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Doneness Preferences, Meat and Meat-derived Heterocyclic Amines Intake, and NAT2 Polymorphisms: Association with Colorectal Adenoma in Japanese Brazilians

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Abstract

Objective: Intake of heterocyclic amines (HCAs) and other mutagenic compounds formed during cooking have been hypothesized to underlie the positive association observed between red meat and colorectal cancer. We evaluated whether well-done/very well-done preferences for various meat and fish items, higher intakes of meat and fish, and meat- and fish-derived HCA are associated with the risk of colorectal adenoma (CRA) in a Japanese-Brazilian population.

Methods: We selected 302 patients with adenoma and 403 control individuals who underwent total colonoscopy between 2007 and 2013 and collected information on aspects of meat intake using a detailed questionnaire. We also estimated HCA intake of study subjects using an HCA database which matched the cooking methods of this population.

Results: Latent class analysis based on response to doneness preferences for different cooking methods of commonly consumed meat and fish items identified 4 distinct subgroups. Compared to the subgroup characterized by a preference for rare/medium well-done cooking for most meat and fish items, the odds ratio of CRA for the well-done/very well-done preference subgroup was 1.19

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(95% confidence interval 0.51–2.75). High intake of mixed-meat dishes was suggestively inversely associated with CRA while a high intake of poultry was positively associated with CRA. No clear association with intake of total or specific HCAs and no effect modification by *NAT2* acetylation genotype was observed.

Conclusion: We found no statistically significant associations between meat and HCA intake and CRA. These findings do not support a positive association between meat and meat-derived HCA intake and risk of CRA.

Keywords

Red and processed meats; fish; heterocyclic amines; *NAT2* polymorphism; colorectal adenoma

Introduction

Intake of heterocyclic amine (HCA) compounds, which are produced from protein precursors in meat cooked at high temperature, is one of the mechanisms that are hypothesized to underlie the positive association between red and processed meat intake and colorectal cancer (CRC) risk (WCRF/AICR, Cross and Sinha, 2004). HCAs are mutagenic compounds, and experimental studies have shown their ability to induce tumors in various organs in rodents and non-human primates (Sugimura, 1997, Gooderham et al., 2001, Sugimura et al., 2004). However, despite this plausible evidence, human epidemiological studies of HCA in relation to the risk of colorectal neoplasia have shown inconsistent results.

Cooking methods and doneness preferences, as surrogate measures of HCA intake, were associated with increased risk of CRC or colorectal adenoma (CRA) in some (Sinha et al., 1999, Gunter et al., 2005, Shin et al., 2007, Fu et al., 2011) but not other studies (Tiemersma et al., 2004, Parr et al., 2013). The use of cooking method or doneness preference as an exposure measure may reflect the putative carcinogenicity of all known and unknown compounds present in cooked meat, not just HCAs. However, earlier studies analyzed a single cooking method or doneness preference for one meat item at a time. Given that preferences for cooking methods and doneness levels are likely to differ among foods in the same individual, the underlying formation of HCAs and other mutagenic compounds is also likely to differ among meals. Thus, it is likely that the collective classification of individuals based on cooking methods or doneness preferences of all (commonly consumed) meat and fish items will more closely reflect their overall exposure to putative carcinogens.

Among studies that quantified the amount of total or type-specific HCAs, an increased risk of colorectal neoplasia associated with one or more types of HCA was observed in at least four studies of CRA (Sinha et al., 2001, Rohrmann et al., 2009, Fu et al., 2011, Barbir et al., 2012) and two studies of CRC (Butler et al., 2008, Helmus et al., 2013), including a recent large prospective study conducted within the NIH-AARP cohort (Cross et al., 2010). In contrast, several other reports failed to find any measurable association between total and type-specific HCAs and risk of CRC or adenoma (Gunter et al., 2005, Gilsing et al., 2012, Ollberding et al., 2012). Estimated HCA concentrations were highly variable among these studies, probably reflecting differences in

dietary questionnaires and cooking methods among populations. This variation may in turn explain the inconsistent findings among these studies, to some extent at least.

Here, using doneness preference profiles for 14 commonly consumed meat and fish items in a Japanese-Brazilian population, we classified participants into subgroups which shared a similar preference for most of the included meat and fish items. We then evaluated the association between subgroup membership and CRA risk. We also examined the associations between meat, fish, and meat and fish-derived HCA intake and risk of CRA. Because N-acetyltransferase 2 (NAT2) enzymes are involved in the bioactivation of HCA carcinogens (Hein, 2002), and because polymorphisms in the *NAT2* gene are reported to affect enzyme activity, we also evaluated the modifying effects of *NAT2* genetic polymorphisms on these associations.

Methods

The Brazil adenoma study was designed to evaluate the association of colorectal adenoma with putative protective or risk factors, such as food consumption, nutrient intake, biological markers, and related-enzymatic genetic polymorphisms, and to explore the interactions between them. Study participants were recruited from among patients undergoing colonoscopy in two hospitals serving patients of Japanese ancestry in São Paulo, Brazil, between May 2007 and August 2013. Eligibility criteria for participation in the study included age of 40–79 years; at least 75% Japanese [for example, parents of pure Japanese origin; at least three of four grandparents of pure Japanese origin (at least 3/4 pure Japanese origin); or at least six of eight great-grandparents of pure Japanese origin (at least 6/8 pure Japanese origin)]; and residency in São Paulo for at least 6 months prior to recruitment. Exclusion criteria for subjects included a previous history of CRA, CRC or other invasive cancer, familial polyposis, inflammatory bowel diseases, previous colon resection, unsatisfactory colon preparation as determined from the endoscopy report, or incomplete examination (cecum not reached for colonoscopy). A total of 1066 subjects met the eligibility criteria of the study. Of these, all but 2 consented to participate, resulting in a study participation rate of 99.8%. We excluded 59 participants who satisfied one or more of the above exclusion criteria. The colonoscopy report and pathological diagnosis results of the remaining participants (n=1005) were obtained from the medical records. 304 participants had one or more adenoma, 15 had CRC, 127 had hyperplastic polyp (HPP) only, 149 had other lesions (e.g. hemorrhoid, diverticulitis) and the remaining 410 had a normal colon. In the present analysis, we further excluded those with very low (<500 Kcal) or high (>5000 Kcal) energy intake (n=15) to minimize the possibility of under- or over-reporting of nutrient intakes, respectively. We also excluded those with CRC, HPP and other lesions. Finally, we considered 302 individuals with colorectal adenoma as cases and 403 individuals with normal colon as controls. All participants gave written informed consent prior to the interview. The study protocol was approved by the institutional review boards of the National Cancer Center, Tokyo, Japan, and University of Hawaii, Committee on Human Studies; as well as by CONEP (Comissao Nacional de Etica em Pesquisa), Ministry of Health, Brazil, and CEP (Comissao de Etica em Pesquisa), Hospital Universitario, University of São Paulo.

Data on lifestyle and other behavioral factors were collected by face-to-face interview using a structured questionnaire. All interviews were conducted by a trained interviewer before the colonoscopy procedure. Usual dietary intake information during the reference period was assessed using a 161-item food frequency questionnaire (FFQ), with the reference period defined as the past 12 months (Sharma et al., 2009; Pakseresht et al., 2013). Frequency of intake in the FFQ ranged from 'never or hardly ever' to 'two or more times per day' in 8 categories. Portion size was determined by asking respondents to evaluate their typical serving compared with culturally appropriate utensils and food models of known weight. Daily nutrient intake was calculated from the FFQ using a modified version of the United States Department of Agriculture's food composition table and local recipes collected from Sao Paulo residents of Japanese ancestry, modified to accommodate dietary characteristics. The questionnaire included 21 meat or meat dishes, 13 chicken or poultry dishes, 12 fish or fish dishes and 2 meat alternatives (Supplemental method 1). Additional questions asked about the 'doneness' levels (ranging from rare to very well cooked) of 14 grilled, *churrasco* or pan-fried meat items (4 beef items, 1 pork item, 6 chicken items and 3 fish items). To capture HCAs from the meat drippings, we also collected information on the frequency and amount of consumption of gravy made with drippings from pan-fried and roasted meats and poultry. These FFQ data, along with an HCA database we previously developed for meat and fish items cooked by Brazilian methods (Iwasaki et al., 2010), were used to estimate the intake of HCA compounds including MeIQx, DiMeIQx, PhIP. Estimated HCA values above or below 3 standard deviations from the age- (4 categories) and sex-specific means for these HCA variables (after log-transformation) were considered as outliers and were not included in the analysis.

We determined three SNPs in the *NAT2* gene, namely T341C (rs1801280), G590A (rs1799930), and G857A (rs1799931)] using TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, California). *NAT2* acetylation phenotype was inferred using these three SNPs. Individuals with none, one and two or more variant alleles for these three SNPs were categorized as having a rapid, intermediate and slow acetylation genotype, respectively.

Statistical analysis

To identify classes (subgroups) of individuals with similar cooking methods and doneness preferences, we performed a latent class analysis (LCA) using information on the doneness levels (dichotomized into well-done/very well-done versus medium/less than medium cooked) of 14 grilled, *churrasco* or pan-fried meat items. Models with increasing numbers of latent classes (from 2 to 8) were fitted, and selection of the best fitting model was based on the Bayesian information criterion (BIC) and interpretability of the obtained classes. The study subjects were then assigned to their most likely class based on their predicted posterior probability. Logistic regression analysis was then applied to examine the association between the presence of CRA and class membership. To analyze meat/fish or HCA intake estimates and CRA association, subjects were divided into quartile categories (based on distribution of intake level among controls), and odds ratios (OR) and corresponding 95% confidence intervals (CI) was calculated using logistic regression analysis. Linear trends were tested in the logistic regression models using the exposure categories as ordinal variables. The interaction effect of *NAT2* acetylation genotype on meat/HCA and CRA

association was tested by inserting a product term of the *NAT2* acetylation genotype (dichotomized to slow/intermediate or rapid acetylation genotype) and meat/HCA (high or low intake group by median intake) in the model. All statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) and R statistical software (version 3.1.2; R Development Core Team, 2014).

Results

The distribution of selected characteristics between CRA cases and controls is shown in Table 1. The number of women participants was larger than men; among the total participants, about 53% in the case group and about 69% in the control group were women. Compared to the controls, cases were older and less physically active; were more likely to be a smoker, alcohol consumer, and NSAID user; and had a higher dietary consumption of total energy but lower consumption of calcium and fish. Apart from these, no other significant differences in lifestyle or dietary variables were seen between CRA cases and controls.

The LCA model with four classes was selected based on the BIC. The estimated and predicted class membership probabilities, as well as the item-specific response patterns in each class, are shown in Supplemental table 1. Class 3 accounted for 19% of the total population, and was characterized by none or only very low endorsement for the well-done/very well-done preference for almost all of the included meat and fish items. Class 2 was characterized by endorsement of a well-done/very well-done preference for almost all the meat and fish items and represented around 5% of the study population. With a population share of 53%, Class 4 represented the most likely preference pattern and was characterized by endorsement of well-done/very well-done preference for beef items but a less strong endorsement of this preference for chicken and fish items. Finally, Class 1 accounted for 24% percent of the study population and exhibited a preference pattern intermediary between class 2 and class 3.

The non-dietary lifestyle factors and some dietary factors including total energy, fiber, fruits, vegetables, processed meat, meat in mixed dishes, and poultry (in controls) showed no significant variation between the classes (Table 2). However, while folate intake was low, intake of calcium, fish and red meat was higher in class 2, although the P value for difference was not significant for all. As expected, the intake of PhIP, MeIQx, DiMeIQx, and total HCA was also highest in class 2 and lowest in class 3. The multivariate-adjusted OR of CRA by class membership is shown in Table 3. Because class 3 participants had a very low endorsement for well-done/very well-done preference for all meat items, this class was taken as the reference. However, the results showed no significant increase in the ORs associated with membership to any of the three other classes.

The multivariate-adjusted OR and corresponding 95% CI for CRA according to intake categories of meat and HCA are shown in Table 4. Intakes of red meat, processed meat and fish, as well as total and specific HCA, were not associated with the risk of CRA. However, higher intake of meat from mixed dishes showed a suggestive inverse association with CRA, while poultry intake was positively associated with CRA.

NAT2 acetylation genotype was not associated with CRA risk (Supplemental table 2). Furthermore, *NAT2* acetylation genotype did not exert any appreciable effect modification on the association between total HCA ($P_{\text{interaction}} = 0.44$) or meat intake ($P_{\text{interaction}} = 0.41$) and CRA risk (Table 5). Exclusion of participants with a family history of CRC (n=97) did not appreciably change the results for the direct or interaction association of *NAT2* and CRA (data not shown).

Discussion

In this study, we used LCA to identify four distinct subgroups/classes of individuals based on their response to doneness preferences for different cooking methods of commonly consumed meat and fish items. The subgroup who endorsed a well-done/very well-done preference for most of the included items compared to the subgroup with low endorsement of the well-done/very well-done preference had increased OR of CRA, albeit that this association did not reach statistical significance. We found no apparent association between red meat, processed meat and HCA intakes and CRA whereas poultry intake appeared to be positively associated with CRA.

Inquiries about behaviors, such as preferences for meat and fish doneness, are usually based on questionnaires which include several categorical items. Analyzing the answers to these items one at a time might fail to reveal general patterns of behavior to which people adhere. In this context, the use of LCA is particularly interesting because it provides a powerful means of identifying distinct subgroups of individuals sharing similar behaviors (Bandeem-Roche et al., 1999). In this study, based on the response profile of doneness preference for cooking methods of a variety of meat and fish items, we identified a subgroup whose participants had a well-done/very well-done preference for most included meat/fish items. The descriptive statistics showed that participants of this subgroup had the highest average intake of HCA among all subgroups. Membership to this subgroup, however, was not significantly associated with the risk of CRA in the present study. Interestingly, along with a high HCA intake, participants in this subgroup also had a higher average intake of calcium, fruits and vegetables, all of which are reported to be protective against CRA (WCRF/AICR). Although adjustment for these factors in the statistical model showed no appreciable difference in the ORs in our study, the possibility of residual confounding from these factors remains, and will likely attenuate the risk estimates towards the null. The lack of association may have also resulted from a lack of statistical power as there were only 16 cases and 19 controls in this class. Implementation of a similar analytical approach in larger populations would overcome the power problem and is warranted.

Contrary to our hypothesis, we observed no clear association between HCA estimates and CRA in the present study. Previous findings regarding total or specific HCA estimates and association with colorectal neoplasia, however, are not all consistent. Only one (Ferrucci et al., 2009; Rohrmann et al., 2009; Wang et al., 2011) or two (Sinha et al., 2005) specific types of HCA or total HCA (Cross et al., 2010) was associated with colorectal neoplasia risk in some studies, while others (Ollberding et al., 2012) showed no association. Because HCA formation varies not only as a result of the temperature and duration of cooking, but also by meat type, one reason for this inconsistency might relate to the difficulty in measuring HCA in the diet.

The estimated amount of HCA also varied among these studies; for example, median PhIP and MeIQx estimates were 38 and 12 ng/d in one study (Sinha et al., 2001) versus 55 and 19 ng/d, respectively, in another (Cross et al., 2005). A Swedish case-control study reported no clear association between overall HCA intake and colon or rectal cancer risk at the usual dietary intake range. However, because 4 cases had an HCA intake in excess of nearly 2000 ng daily, this study was suggestive of carcinogenic potential at an extremely high intake level (Augustsson et al., 1999). In comparison to these studies, PhIP and MeIQx estimates in the present population were much lower, and might have been one of the reasons for the overall lack of an association in our study.

We observed a suggestive positive association between poultry intake and CRA in the present study, but no association with red or processed meat intake. Our previous study in a Japanese population in Tokyo showed a positive association between poultry intake and CRA in women (Q4 vs Q1 OR=1.75, 95% CI=1.00–3.06) (Budhathoki et al., 2015). The poultry group in that study was composed of only four chicken items and the intake of poultry was lower (8.7 g/day). In the present study, however, even though we included 13 chicken/poultry items in the poultry group and also had a greater intake (19.4 g/day), ORs were comparable to those of our earlier study. Our findings for poultry, however, do differ from those of an earlier meta-analysis that included seven studies and produced a summary effect size estimate of 0.98 (95% CI 0.82–1.18) (Xu et al., 2013). The difference in intake range, population composition, and varying definitions of poultry among studies might have contributed to the null finding in the meta-analysis.

NAT2 is an important enzyme which is involved in the activation of HCA and PAH through O-acetylation (Hein, 2002). *NAT2* rapid acetylation status has been hypothesized to increase the risk of cancer, particularly in those with higher exposure to HCAs (Le Marchand et al., 2001). Voutsinas et al. reported a statistically significant increased risk of CRA in rapid *NAT2* acetylators with high HCA intake (Voutsinas et al., 2013), while Nöthlings et al. reported a similar positive association of CRC among rapid *NAT2* acetylators with a dietary pattern that includes a high meat intake (Nöthlings et al., 2009). Le Marchand et al. found a significantly increased risk among individuals with the rapid phenotype for *NAT2* and *CYP1A2* and a preference for well-done or very well-done red meat (Le Marchand et al., 2001). In the present study, both the main effect and interaction analyses of the *NAT2* acetylation genotype revealed no clear association with CRA risk, which is consistent with our earlier finding in a Japanese population in Japan (Budhathoki et al., 2015).

Our study has several strengths. First, all adenoma cases were pathologically verified, whereas controls were identified from individuals with a negative colonoscopy. Second, comprehensive assessment of several aspects of meat intake (type of meat, cooking methods and conditions, doneness levels etc.) likely facilitated the accurate estimation of HCAs. In addition, the HCA intake amount was computed based on a database that was specially prepared to match the cooking habits of this population. Among the limitations, this study was conducted under a cross-sectional design with retrospective assessment of dietary and other lifestyle factors. However, it is unlikely that participants changed their dietary habits because of the presence of adenoma as these lesions are generally asymptomatic. Although the study sample size was moderately large, our study lacked power to detect interaction

effects of small size. Controls were selected from patients with a normal colon at colonoscopy. However, this hospital-based selection of participants means that the findings may not be generalizable to the entire Japanese Brazilian population, as their characteristics might have differed from those of the background population. Although our eligibility criteria required participants to be at least 75% Japanese, the fulfillment of this criterion was based on participants' self-report and not on assessing genetic ancestry based on DNA analysis. Although we quantified only the intake of HCAs, PAHs are also formed in cooked meat, which is also a known colon carcinogen. Nevertheless, ORs according to class membership in the present study, which likely reflect all carcinogenic compounds, including PAH, showed no clear association. The *NAT2* acetylation genotypes were inferred from a panel of three SNPs only. Nevertheless, these three SNPs have been shown to infer acetylation phenotypes with 100% accuracy among a Japanese population (Suarez-Kurtz et al., 2012), and any misclassification error due to the use of a three-SNP panel is therefore likely to be minimal.

In conclusion, the present study also revealed no clear association between higher red and processed meat and HCA intake estimates and CRA, except for poultry intake, for which a positive association with CRA was suggested.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Characteristics of colorectal adenoma cases and controls.

Variable	Colorectal adenoma (n=302)	Controls (n=403)	P*
Men, n (%)	141 (46.7)	124 (30.8)	
Age (years), mean (SD)	64.6 (8.7)	60.2 (9.9)	<0.001
BMI (kg/m ²), mean (SD)	23.8 (3.4)	23.6 (3.4)	0.31
Physical activity, mean (SD)	38.3 (7.2)	39.5 (7.7)	0.03
Ever smoker, n%	89 (29.5)	79 (19.6)	0.002
Alcohol consumer, n%	130 (43.0)	137 (34.0)	0.01
Supplement vitamin use, (%)	93 (30.8)	110 (27.3)	0.31
Familial CRC history, (%)	35 (11.6)	62 (15.4)	0.15
NSAID use, (%)	46 (15.2)	36 (8.9)	0.01
Dietary intake, Median (IQR)			
Total energy (kcal/day)	2264 (1828–2969)	2141 (1562–2781)	0.01
Fiber (g/day)	26.3 (21.7–32.6)	25.9 (20.7–30.8)	0.19
Folate (mg/day)	628.7 (554.6–718.4)	618.8 (548.1–711.9)	0.12
Calcium (mg/day)	1031 (798–1241)	1059 (861–1337)	0.02
Fruits (g/day)	582.7 (379.2–866.9)	582.9 (360.0–870.0)	0.56
Vegetables (g/day)	268.7 (185.6–399.7)	281.4 (186.6–410.6)	0.65
Red meat (g/day)	25.7 (10.6–49.8)	31.0 (13.4–56.4)	0.10
Processed meat (g/day)	5.7 (1.9–14.8)	7.0 (1.4–14.3)	0.88
Mixed dish meat (g/day)	42.6 (24.1–64.6)	46.0 (29.3–66.2)	0.10
Poultry (g/day)	16.4 (9.3–28.5)	19.4 (8.3–31.5)	0.19
Fish (g/day)	25.6 (12.3–50.5)	32.5 (15.2–60.6)	0.03
PhIP (ng/day)	0.28 (0.02–21.89)	0.18 (0.03–21.42)	0.83
MeIQx (ng/day)	0.71 (0.02–5.78)	0.49 (0.03–5.24)	0.61
DiMeIQx (ng/day)	0.01 (0.009–0.67)	0.014 (0.010–1.013)	0.05
Total HCA (ng/day)	1.49 (0.02–27.58)	0.88 (0.03–28.78)	0.83

Abbreviations: BMI, body mass index; CRC, colorectal cancer; NSAID, non-steroidal anti-inflammatory drug; IQR, inter quartile range; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; and HCA, heterocyclic amine.

* *t*-test, Wilcoxon rank sum test or χ^2 test as appropriate.

Table 2.

Distribution of selected characteristics among controls according to class membership.

Variable	Class 1 (n=88)	Class 2 (n=19)	Class 3 (n=95)	Class 4 (n=201)	P*
Men, n (%)	26 (29.6)	7 (36.8)	35 (36.8)	56 (27.9)	
Age (years), mean (SD)	59.2 (9.3)	61.4 (11.0)	61.2 (9.6)	60.0 (10.1)	0.52
BMI (kg/m ²), mean (SD)	23.9 (3.6)	24.7 (2.4)	23.5 (3.3)	23.4 (3.5)	0.33
Physical activity, mean (SD)	39.6 (6.6)	38.7 (7.1)	39.0 (7.5)	39.8 (8.4)	0.81
Ever smoker, n%	19 (21.6)	5 (26.3)	23 (24.2)	32 (15.9)	0.20
Alcohol consumption, n%	33 (37.5)	7 (36.8)	30 (31.6)	67 (33.3)	0.48
Supplement use, (%)	30 (34.1)	5 (26.3)	20 (21.1)	55 (27.4)	0.27
Familial CRC history, (%)	14 (15.9)	3 (15.8)	11 (11.6)	34 (16.9)	0.81
NSAID use, (%)	7 (8.0)	2 (10.5)	10 (10.5)	17 (8.5)	0.95
Dietary intake					
Total energy (kcal/day)	2240 (1805–2877)	2028 (1601–3051)	1981 (1484–2719)	2143 (1520–2707)	0.22
Fiber (g/day)	27.1 (22.5–31.9)	23 (20.6–29.1)	25.5 (20.2–29.4)	25.6 (20.6–31.7)	0.27
Folate (mg/day)	609 (548–713)	560 (479–644)	618 (509–724)	626 (559–713)	0.08
Calcium (mg/day)	1072 (838–1374)	1295 (1127–1585)	1155 (917–1450)	1021 (832–1246)	0.002
Fruits (g/day)	633 (432–919)	621 (452–773)	542 (381–825)	549 (319–872)	0.18
Vegetables (g/day)	279 (193–377)	292 (193–411)	293 (228–375)	280 (180–419)	0.96
Red meat (g/day)	32.3 (14.1–56.2)	35.4 (8.1–58.7)	24.6 (5.9–51.6)	34.4 (16.1–58.6)	0.04
Processed meat (g/day)	6.6 (1.9–13.6)	8.4 (2.9–19.1)	5.6 (0.1–13.5)	7.9 (1.8–14.7)	0.19
Mixed dish meat (g/day)	47.4 (33.5–65.2)	53.1 (27.5–78.5)	39.0 (25.4–68.1)	46.0 (29.8–65.0)	0.43
Poultry (g/day)	20.8 (8.3–34.8)	29.6 (13–42.1)	18.9 (7.6–33.2)	18.7 (8.3–29.9)	0.13
Fish (g/day)	29.5 (13–60.6)	64.8 (25–104.3)	40.5 (20.7–64.8)	29.5 (12.0–47.8)	0.001
PhIP (ng/day)	10.2 (0.04–59.2)	53.0 (14.9–190.7)	0.05 (0.02–3.31)	0.16 (0.03–11.9)	<0.0001
MeIQx (ng/day)	2.30 (0.09–11.4)	19.1 (3.2–24.5)	0.05 (0.02–0.91)	0.43 (0.03–3.03)	<0.0001
DiMeIQx (ng/day)	0.30 (0.01–6.3)	9.4 (1.4–16.9)	0.012 (0.01–0.02)	0.014 (0.01–0.45)	<0.0001
Total HCA (ng/day)	11.9 (0.13–78.0)	69.8 (16.0–238.7)	0.07 (0.02–3.64)	0.80 (0.04–17.15)	<0.0001

Abbreviations: BMI, body mass index; CRC, colorectal cancer; NSAID, non-steroidal anti-inflammatory drug; IQR, inter quartile range; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; and HCA, heterocyclic amines.

* Cochran Armitage test, ANOVA or Kruskal Wallis test as appropriate.

Table 3.

Odds ratio (OR) and 95% confidence interval (CI) of colorectal adenoma according to class membership.

Class membership	Cases/controls	OR (95% CI) ^a
Class 1	64/88	1.05 (0.63–1.73)
Class 2	16/19	1.19 (0.51–2.75)
Class 3	63/95	1.00 (Reference)
Class 4	159/201	1.04 (0.68–1.58)

^a Adjusted for age (<50, 50–<60, 60–<70, 70 years), sex, hospital, body mass index (<18.5, 18.5–<25, 25–<30, 30 kg/m²), total physical activity (METs/tertile), pack-years of smoking (none, <20, 20–<40, 40), life-term alcohol intake (none, <50, 50–<100, 100 kg of ethanol), non-steroidal anti-inflammatory drug use, family history of CRC, and supplemental vitamin use.

Table 4.

Odds ratio (OR) and 95% confidence interval (CI) of colorectal adenoma according to intake of meat and fish, and type-specific and total HCAs.

	Quartile category				P trend
	Q1	Q2	Q3	Q4	
Red meat					
Case (n)	91	73	75	63	
OR (95% CI) ^a	1.00 (ref.)	0.86 (0.55–1.35)	0.95 (0.60–1.49)	0.94 (0.59–1.52)	0.89
Processed meat					
Case (n)	65	105	55	77	
OR (95% CI) ^a	1.00 (ref.)	1.34 (0.84–2.12)	0.65 (0.39–1.09)	1.08 (0.67–1.76)	0.56
Mixed dish meat					
Case (n)	88	83	59	72	
OR (95% CI) ^a	1.00 (ref.)	0.96 (0.61–1.52)	0.59 (0.36–0.94)	0.69 (0.43–1.09)	0.03
Poultry					
Case (n)	67	110	56	69	
OR (95% CI) ^a	1.00 (ref.)	1.83 (1.17–2.88)	0.99 (0.60–1.62)	1.79 (1.08–2.95)	0.20
Fish					
Case (n)	100	72	69	61	
OR (95% CI) ^a	1.00 (ref.)	0.65 (0.42–1.02)	0.78 (0.49–1.23)	0.73 (0.45–1.18)	0.25
PhIP					
Case (n)	91	51	81	77	
OR (95% CI) ^a	1.00 (ref.)	0.71 (0.44–1.14)	0.85 (0.54–1.32)	0.76 (0.48–1.20)	0.34
MeIQx					
Case (n)	82	51	87	80	
OR (95% CI) ^a	1.00 (ref.)	0.67 (0.41–1.09)	1.01 (0.64–1.58)	0.90 (0.57–1.43)	0.97
DeMeIQx					
Case (n)	95	86	49	70	
OR (95% CI) ^a	1.00 (ref.)	1.11 (0.71–1.75)	0.63 (0.39–1.03)	0.84 (0.53–1.34)	0.16
Total HCA					
Case (n)	81	62	83	74	
OR (95% CI) ^a	1.00 (ref.)	0.89 (0.56–1.43)	0.88 (0.56–1.38)	0.84 (0.53–1.34)	0.48

Abbreviation: PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; HCA, heterocyclic amine.

The number of controls from quartile 1 to 4 is 100, 101, 101, and 101, respectively, for meat and fish items and 99, 100, 100, and 100, respectively, for type-specific or total HCAs.

^aAdjusted for age (<50, 50–<60, 60–<70, 70 years), sex, hospital, body mass index (<18.5, 18.5–<25, 25–<30, 30 kg/m²), total physical activity (METs/tertile), pack-years of smoking (none, <20, 20–<40, 40), life-term alcohol intake (none, <50, 50–<100, 100 kg of ethanol), non-steroidal anti-inflammatory drug use, family history of CRC, and supplemental vitamin use.

Table 5.

Association between total heterocyclic amine (HCA) and meat intake and colorectal adenoma risk according to *N-acetyltransferase2* (*NAT2*) acetylation genotype.

	<u>Slow/intermediate</u>		<u>Rapid</u>	
	N	OR (95% CI) ^a	N	OR (95% CI) ^a
Total HCA				
Low (< 0.88 ng/day)	75/103	1.00 (ref.)	68/96	1.02 (0.64–1.63)
High (≥ 0.88 ng/day)	75/109	0.80 (0.50–1.27)	82/91	1.06 (0.67–1.68)
<i>P</i> _{interaction}		0.44		
Total meat				
Low (< 95 g/day)	77/102	1.00 (ref.)	81/96	1.02 (0.64–1.60)
High (≥ 95 g/day)	73/110	0.76 (0.48–1.23)	69/91	1.03 (0.64–1.65)
<i>P</i> _{interaction}		0.41		

N, number of cases/controls; OR, odds ratio; CI, confidence interval.

^a Adjusted for age (<50, 50–<60, 60–<70, ≥70 years), sex, hospital, body mass index (<18.5, 18.5–<25, 25–<30, ≥30 kg/m²), total physical activity (METs/tertile), pack-years of smoking (none, <20, 20–<40, ≥40), life-term alcohol intake (none, <50, 50–<100, ≥100 g of ethanol), non-steroidal anti-inflammatory drug use, family history of CRC, and supplemental vitamin use.