHS-recorded doneness preference as a predictor of HCA concentration in the diet, repeated measurement of this response, as well as of cooked meat samples over time, is needed so that inherent variability in dietary preference and HCA concentrations in cooked meat can be accounted for.

In conclusion, we found significant differences in HCA levels when the cooked meat samples were visually assessed for their degree of doneness, but not when doneness preference was used to classify the degree of doneness. We conclude that visual aids will improve exposure assessment in epidemiological investigations of HCA intake.

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