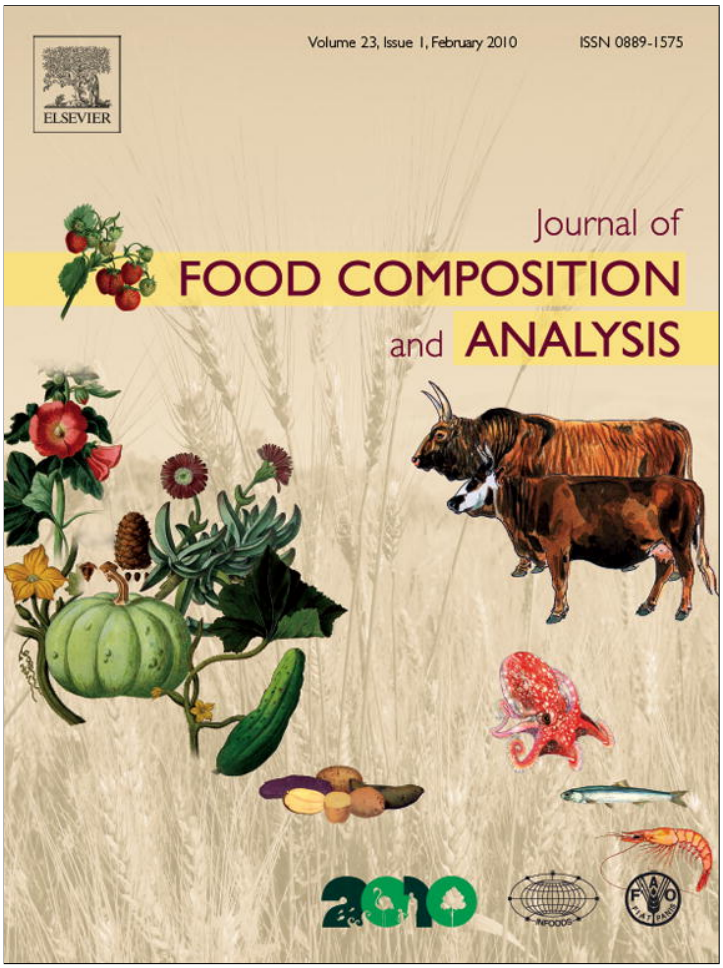

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Original Article

Heterocyclic amines content of meat and fish cooked by Brazilian methods

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ABSTRACT

Heterocyclic amine (HCA) concentrations were measured in meat and fish samples cooked by pan-frying, grilling and *churrasco* (Brazilian barbecue) to various levels of doneness in accordance with the cooking methods most commonly used in Brazil. HCAs were extracted by the Blue-rayon® absorption method and measured by liquid chromatography–mass spectrometry. 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), and 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx) were sharply increased in very well-done meats and fish. HCA levels varied somewhat across cooking methods: levels of PhIP (ng/g) in very well-done, non-marinated samples were particularly high for *churrasco* (31.8 in the exterior of the sample), compared to lower levels for grilled (16.3), and pan-fried beef (0.58). On comparison across foods, chicken contained higher HCA levels than other non-marinated samples. For example, PhIP levels (ng/g) in very well-done pan-fried foods were 34.6 for chicken with the skin, 0.58 for beef, 7.25 for pork, 2.28 for sardines, and 7.37 for salmon cooked with the skin. HCA levels were lower in marinated meats and fish than in non-marinated samples, except for pan-fried salmon. This study provides valuable information which will allow the estimation of dietary HCA exposure using an epidemiologic questionnaire and the investigation of the association of HCA intake with cancer risk in Brazil.

1. Introduction

Heterocyclic amines (HCAs) are formed from the reaction of creatine or creatinine, amino acids, and sugars in meat and fish cooked at high temperatures. The formation of HCAs increases with the temperature and duration of cooking and varies with the type of meat and cooking method, with the highest levels produced by pan-frying, barbecuing, and grilling (Skog et al., 1998). These cooking methods are not equivalent across countries and populations, however. One example is *churrasco*, or Brazilian barbecue. Whereas barbecuing is usually defined as cooking meat or fish for a relatively short time over direct heat, usually from hot charcoals or an open fire, *churrasco* is typically characterized by cooking meat or fish using indirect heat or low-level direct radiant heat from charcoals or embers, at lower temperatures and over longer cooking times. This method might result in different, possibly lower, HCA levels.

Following experimental studies showing that HCAs were mutagenic, and carcinogenic to non-human primates (Sugimura et al., 2004), it has been hypothesized that high HCA intake may be associated with an increased risk of cancer at several sites. In particular, the role of HCA has been most extensively studied for colorectal cancer, following findings that red and processed meats increase the risk of this disease (World Cancer Research Fund and American Institute for Cancer, 2007). However, the findings from the few epidemiological studies that have specifically tested the association of HCA intake and cancer risk have been inconsistent (Butler et al., 2003; Gunter et al., 2005; Sinha et al., 2005; Wu et al., 2006). This might reflect the difficulties in assessing exposure since there is only limited information on the levels of these compounds in foods.

Colorectal cancer incidence in Brazil increased two-fold between 1969 and 1993, but as of 2002 was still lower than that in the US and Japan (Curado et al., 2007; Instituto Nacional do Cancer, 2003). Meat consumption (g/capita/day) in Brazil increased from 82.2 in 1970 to 221.9 in 2003, and was higher than

Abbreviations: AP, atmospheric pressure; ESI, electrospray ionization; HCA, heterocyclic amine; IS, internal standard; LC–MS, liquid chromatography–mass spectrometry; QFFQ, quantitative food frequency questionnaire; SIM, selected ion monitoring; 4,7,8-TriMeIQx, 2-amino-3,4,7,8-tetramethyl-3H-imidazo[4,5-f]-quinoxaline.

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that in Japan (117.8 in 2003) and lower than that in the US (337 in 2003) (FAOSTAT, 2009). Of interest, in contrast to the marked increase in colorectal cancer incidence observed among Japanese who migrated to Hawaii and California (Shimizu et al., 1987; Tominaga, 1985), 1969–1979 colorectal cancer rates did not increase among first-generation Japanese migrants to São Paulo, despite a high red meat intake and a higher body mass index, compared to Japanese in Japan (Tsugane et al., 1990, 1994, 1996). Although more recent data from 2000 showed that mortality from colorectal cancer among first-generation Japanese migrants to São Paulo had become similar to that of Japanese in Japan (Iwasaki et al., 2004, 2008), these descriptive epidemiological data led to the hypothesis that Japanese Brazilians might consume smaller amounts of HCAs, despite a high red meat intake, compared to Japanese in Japan; or might be more likely exposed to protective factors, as suggested by their high intake of fruits and vegetables (Tsugane et al., 1996); or both.

Clarification of the role of HCAs in the etiology of human cancer requires the accurate assessment of HCA exposure. Here, since information on the HCA content of meat and fish in Brazil has not been available, HCA concentrations were measured in commonly consumed meats and fish cooked by the methods typically used in Brazil.

2. Materials and methods

2.1. Food samples

In this study, HCA concentrations were analyzed in beef, chicken, pork, hamburger, sausage, sardine, and salmon cooked by various methods typically used in Brazil and to various levels of doneness (Table 1). They were selected from the food list included in a quantitative food frequency questionnaire (QFFQ) developed as part of a colonoscopy-based case–control study of colorectal adenoma among Japanese Brazilians to assess the intake of specific foods, nutrients, and HCAs (Sharma et al., 2009).

In accordance with the cooking practices of Japanese Brazilians, the cooking methods in this study were defined as follows: pan-fried foods were cooked in a frying-pan or griddle with oil, grilled foods were cooked in a frying-pan or griddle without oil, and *churrasco* was cooked on a grid over charcoal without oil for a long period of time. For the HCA analyses, food samples were purchased from a local supermarket. Beef (top sirloin, about 1 cm thick, 134 g on average) was pan-fried with a half-tablespoon of soy and rapeseed-blended oil per slice and grilled, and beef (rump steak, about 10 cm thick, 344 g on average) was cooked by *churrasco*. Chicken (breast, 5 cm square size, 60 g on average) with and without skin was pan-fried with one cup of soy and rapeseed-blended oil per 500 g and grilled, chicken (breast, fillet, 155 g on average) with and without skin was cooked by *churrasco*. Pork (tenderloin, about 1.5 cm thick, 42 g on average) was pan-fried with a half-tablespoon of soy and rapeseed-blended oil per piece and pork (rib) was cooked by *churrasco*. Hamburger (partially thawed hamburger, 161 g on average) was pan-fried with one tablespoon of soy and rapeseed-blended oil per slice and sausage (pork wiener sausage, 65 g on average) was cooked by *churrasco*. Whole sardine (fresh, 125 g on average) was pan-fried with two cups of soy and rapeseed-blended oil per 500 g. Salmon fillet with and without skin (fresh) was pan-fried with two cups of soy and rapeseed-blended oil per 500 g and whole half salmon (fresh, 1501 g on average) was cooked by *churrasco*. Some of the samples of beef, pork, chicken, sardine and salmon were marinated according to typical recipes, which were obtained from a Japanese Brazilian dietitian (Table 2). Food samples were pan-fried and grilled using a Teflon-coated frying-pan (Fujimaru Co., Ltd., Kanagawa, Japan) and gas stoves (Maruzen Co., Ltd., Tokyo, Japan) and were cooked by *churrasco* using a *churrasco* grill (Metalúrgica Mor S.A., Rio Grande do Sul, Brazil) out of doors. Drippings from grilling and left-over pan-frying oil were disposed of. The cooking of the samples was performed mainly by Japanese dietitians in the Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo under the supervision of a Japanese Brazilian, except for *churrasco* pork, which was provided by a Brazilian restaurant in Tokyo.

The degree of doneness for the different types of foods was primarily defined based on the maximum internal temperature, as follows: an internal temperature of 60 °C was rare, 70 °C was medium, 80 °C was well-done, and 90 °C was very well-done. Four levels of doneness were applied to beef, from rare to very well-done, and three levels to chicken, pork, hamburger, sardine and salmon, from medium to very well-done. No doneness level was applied to *churrasco* sausage and pork. Internal temperature was monitored during cooking using a digital thermometer (Sato Keiryoki Mfg. Co., Ltd., Tokyo, Japan). In addition, the level of surface browning was assessed as one of the following categories: not browned; moderately browned; well browned; and very well browned/charred (Sinha et al., 1998b).

Information was collected on the weight of food samples before and after cooking to calculate the percentage weight loss due to cooking, as well as on total cooking time. The surfaces of the cooked foods were photographed to record the level of browning. Since *churrasco* cooking is done using a large cut of beef which is

Table 1
Summary of food items and cooking methods.

Food item	Skin	Cooking method	Marinade	Doneness level
Beef	Not applicable	Pan-fried	With or without	Rare, medium, well-done, very well-done
	Not applicable	Grilled	With or without	Rare, medium, well-done, very well-done
	Not applicable	Churrasco	With or without	Rare, medium, well-done, very well-done
Hamburger	Not applicable	Pan-fried	Without	Medium, well-done, very well-done
Sausage	Not applicable	Churrasco	Without	Not applicable
Pork	Not applicable	Pan-fried	With or without	Medium, well-done, very well-done
	Not applicable	Churrasco	Without	Not applicable
Chicken	With or without	Pan-fried	With or without	Medium, well-done, very well-done
	With or without	Grilled	With or without	Medium, well-done, very well-done
	With or without	Churrasco	With or without	Medium, well-done, very well-done
Sardine	Not applicable	Pan-fried	With or without	Medium, well-done, very well-done
Salmon	With or without	Pan-fried	With or without	Medium, well-done, very well-done
	With or without	Churrasco	Without	Medium, well-done, very well-done

Table 2
Recipe for marinated samples.

Food item	Cooking method	Marinade
Beef	Pan-fried	Four slices of beef marinated with four cloves of garlic, half onion, one tablespoon of salt, and three teaspoons of black pepper for half an hour in a refrigerator before pan-frying.
	Grilled	Four slices of beef marinated with four cloves of garlic, three tablespoons of vinegar, one tablespoon of salt, and three teaspoons of black pepper for half an hour in a refrigerator before grilling.
	Churrasco	Beef per kg marinated with five cloves of garlic, one onion, one tomato, ten bunches of parsley, one tablespoon of salt, half-tablespoon of black pepper, and two tablespoons of soy oil for over night in a refrigerator.
Pork	Pan-fried	Pork per 500 g marinated with one clove of garlic, half-tablespoon of salt, one teaspoon of black pepper, quarter cup of white wine and juice from one lime for half hour in a refrigerator before pan-frying.
Chicken	Pan-fried	Chicken per 500 g marinated with five cloves of garlic, half onion, half-tablespoon of salt, and one teaspoon of black pepper for 1 h in a refrigerator before pan-frying.
	Grilled	Chicken per kg marinated with one and quarter cup of vinegar and half-tablespoon of salt for half hour in a refrigerator before grilling.
	Churrasco	Chicken per kg marinated with two tomatoes, ten bunches of parsley, juice from a half lime, three tablespoons of pinga (a distilled alcoholic beverage in Brazil made from sugarcane), one tablespoon of salt and two teaspoons of black pepper for 2 h in a refrigerator before churrasco.
Sardine	Pan-fried	Sardine per 500 g marinated with a half-tablespoon of salt, one teaspoon of black pepper, one tablespoon of pinga, and juice from one-quarter of lime for 2 h in a refrigerator before pan-frying.
Salmon	Pan-fried	Salmon per 500 g marinated with three cloves of garlic, a half-tablespoon of salt, one teaspoon of black pepper, three tablespoons of pinga, and juice from quarter lime for 1 h in a refrigerator before pan-frying.

usually carved on serving, *churrasco* beef was divided into exterior and interior portions for analysis. All cooked food samples were stored at −20 °C until analysis.

2.2. Laboratory analysis

2.2.1. Reagents and standards

In this study, the following ten HCA standards were used for analysis (Table 3). 2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ) was purchased from Toronto Research Chemicals (Downsview, Canada). 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 (4,8-DiMeIQx), 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline (7,8-DiMeIQx), 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC), and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) were purchased from Funakoshi Pharmaceutical Co. Ltd. (Tokyo, Japan). 3-Amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) was purchased from Wako Pure Chemical Industries (Osaka, Japan). 3-Amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2) and 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1) were kindly provided by Dr. H. Hayatsu, Professor Emeritus of Okayama University. 2-Amino-3,4,7,8-tetramethyl-3*H*-imidazo[4,5-*f*]-quinoxaline (4,7,8-TriMeIQx: Toronto Research Chemicals) was used as an internal standard (IS). Each HCA was dissolved in methanol to make a stock solution at a concentration of 0.1 mg/mL, except 4,7,8-TriMeIQx, which was dissolved at a concentration of 1 mg/mL. Blue-rayon® was obtained from Funakoshi Pharmaceutical Co. Ltd. (Tokyo, Japan). 0.1 M HCl, 6 M NaOH, MeOH, n-hexane, and CH_2Cl_2 were purchased from Sigma–Aldrich Japan (Tokyo, Japan), and trichloroacetic acid and 28% NH_3, from Wako Pure Chemical Industries (Osaka, Japan). Ammonium acetate was purchased from Nacalai Tesque (Kyoto, Japan). LC–MS grade acetonitrile and water used as mobile phase were purchased from Kanto Chemicals (Tokyo, Japan). MeOH, n-hexane, and CH_2Cl_2 were of HPLC grade and other chemicals were of analytical-reagent grade.

Table 3
List of heterocyclic amines measured in this study.

Name	Abbreviation
2-Amino-9*H*-pyrido [2,3-b] indole	AαC
2-Amino-3,4,8-trimethylimidazo [4,5-*f*] quinoxaline	4,8-DiMeIQx
2-Amino-3,7,8-trimethylimidazo [4,5-*f*] quinoxaline	7,8-DiMeIQx
2-Amino-6-methyldipyrido [1,2-a: 3′,2′-d] imidazole	Glu-P1
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-1-methyl-6-phenylimidazo[4,5-*b*]pyridine	PhIP
3-Amino-1,4-dimethyl-5*H*-pyrido[4,3-b]indole	Trp-P-1
3-Amino-1-methyl-5*H*-pyrido [4,3-b]indole	Trp-P-2

2.2.2. Sample preparations

Extraction of HCAs from food samples was carried out by modification of a previously reported method (Kataoka et al., 2002). Whole cooked foods were chopped and ground in a food processor (National, Osaka, Japan) for most samples, while half or one-third of cooked foods were used for extraction in several large samples. Three aliquots (5 g each) were taken from well-grounded and blended samples and each aliquot was homogenized with 500 mL of 0.1 M HCl containing 100 ng of 4,7,8-TriMeIQx (IS) with a food mixer (National, Osaka, Japan). After centrifugation (Beckman Coulter, Inc., California, USA) at 3100 rpm for 30 min, the precipitate was re-extracted with 300 mL of 0.1 M HCl. Trichloroacetic acid was added to the combined supernatants at a final concentration of 5% and the mixture was stored at 4 °C overnight. After centrifugation at 3,100 rpm for 30 min, the supernatant was neutralized with 6 M NaOH, and insoluble materials were removed by filtration. HCAs in the filtrate were extracted by the Blue-rayon® adsorption method (Hayatsu, 1992). Briefly, one 500-mg portion of Blue-rayon® was added to the filtrate and the mixture was shaken for 20 min. After removal of the Blue-rayon® and filtration through a nylon mesh, the second portion was added to the filtrate and the mixture was shaken for a further 20 min. After removal of the Blue-rayon® and filtration through a nylon mesh, the combined Blue-rayon® was washed twice with 150 mL of distilled water and dried with a paper towel. HCAs adsorbed on the Blue-rayon® were then eluted twice with 100 mL of MeOH-28% NH_3 (50:1) and once with 50 mL of MeOH–28% NH_3 (50:1). The combined eluate was evaporated to dryness with a rotary evaporator (Iwaki Co., Ltd., Tokyo, Japan) at 37 °C, and the residue was dissolved in 6 mL of MeOH, transferred to a 10 mL Pyrex glass tube with a PTFE-lined screw-cap, and concentrated under vacuum (Genevac Ltd., Ipswich, England). The residue was dissolved in 1 mL of MeOH, centrifuged at 3100 rpm for 10 min, and the supernatant was collected and concentrated under vacuum. The residue was dissolved in 2 mL of 0.1 M HCl. After washing with

2 mL of n-hexane, the aqueous layer was adjusted to pH > 10 with 28% NH_3, extracted twice with 2 mL of CH_2Cl_2, and the organic layer was concentrated under vacuum. The residue was dissolved in 0.2 mL of MeOH and used for liquid chromatography–mass spectrometry (LC–MS) analysis.

2.2.3. Identification and quantification of HCAs

HCA levels were determined in triplicate samples of each cooked food by modification of a previously reported LC–MS method (Kataoka and Pawliszyn, 1999). The LC–MS system was a Model 1100 series LC coupled with an atmospheric pressure (AP) electrospray ionization (ESI) MS (Agilent Technologies, Boeblingen, Germany). A Chromolith RP-18e (100 mm × 4.6 mm; Merck, Darmstadt, Germany) was used for LC separation under the following conditions: column temperature, 30 °C; mobile phase, 10 mM ammonium acetate (solvent A)/acetonitrile (solvent B); and flow rate, 1.0 mL/min with a run time of 19 min. The gradient program was 15% B in A, from 0 to 4 min; 15–40% B in A, from 4 to 14 min; 40% B in A, from 14 to 17 min; and a return to initial conditions in 2 min. ESI-MS conditions were as follows: nebulizer gas N_2 (40 psi); drying gas, N_2 (10 L/min, 350 °C); fragmentor voltage, 90 V; capillary voltage, 3500 V; ionization mode, positive mode; selected ion monitoring (SIM) for $[M+H]^+$ of each compound, *m/z* 184 (AαC), *m/z* 198 (Glu-P-1 and Trp-P-2), *m/z* 199 (IQ), *m/z* 212 (Trp-P-1), *m/z* 213 (MeIQ), *m/z* 214 (MeIQx), *m/z* 225 (PhIP), *m/z* 228 (7,8-DiMeIQx and 4,8-DiMeIQx) and *m/z* 242 (4,7,8-TriMeIQx); and dwell times for the ions in SIM, 63 ms. LC–MS data were processed with an HP ChemStation (Agilent Technologies, Waldbronn, Germany). The calibration curves for HCAs were constructed from the peak height ratios of analyte to the IS (4,7,8-TriMeIQx), and the correlation coefficients were above 0.9990. The detection limits giving a signal-to-noise ratio of 3 under LC–MS conditions in this study were 0.11 ng/mL (AαC), 0.21 ng/mL (Glu-P-1), 0.68 ng/mL (Trp-P-1), 0.88 ng/mL (Trp-P-2), 0.17 ng/mL (IQ), 0.42 ng/mL (MeIQ), 0.14 ng/mL (MeIQx), 0.12 ng/mL (PhIP), 0.33 ng/mL (7,8-DiMeIQx) and 0.45 ng/mL (4,8-DiMeIQx). The intra-day and inter-day relative standard deviations were below 4.0% and 6.9%, respectively (n = 5).

2.3. Statistical analysis

Mean values for PhIP, MeIQx, and 4,8-DiMeIQx (ng/g) were calculated based on triplicate samples per single cooked food which were analyzed independently from extraction. Total HCA level was defined for this study as the sum of PhIP, MeIQx, and 4,8-DiMeIQx. Since PhIP was a major HCA in cooked foods, PhIP levels in very well-done, non-marinated samples were compared across cooking methods and food items using a regression model. In addition, PhIP levels were also compared between very well-done, non-marinated samples with and without skin, and between very well-done samples with and without marinade. All p-values reported are two-sided, and significance level was set at $p < 0.10$. All statistical analyses were performed with SAS software version 9.1 (SAS Institute, Inc., Cary, NC).

3. Results

Proportions of detected samples and HCA concentrations in cooked meats and fish are presented in Tables 4–8. Overall, PhIP, MeIQx, and 4,8-DiMeIQx were detected in the majority of very well-done meats and fish (Table 4), although values varied by food type and cooking conditions (Tables 5–8). Levels of PhIP were considerably higher than those of MeIQx and 4,8-DiMeIQx, with the highest detected level of PhIP of 47.3 ng/g in very well-done, non-marinated *churrasco* chicken with skin (Table 7) versus the highest detected levels of MeIQx and 4,8-DiMeIQx of 15.4 and 3.67 ng/g, respectively, for very well-done, non-marinated *churrasco* beef (Table 5). PhIP, MeIQx, and 4,8-DiMeIQx were also detected in some of the medium and well-done foods, but at substantially lower levels than in the very well-done foods (Table 4). In contrast, IQ, MeIQ, 7,8-DiMeIQx, AαC, Glu-P1, Trp-P-1 and Trp-P-2 were not detected in any of the cooked foods (data not shown).

HCA content differed somewhat by cooking method. PhIP levels (ng/g) in very well-done, non-marinated beef were significantly higher for *churrasco* (31.8 for the exterior portion), than for grilling (16.3) and pan-frying (0.58) (Tables 5 and 9). Similarly, PhIP values were also significantly higher for *churrasco* than pan-frying and grilling for chicken (Tables 7 and 9), and than pan-frying for fish (Tables 8 and 9).

When HCA content was compared across foods, chicken contained higher HCA levels than beef, pork, sardine and salmon in the non-marinated samples (Tables 5–8 and 10). In particular, PhIP levels in very well-done, non-marinated samples were significantly higher for chicken with skin than for beef, pork, sardine and salmon with skin (Table 10). For example, PhIP levels (ng/g) in the very well-done pan-fried samples were 34.6 for chicken with skin, 0.58 for beef, 7.25 for pork, 2.28 for sardine, and 7.37 for salmon with skin. In addition, among very well-done, non-marinated samples, pan-fried and *churrasco* chicken cooked with skin contained significantly higher PhIP levels than samples cooked without skin, although no difference was found between chicken grilled with and without skin (Table 11). Meanwhile, PhIP levels did not differ between pan-fried salmon with and without skin, while *churrasco* salmon cooked with skin contained significantly lower PhIP levels than samples cooked without skin (Table 11).

Interestingly, we also saw an effect of marinade on HCA levels in cooked meats and fish (Tables 5–8 and 12). PhIP levels were significantly lower in marinated than non-marinated meats and fish, except for pan-fried salmon. A marked difference was observed for PhIP levels in very well-done foods: the PhIP level in very well-done chicken pan-fried with skin was 34.6 ng/g when not marinated, for example, versus 0.79 ng/g when marinated (Tables 7 and 12).

Table 4
Proportions of detected samples and summary of heterocyclic amine levels according to doneness level.

Doneness level	No. of samples	MeIQx		4,8-DiMeIQx		PhIP	
		Detected sample (%)	Median (minimum, maximum)[a] (ng/g)	Detected sample (%)	Median (minimum, maximum)[a] (ng/g)	Detected sample (%)	Median (minimum, maximum)[a] (ng/g)
Rare	7	0	–	0	–	29	0.30 (0.10, 0.49)
Medium	30	17	0.19 (0.02, 0.34)	30	0.10 (0.05, 0.26)	33	0.29 (0.02, 1.73)
Well-done	30	57	0.14 (0.06, 1.09)	33	0.20 (0.11, 0.39)	60	0.63 (0.01. 12.0)
Very well-done	30	100	1.61 (0.22, 15.4)	87	0.70 (0.21, 3.67)	97	6.17 (0.05, 47.3)

[a] Among detected samples MeIQx: 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; 4,8-DiMeIQx: 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.

Table 5
Heterocyclic amine levels (HCAs) in cooked beef.

Food item	Cooking method	Marinade	Doneness level	Weight loss (%)	Duration of cooking (min)	Internal temperature (°C)	MeIQx (ng/g)[a]	4,8-DiMeIQx (ng/g)[a]	PhIP (ng/g)[a]	Total HCA (ng/100 g)[a,b]
Beef	Pan-fried	Without	Rare	9	1.4	55	ND	ND	ND	ND
			Medium	21	2.1	68	ND	ND	0.04	4.3
			Well-done	33	9.6	78	0.07	ND	0.04	10.5
			Very well-done	46	16.4	104	1.43	0.39	0.58	239.4
		With	Rare	0	1.5	62	ND	ND	ND	ND
			Medium	2	2.5	61	ND	ND	ND	ND
			Well-done	23	9.9	76	ND	ND	ND	ND
			Very well-done	45	23.3	85	0.33	ND	0.05	37.9
Beef	Grilled	Without	Rare	7	1.1	54	ND	ND	ND	ND
			Medium	24	6.1	55	ND	ND	ND	ND
			Well-done	24	9.0	73	0.24	ND	0.70	94.1
			Very well-done	41	13.2	85	5.41	1.92	16.27	2360.1
		With	Rare	0	1.1	57	ND	ND	ND	ND
			Medium	18	2.0	67	ND	ND	ND	ND
			Well-done	28	7.4	83	ND	ND	ND	ND
			Very well-done	48	17.2	95	4.86	2.35	4.64	1185.5
Beef	Churrasco	Without	Rare (interior)	5	6.4	31	ND	ND	0.10	10.0
			Medium	31	33.7	56	ND	ND	0.43	42.7
			Well-done	48	59.3	60	ND	ND	0.56	55.6
			Very well-done	52	68.6	92	0.53	ND	1.13	165.6
			Rare (exterior)	5	6.4	31	ND	ND	0.49	49.4
			Medium	31	33.7	56	0.34	ND	1.61	194.9
			Well-done	48	59.3	60	0.56	ND	4.07	463.3
			Very well-done	52	68.6	92	15.4	3.67	31.8	5086.1
		With	Rare	23	9.4	58	ND	ND	ND	ND
			Medium	27	15.7	56	ND	ND	ND	ND
			Well-done	50	28.1	73	0.21	0.28	0.14	62.8
			Very well-done	49	37.3	73	0.43	0.26	0.56	124.7

ND: not detected (limits of detection [ng/g] were 0.0057 for MeIQx, 0.018 for 4,8-DiMeIQx, and 0.0049 for PhIP). MeIQx: 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; 4,8-DiMeIQx: 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.
[a] Mean value of triplicate samples.
[b] Total HCA level was defined as the sum of PhIP, MeIQx, and 4,8-DiMeIQx.

Table 6
Heterocyclic amine levels (HCAs) in cooked hamburger, sausage and pork.

Food item	Cooking method	Marinade	Doneness level	Weight loss (%)	Duration of cooking (min)	Internal temperature (°C)	MeIQx (ng/g)[a]	4,8-DiMeIQx (ng/g)[a]	PhIP (ng/g)[a]	Total HCA (ng/100 g)[a,b]
Hamburger	Pan-fried	Without	Medium	19	6.3	70	ND	ND	ND	ND
			Well-done	27	10.0	84	0.13	ND	0.01	14.3
			Very well-done	42	13.5	100	2.15	0.67	0.33	314.3
Sausage	Churrasco	Without	NA	36	33.8	NA	0.22	ND	0.04	26.2
Pork	Pan-fried	Without	Medium	9	6.4	68	ND	0.23	ND	23.1
			Well-done	41	10.3	88	0.16	0.34	ND	49.7
			Very well-done	43	15.5	101	5.43	2.81	7.25	1549.2
		With	Medium	25	13.8	81	ND	0.26	ND	26.2
			Well-done	33	14.9	86	0.10	0.13	2.22	244.2
			Very well-done	43	18.7	98	1.82	0.47	0.30	259.5
Pork	Churrasco	Without	NA	NA	NA	NA	1.16	ND	6.27	743.1

ND: not detected (limits of detection [ng/g] were 0.0057 for MeIQx, 0.018 for 4,8-DiMeIQx, and 0.0049 for PhIP.) NA: not applicable; MeIQx: 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; 4,8-DiMeIQx: 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.
[a] Mean value of triplicate samples.
[b] Total HCA level was defined as the sum of PhIP, MeIQx, and 4,8-DiMeIQx.

4. Discussion

In this study, the first to measure HCA concentrations in meat and fish cooked by typical Brazilian cooking methods and to various levels of doneness, PhIP, MeIQx, and 4,8-DiMeIQx were detected in most meat and fish samples, and levels were much higher in very well-done cooked samples. This general pattern is consistent with those of previous studies (Sinha et al., 1995, 1998b; Skog et al., 1995, 1997), although levels varied by food type and cooking methods. These data provide important information in estimating HCA exposure and will facilitate investigation of the role of HCAs in the etiology of cancer in Japanese Brazilians.

As an initial comment, it is noted that HCA concentrations in this study were obtained from mean values of three aliquots collected from a single food sample. The results might therefore have been affected by sampling variation and should be interpreted with caution. In addition, although comparison of HCA concentrations across published studies is informative, consideration should be given to differences between studies in food samples, cooking and analytical methods.

Contrary to expectations, PhIP levels in the very well-done, non-marinated beef, chicken and salmon were significantly higher in the *churrasco* samples than in those cooked by other methods in this study. For example, PhIP levels (ng/g) in the non-marinated

Table 7
Heterocyclic amine levels (HCAs) in cooked chicken.

Food item	Skin	Cooking method	Marinade	Doneness level	Weight loss (%)	Duration of cooking (min)	Internal temperature (°C)	MeIQx (ng/g)[a]	4,8-DiMeIQx (ng/g)[a]	PhIP (ng/g)[a]	Total HCA (ng/100 g)[a,b]
Chicken	With skin	Pan-fried	Without	Medium	18	9.5	60	0.02	0.05	0.76	83.2
				Well-done	21	15.3	78	0.06	0.11	0.35	52.0
				Very well-done	50	24.1	98	2.03	2.85	34.6	3952.1
			With	Medium	12	10.9	67	ND	0.08	ND	8.4
				Well-done	24	18.6	77	ND	0.16	ND	15.7
				Very well-done	43	21.3	98	2.13	0.86	0.79	378.7
	Without skin		Without	Medium	20	7.9	67	ND	0.11	ND	11.2
				Well-done	26	10.0	75	0.09	0.33	0.59	101.5
				Very well-done	53	27.3	100	2.21	2.58	20.7	2543.8
			With	Medium	15	10.6	66	ND	ND	ND	ND
				Well-done	18	13.3	77	ND	ND	ND	ND
				Very well-done	27	18.4	91	0.44	0.21	ND	64.4
Chicken	With skin	Grilled	Without	Medium	15	10.2	65	ND	0.25	ND	24.5
				Well-done	22	14.9	67	0.09	0.16	0.67	91.7
				Very well-done	39	25.8	90	1.63	3.33	27.38	3233.9
			With	Medium	17	8.8	60	ND	0.09	ND	8.8
				Well-done	19	13.5	74	ND	0.15	ND	14.8
				Very well-done	29	18.5	85	0.65	0.32	0.29	126.3
	Without skin		Without	Medium	19	9.8	62	ND	0.10	ND	10.4
				Well-done	20	12.3	77	0.22	0.39	2.36	297.5
				Very well-done	35	20.3	92	1.76	3.53	29.47	3475.7
			With	Medium	18	8.8	68	ND	0.10	ND	9.9
				Well-done	21	10.8	74	ND	0.24	ND	23.6
				Very well-done	29	16.3	87	1.01	0.67	0.58	226.0
Chicken	With skin	Churrasco	Without	Medium	NA	22.1	66	0.19	ND	1.73	192.3
				Well-done	NA	30.0	80	0.63	ND	12.0	1267.7
				Very well-done	NA	68.2	85	2.34	1.20	47.3	5082.9
			With	Medium	NA	33.4	64	0.25	ND	0.22	46.9
				Well-done	NA	44.3	71	1.09	ND	0.78	186.8
				Very well-done	NA	64.0	91	2.15	0.55	1.68	437.8
	Without skin		Without	Medium	NA	22.1	66	0.09	ND	0.37	46.6
				Well-done	NA	30.0	80	0.33	ND	2.37	270.1
				Very well-done	NA	68.2	85	1.69	1.18	34.8	3763.4
			With	Medium	NA	33.4	64	ND	ND	ND	ND
				Well-done	NA	44.3	71	ND	ND	ND	ND
				Very well-done	NA	64.0	91	2.68	0.73	3.69	709.7

ND: not detected (limits of detection [ng/g] were 0.0057 for MeIQx, 0.018 for 4,8-DiMeIQx, and 0.0049 for PhIP); NA: not available due to incomplete information; MeIQx: 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; 4,8-DiMeIQx: 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.
[a] Mean value of triplicate samples.
[b] Total HCA level was defined as the sum of PhIP, MeIQx, and 4,8-DiMeIQx.

churrasco beef (exterior portion) were 0.49, 1.61, 4.07, and 31.8 in order from rare to very well-done, versus corresponding levels in the non-marinated grilled beef of undetectable, undetectable, 0.70, and 16.3. Sinha et al. (1998b) reported corresponding PhIP levels (ng/g) in rare to very well-done grilled/barbecued beef of 2.5, 4.7, 7.3, and 30.0, which were comparable with the values for non-marinated *churrasco* beef in the present study, for the very well-done samples at least. As mentioned in the Introduction, *churrasco* requires a longer cooking time than grilling or barbecuing. Even though *churrasco* uses indirect heat, and therefore a lower temperature, its longer cooking time might result in the same or higher levels of HCAs as regular grilling or barbecuing.

The formation of HCAs may be dependent on the type of meat or fish. In the present study, PhIP levels in very well-done, non-marinated foods cooked by the same method were significantly higher in chicken than in beef, pork, sardine and salmon. Sinha et al. (1995, 1998b) reported PhIP levels in very well-done samples of 480 ng/g for grilled/barbecued chicken breasts without skin and bone and 30 ng/g for grilled/barbecued beef steak. Although the pattern was similar, PhIP levels in the very well-done grilled/barbecued chicken in the present study were substantially lower: 29.5 ng/g for very well-done, non-marinated grilled chicken without skin, and 34.8 ng/g for very well-done, non-marinated *churrasco* chicken without skin (Sinha et al., 1995). Regarding PhIP levels in chicken with and without skin, no difference was found in PhIP levels between grilled samples but higher levels among pan-fried and *churrasco* samples with skin were found than in those without skin in the present study. In contrast, Sinha et al. (1995) observed lower PhIP levels in chicken with skin regardless of cooking method.

Inconsistencies in HCA levels among studies might reflect differences in cooking conditions, even for the same foods and cooking methods. For example, the present study found PhIP, MeIQx and 4,8-DiMeIQx levels (all ng/g) in very well-done, non-marinated pan-fried meats of 0.58, 1.43, and 0.39, respectively, for beef, and 7.25, 5.43, and 2.81, respectively, for pork. Skog et al. (1995, 1997) reported similar results, at 1.8, 1.6 and 0.6, respectively, for sirloin steak (Skog et al., 1995), and 13.4, 4.6 and 3.3, respectively, for pork fillet, both cooked by pan-frying at 225 °C (Skog et al., 1997). In contrast, Sinha et al. (1998a,b) reported PhIP and MeIQx levels (ng/g) in very well-done, pan-fried meats of 23.2, and 8.2, respectively, for beef (Sinha et al., 1998b), and undetectable and 3.8, respectively, for pork (Sinha et al., 1998a).

Marinating prior to cooking is used to improve the flavor, tenderness and moistness of the cooked foods. Here, in agreement with previous observations (Nerurkar et al., 1999; Salmon et al., 1997), the present study showed that marination before cooking had an overall reducing effect on HCA formation regardless of food items and cooking methods. Given that recipes for marinade varied

Table 8
Heterocyclic amine levels (HCAs) in cooked fish.

Food item	Skin	Cooking method	Marinade	Doneness level	Weight loss (%)	Duration of cooking (min)	Internal temperature (°C)	MeIQx (ng/g)[a]	4,8-DiMeIQx (ng/g)[a]	PhIP (ng/g)[a]	Total HCA (ng/100 g)[a,b]
Sardine	NA	Pan-fried	Without	Medium	38	5.9	77	ND	ND	ND	ND
				Well-done	47	8.8	90	0.14	ND	0.06	19.7
				Very well-done	54	13.7	101	0.70	0.35	2.28	332.5
			With	Medium	43	5.8	70	ND	ND	ND	ND
				Well-done	37	8.6	77	0.09	ND	ND	9.3
				Very well-done	49	11.3	100	0.36	0.26	0.53	115.1
Salmon	With skin	Pan-fried	Without	Medium	NA	8.0	67	ND	ND	ND	ND
				Well-done	NA	11.6	83	ND	ND	ND	ND
				Very well-done	NA	18.3	102	0.66	ND	7.37	803.2
			With	Medium	NA	9.4	63	ND	ND	0.02	1.7
				Well-done	NA	11.8	74	0.09	ND	0.04	13.4
				Very well-done	NA	17.3	92	1.07	0.45	6.17	769.3
	Without skin		Without	Medium	NA	8.0	67	ND	ND	ND	ND
				Well-done	NA	11.6	83	ND	ND	ND	ND
				Very well-done	NA	18.3	102	0.74	0.26	7.31	831.1
			With	Medium	NA	9.4	63	ND	ND	0.03	3.3
				Well-done	NA	11.8	74	ND	ND	0.03	2.6
				Very well-done	NA	17.3	92	1.59	0.75	7.41	975.0
Salmon	With skin	Churrasco	Without	Medium	21	60.0	68	ND	ND	0.16	16.4
				Well-done	21	90.0	80	ND	ND	2.04	204.5
				Very well-done	32	72.0	84	0.87	0.42	22.55	2383.9
	Without skin		Without	Medium	24	60.0	61	ND	ND	ND	ND
				Well-done	33	90.0	73	ND	ND	ND	ND
				Very well-done	37	72.0	87	0.22	ND	28.8	2899.3

ND: not detected (limits of detection [ng/g] were 0.0057 for MeIQx, 0.018 for 4,8-DiMeIQx, and 0.0049 for PhIP); NA: not available due to incomplete information; MeIQx: 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; 4,8-DiMeIQx: 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.
[a] Mean value of triplicate samples.
[b] Total HCA level was defined as the sum of PhIP, MeIQx, and 4,8-DiMeIQx.

Table 9
PhIP levels in very well-done, non-marinated samples according to cooking method.

Food item	Skin	Cooking method				
		Churrasco	Pan-fried		Grilled	
		PhIP (ng/g)[a]	PhIP (ng/g)[a]	*p*-Value[b]	PhIP (ng/g)[a]	*p*-Value[b]
Beef	Not applicable	31.8[c]	0.58	<0.01	16.3	<0.01
Chicken	With	47.3	34.6	0.04	27.4	<0.01
	Without	34.8	20.7	<0.01	29.5	0.09
Salmon	With	22.5	7.37	<0.01	–	–
	Without	28.8	7.31	<0.01	–	–

PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.
[a] Mean value of triplicate samples.
[b] *p*-Values were calculated based on comparison with churrasco samples.
[c] From the exterior part.

across food items and cooking methods in this study, marinade might decrease HCA formation regardless of marinade type. Interestingly, on the other hand, Nerurkar et al. (1999) found a reducing effect of teriyaki and turmeric–garlic sauces on HCA formation but an enhancing effect of commercial honey barbecue sauce, suggesting that marinades vary in their effect on HCA formation. This would be unlikely to explain the lack of difference in HCA levels between marinated and non-marinated pan-fried salmon in the present study, however, because pan-fried pork marinated with a similar marinade showed a decrease in HCA

Table 10
PhIP levels in very well-done, non-marinated samples according to food items.

Cooking method	Food items								
	Chicken with skin	Beef		Pork		Sardine		Salmon with skin	
	PhIP (ng/g)[a]	PhIP (ng/g)[a]	*p*-Value[b]	PhIP (ng/g)[a]	*p*-Value[b]	PhIP (ng/g)[a]	*p*-Value[b]	PhIP (ng/g)	*p*-Value[b]
Pan-fried	34.6	0.58	<0.01	7.25	<0.01	2.28	<0.01	7.37	<0.01
Grilled	27.4	16.3	<0.01	–	–	–	–	–	–
Churrasco	47.3	31.8[c]	0.03	–	–	–	–	22.5	<0.01

PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.
[a] Mean value of triplicate samples.
[b] *p*-Values were calculated based on comparison with chicken with skin.
[c] From exterior part.

Table 11
PhIP levels in very well-done, non-marinated samples with or without skin.

Food item	Cooking method	Skin		
		With	Without	
		PhIP (ng/g)[a]	PhIP (ng/g)[a]	*p*-Value[b]
Chicken	Pan-fried	34.6	20.7	<0.01
	Grilled	27.4	29.5	0.51
	Churrasco	47.3	34.8	0.09
Salmon	Pan-fried	7.37	7.31	0.94
	Churrasco	22.5	28.8	0.03

PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.
[a] Mean value of triplicate samples.
[b] *p*-Values were calculated based on comparison with samples with skin.

Table 12
PhIP levels in very well-done samples with or without marinade.

Food item	Skin	Cooking method	Marinade		
			Without	With	
			PhIP (ng/g)[a]	PhIP (ng/g)[a]	*p*-Value[b]
Beef	Not applicable	Pan-fried	0.58	0.05	<0.01
	Not applicable	Grilled	16.3	4.64	<0.01
	Not applicable	Churrasco	31.8[c]	0.56	<0.01
Pork	Not applicable	Pan-fried	7.25	0.30	<0.01
Chicken	With	Pan-fried	34.6	0.79	<0.01
	With	Grilled	27.4	0.29	<0.01
	With	Churrasco	47.3	1.68	<0.01
	Without	Pan-fried	20.7	ND	–
	Without	Grilled	29.5	0.58	<0.01
	Without	Churrasco	34.8	3.69	<0.01
Sardine	Not applicable	Pan-fried	2.28	0.53	<0.01
Salmon	With	Pan-fried	7.37	6.17	0.20
	Without	Pan-fried	7.31	7.41	0.81

PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.
[a] Mean value of triplicate samples.
[b] *p*-Values were calculated based on comparison with samples with skin.
[c] From the exterior part.

formation over non-marinated samples. In addition, the length of exposure to the marinade varied from thirty minutes to overnight in this study. Given Salmon et al.'s (1997) finding that the lowering effect of marination on HCA formation was similar regardless of the length of exposure to the marinade, even when the food was dipped in marinade just prior to cooking, this is also unlikely to have affected HCA levels.

The present finding, that chicken contained higher HCA levels than beef and pork, suggests that poultry is an important source of HCA intake. If HCA plays an important role in the aetiology of colorectal cancer, this is inconsistent with current epidemiological evidence that red and processed meats increase the risk of colorectal cancer (World Cancer Research Fund and American Institute for Cancer, 2007) and that poultry intake was not associated with the risk of this disease (Norat et al., 2005). Moreover, nitrate/nitrite intake from processed meats was associated with an increased risk of colorectal adenoma independent of HCA intake (Ward et al., 2007). These findings imply that HCA intake from the usual diet might not substantially contribute to the risk of colorectal cancer. As mentioned in the Introduction, however, few studies have examined the association between HCA intake and the risk of this disease, and therefore further accumulation of evidence is required. In particular, the present finding that marination before cooking had an overall reducing effect on HCA formation indicates that further studies should take the use of marinades into consideration in estimating HCA intake.

Assessment of dietary HCA exposure at the individual level requires information on the dietary intake of various food items and corresponding food composition tables. An HCA database was previously developed for cooked foods prepared by customary Japanese cooking methods and doneness levels (Kataoka et al., 2002). This database was then used to estimate dietary HCA intake in subjects of a large-scale population-based prospective study in Japan, the Japan Public Health Center-based Prospective Study (JPHC study), using a FFQ developed for the 5-year follow-up survey of the JPHC study (Kobayashi et al., 2002). Further, the validity of this FFQ in estimating HCA intake was also assessed by comparison with HCA levels in hair samples (Kobayashi et al., 2007). Similarly, since the results of the present study are directly applicable to Japanese Brazilians, they are intended to be used to calculate HCA intake with a QFFQ developed for Japanese Brazilians (Sharma et al., 2009), after which the validity of this approach will be evaluated. Moreover, they may be applicable to Brazilians with similar cooking practices to Japanese Brazilians, such as churassco, to some extent at least.

5. Conclusions

HCA contents were measured in meat and fish cooked using typical Brazilian cooking methods to varying degrees of doneness. These data provide important information needed to estimate

dietary HCA exposure and will allow us to investigate the role of HCAs in the aetiology of cancer in Japanese Brazilians.

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References

Butler, L.M., Sinha, R., Millikan, R.C., Martin, C.F., Newman, B., Gammon, M.D., Ammerman, A.S., Sandler, R.S., 2003. Heterocyclic amines, meat intake, and association with colon cancer in a population-based study. American Journal of Epidemiology 157, 434–445.

Curado, M.P., Edwards, B., Shin, H.R., Storm, H., Ferlay, J., Heanue, M., Boyle, P. (Eds.), 2007. Cancer Incidence in Five Continents, vol. IX. IARC Scientific Publications No. 160. IARC, Lyon.

FAOSTAT, 2009. The Agricultural Consumption Domain. Retrieved May 21, 2009 from http://faostat.fao.org/site/345/default.aspx.

Gunter, M.J., Probst Hensch, N.M., Cortessis, V.K., Kulldorff, M., Haile, R.W., Sinha, R., 2005. Meat intake, cooking-related mutagens and risk of colorectal adenoma in a sigmoidoscopy-based case–control study. Carcinogenesis 26, 637–642.

Hayatsu, H., 1992. Cellulose bearing covalently linked copper phthalocyanine trisulphonate as an adsorbent selective for polycyclic compounds and its use in studies of environmental mutagens and carcinogens. Journal of Chromatography 597, 37–56.

Instituto Nacional do Cancer, 2003. Cancer no Brazil. Dados dos registros de base populacional, vol. III. Instituto Nacional do Cancer, Ministerio da Saude, Rio de Janeiro (In Portuguese).

Iwasaki, M., Mameri, C.P., Hamada, G.S., Tsugane, S., 2004. Cancer mortality among Japanese immigrants and their descendants in the state of São Paulo, Brazil, 1999–2001. Japanese Journal of Clinical Oncology 34, 673–680.

Iwasaki, M., Mameri, C.P., Hamada, G.S., Tsugane, S., 2008. Secular trends in cancer mortality among Japanese Immigrants in the State of São Paulo, Brazil, 1979–2001. European Journal of Cancer Prevention 17, 1–8.

Kataoka, H., Pawliszyn, J., 1999. Development of in-tube solid-phase microextraction/liquid chromatography/electrospray ionization mass spectrometry for the analysis of mutagenic heterocyclic amines. Chromatographia 50, 532–538.

Kataoka, H., Nishioka, S., Kobayashi, M., Hanaoka, T., Tsugane, S., 2002. Analysis of mutagenic heterocyclic amines in cooked food samples by gas chromatography with nitrogen-phosphorus detector. Bull Environ Contam Toxicol 69, 682–689.

Kobayashi, M., Hanaoka, T., Nishioka, S., Kataoka, H., Tsugane, S., 2002. Estimation of dietary HCA intakes in a large-scale population-based prospective study in Japan. Mutation Research 506–507, 233–241.

Kobayashi, M., Hanaoka, T., Tsugane, S., 2007. Validity of a self-administered food frequency questionnaire in the assessment of heterocyclic amine intake using 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) levels in hair. Mutation Research 630, 14–19.

Nerurkar, P.V., Le Marchand, L., Cooney, R.V., 1999. Effects of marinating with Asian marinades or western barbecue sauce on PhIP and MeIQx formation in barbecued beef. Nutrition and Cancer 34, 147–152.

Norat, T., Bingham, S., Ferrari, P., Slimani, N., Jenab, M., Mazuir, M., Overvad, K., Olsen, A., Tjønneland, A., Clavel, F., Boutron-Ruault, M.C., Kesse, E., Boeing, H., Bergmann, M.M., Nieters, A., Linseisen, J., Trichopoulou, A., Trichopoulos, D., Tountas, Y., Berrino, F., Palli, D., Panico, S., Tumino, R., Vineis, P., Bueno-de-Mesquita, H.B., Peeters, P.H., Engeset, D., Lund, E., Skeie, G., Ardanaz, E., González, C., Navarro, C., Quirós, J.R., Sanchez, M.J., Berglund, G., Mattisson, I., Hallmans, G., Palmqvist, R., Day, N.E., Khaw, K.T., Key, T.J., San Joaquin, M., Hémon, B., Saracci, R., Kaaks, R., Riboli, E., 2005. Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition. Journal of the National Cancer Institute 97, 906–916.

Salmon, C.P., Knize, M.G., Felton, J.S., 1997. Effects of marinating on heterocyclic amine carcinogen formation in grilled chicken. Food and Chemical Toxicology 35, 433–441.

Sharma, S., Iwasaki, M., Kunieda, C., Cao, X., Ishihara, J., Hamada, G., Miyajima, N., Tsugane, S., Le Marchand, L., 2009. Development of a Quantitative Food Frequency Questionnaire for assessing food, nutrient, and heterocyclic aromatic amine intake in Japanese Brazilians for a colorectal adenoma case–control study. International Journal of Food Sciences and Nutrition 60, 128–139.

Shimizu, H., Mack, T.M., Ross, R.K., Henderson, B.E., 1987. Cancer of the gastrointestinal tract among Japanese and white immigrants in Los Angeles County. Journal of the National Cancer Institute 78, 223–228.

Sinha, R., Rothman, N., Brown, E.D., Salmon, C.P., Knize, M.G., Swanson, C.A., Rossi, S.C., Mark, S.D., Levander, O.A., Felton, J.S., 1995. High concentrations of the carcinogen 2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine (PhIP) occur in chicken but are dependent on the cooking method. Cancer Research 55, 4516–4519.

Sinha, R., Knize, M.G., Salmon, C.P., Brown, E.D., Rhodes, D., Felton, J.S., Levander, O.A., Rothman, N., 1998a. Heterocyclic amine content of pork products cooked by different methods and to varying degrees of doneness. Food and Chemical Toxicology 36, 289–297.

Sinha, R., Rothman, N., Salmon, C.P., Knize, M.G., Brown, E.D., Swanson, C.A., Rhodes, D., Rossi, S., Felton, J.S., Levander, O.A., 1998b. Heterocyclic amine content in beef cooked by different methods to varying degrees of doneness and gravy made from meat drippings. Food and Chemical Toxicology 36, 279–287.

Sinha, R., Peters, U., Cross, A.J., Kulldorff, M., Weissfeld, J.L., Pinsky, P.F., Rothman, N., Hayes, R.B., 2005. Meat, meat cooking methods and preservation, and risk for colorectal adenoma. Cancer Research 65, 8034–8041.

Skog, K., Steineck, G., Augustsson, K., Jagerstad, M., 1995. Effect of cooking temperature on the formation of heterocyclic amines in fried meat products and pan residues. Carcinogenesis 16, 861–867.

Skog, K., Augustsson, K., Steineck, G., Stenberg, M., Jagerstad, M., 1997. Polar and non-polar heterocyclic amines in cooked fish and meat products and their corresponding pan residues. Food and Chemical Toxicology 35, 555–565.

Skog, K.I., Johansson, M.A., Jagerstad, M.I., 1998. Carcinogenic heterocyclic amines in model systems and cooked foods: a review on formation, occurrence and intake. Food and Chemical Toxicology 36, 879–896.

Sugimura, T., Wakabayashi, K., Nakagama, H., Nagao, M., 2004. Heterocyclic amines: mutagens/carcinogens produced during cooking of meat and fish. Cancer Sciences 95, 290–299.

Tominaga, S., 1985. Cancer incidence in Japanese in Japan, Hawaii, and western United States. National Cancer Institute Monographs 69, 83–92.

Tsugane, S., de Souza, J.M., Costa Jr, M.L., Mirra, A.P., Gotlieb, S.L., Laurenti, R., Watanabe, S., 1990. Cancer incidence rates among Japanese immigrants in the city of São Paulo, Brazil, 1969–1978. Cancer Causes Control 1, 189–193.

Tsugane, S., Hamada, G.S., Souza, J.M., Gotlieb, S.L.D., Takashima, Y., Todoriki, H., Kabuto, M., Karita, K., Yamaguchi, M., Watanabe, S., Laurenti, R., 1994. Lifestyle and health related factors among randomly selected Japanese residents in the city of São Paulo, Brazil, and their comparisons with Japanese in Japan. Journal of Epidemiology 4, 37–46.

Tsugane, S., Hamada, G.S., Karita, K., Tsubono, Y., Laurenti, R., 1996. Cancer patterns and lifestyle among Japanese immigrants and their descendants in the city of São Paulo, Brazil. Gann Monographs Cancer Research 44, 43–50.

Ward, M.H., Cross, A.J., Divan, H., Kulldorff, M., Nowell-Kadlubar, S., Kadlubar, F.F., Sinha, R., 2007. Processed meat intake, CYP2A6 activity and risk of colorectal adenoma. Carcinogenesis 28, 1210–1216.

World Cancer Research Fund and American Institute for Cancer Research, 2007. Food, Nutrition, Physical Activity and the Prevention of Cancer: A Global Perspective. American Institute for Cancer Research, Washington, DC.

Wu, K., Giovannucci, E., Byrne, C., Platz, E.A., Fuchs, C., Willett, W.C., Sinha, R., 2006. Meat mutagens and risk of distal colon adenoma in a cohort of U.S. men. Cancer Epidemiology, Biomarkers & Prevention 15, 1120–1125.