**Tobacco Smoking and Urinary Levels of 2-Amino-9H-Pyrido[2,3-b]Indole in Men of Shanghai, China**

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**Abstract**

Carcinogenic heterocyclic aromatic amines (HAA) are formed in cooked meats, poultry, and fish and arise in tobacco smoke. We measured the concentrations of four prevalent HAAs in spot urine samples collected at baseline from 170 participants of the Shanghai Cohort study, a population-based cohort study of adult men recruited during 1986 to 1989 in Shanghai, China. Sixteen (18.6%) of 86 nonsmokers were positive for urinary 2-amino-9H-pyrido[2,3-b]indole (A9C) versus 41 (48.8%) of 84 cigarette smokers; the difference was statistically significant (P < 0.001). The number of cigarettes smoked per day was positively and significantly related to urinary levels of A9C in study subjects (P < 0.001); the mean level among nonsmokers was 2.54 ng/g creatinine, whereas the means for light (1-19 cigarettes per day) and heavy (20+ cigarettes per day) smokers were 7.50 and 11.92 ng/g creatinine, respectively. 2-Amino-3,4,8-trimethylimidazo[4,5-f]quinoline was undetected in the urine of the 170 subjects. Only 5 (2.9%) and 6 (3.5%) subjects, respectively, showed detectable levels of urinary 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and 2-amino-3,8-dimethylimidazo[4,5-f]quinoline, and smoking status was unrelated to levels of either HAA. Quantitative measurements of HAAs in commonly eaten pork and chicken dishes in Shanghai showed low concentrations of HAAs (<1 ng/g meat). Our data indicate that A9C represents a major HAA exposure in adult men of Shanghai, China, and that tobacco smoke is an important point source of their A9C exposure. (Cancer Epidemiol Biomarkers Prev 2007;16(8):1554–60)

**Introduction**

More than 20 known heterocyclic aromatic amines (HAA) are formed in cooked meats, poultry, and fish and arise in tobacco smoke. We measured the concentrations of four prevalent HAAs in spot urine samples collected at baseline from 170 participants of the Shanghai Cohort study, a population-based cohort study of adult men recruited during 1986 to 1989 in Shanghai, China. Sixteen (18.6%) of 86 nonsmokers were positive for urinary 2-amino-9H-pyrido[2,3-b]indole (A9C) versus 41 (48.8%) of 84 cigarette smokers; the difference was statistically significant (P < 0.001). The number of cigarettes smoked per day was positively and significantly related to urinary levels of A9C in study subjects (P < 0.001); the mean level among nonsmokers was 2.54 ng/g creatinine, whereas the means for light (1-19 cigarettes per day) and heavy (20+ cigarettes per day) smokers were 7.50 and 11.92 ng/g creatinine, respectively. 2-Amino-3,4,8-trimethylimidazo[4,5-f]quinoline was undetected in the urine of the 170 subjects. Only 5 (2.9%) and 6 (3.5%) subjects, respectively, showed detectable levels of urinary 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and 2-amino-3,8-dimethylimidazo[4,5-f]quinoline, and smoking status was unrelated to levels of either HAA. Quantitative measurements of HAAs in commonly eaten pork and chicken dishes in Shanghai showed low concentrations of HAAs (<1 ng/g meat). Our data indicate that A9C represents a major HAA exposure in adult men of Shanghai, China, and that tobacco smoke is an important point source of their A9C exposure. (Cancer Epidemiol Biomarkers Prev 2007;16(8):1554–60)
pretreated with acid or base (such treatment enables the detection of the parent compound plus cleaved N-glucuronide and sulfamate conjugates; ref. 27). Our findings reveal that the amounts of HAAs formed in commonly eaten meats cooked by Shanghai-style cuisine are low. AoC represents the major HAA exposure in this population and it occurs predominantly through tobacco smoke.

Materials and Methods

Study Subjects. The design of the Shanghai Cohort study has been described previously (28, 29). In brief, between January 1, 1986 and September 30, 1989, all male residents of four, small, geographically defined communities in Shanghai, China, who were ages 45 to 64 years and had no history of cancer, were invited to participate in a prospective study of diet and cancer. At enrollment, participants were interviewed in person using a structured questionnaire that included lifetime history of tobacco use, and each participant provided a 10-mL blood and a single-void urine sample. Urine samples were stored at −20°C. A total of 18,244 men (80% of eligible subjects) were enrolled in the study. Follow-up has been maintained via personal contacts with all surviving cohort members, routine reviews of records from the population-based Shanghai Cancer Registry, and death certificates from the local vital statistics offices. To date, 479 subjects have been lost to follow-up.

The present study included 186 cohort participants who were part of a nested case-control study of colorectal cancer. For each case cancer, an individually matched control was randomly selected from the pool of cohort subjects who were free of cancer and alive at the time of the cancer diagnosis of the index case. The matching criteria were date of birth (within 2 years), date of biospecimen collection (within 1 month), and neighborhood of residence at recruitment. Sixteen subjects (5 cases and 11 controls) were excluded due to either poor recovery of the analyte or isobaric interferences in the tandem mass spectrometry (MS) transition of AoC, resulting in a poor ratio for the qualifier/target ion \([M + H - 44]^+ \) / \([M + H - 17]^+\) and uncertainty in the purity of the analyte. Therefore, 170 subjects (88 colorectal cancer cases and 82 controls) formed the final study data set.

Chemicals. The following chemicals were purchased from Toronto Research Chemicals: 8-MeIQx and the trideuterated isotopic purity, >99%); and AoC, 4,8-DiMeIQx and 3-[13C6]-4,8-DiMeIQx (isotopic purity, >99%). The compounds were purchased from Toronto Research Chemicals.

Measurement of HAAs in Shanghai-Style Cooked Meats. Pork and chicken samples were cooked, by means of deep, pan-, or stir-frying Shanghai style and then assayed for HAA content. Two independent samples 1 and 2: pork loin with bone (173 g), Wesson vegetable cooking oil (16 g), soy sauce (6.6 g), yellow wine (2.0 g), salt (0.1 g), sugar (0.5 g), and wheat flour (12 g). The meat was deep fried for 7 min in a Teflon-coated wok, at a surface temperature of 200°C. Sample 3: pork loin with bone (227 g), Wesson vegetable cooking oil (21 g), soy sauce (8.7 g), yellow wine (2.6 g), salt (0.1 g), sugar (0.5 g), and wheat flour (15.7 g). The meat was pan fried for 4 min in a Teflon-coated wok, at a surface temperature of 220°C. Sample 4: shredded, sliced pork (245 g), salt (2 g), yellow wine (10 g), and Wesson vegetable cooking oil (26.1 g). The meat was stir-fried for 2 min in a Teflon-coated wok, at a surface temperature of 140°C. Sample 5: shredded, sliced chicken (146 g), salt (1.5 g), and Wesson vegetable cooking oil (25.0 g). The meat was stir-fried for 2 min in a Teflon-coated wok, at a surface temperature of 135°C.

Isolation of HAAs from Cooked Meats. Cooked meat samples were isolated by tandem SPE, using an Extrelut-20 resin placed in series with a Waters Oasis MCX SPE resin (150 mg; refs. 6, 11). All of the meat samples were added to isotopically labeled HAAs (5 ng/g of cooked meat) in 1 N NaOH (8 mL) and processed by SPE as reported previously (6, 11).

Isolation of Unmetabolized HAAs from Urine. The isolation of HAAs is based on a tandem solvent-SPE method that had been described previously (26). In brief, urine (1 mL) was placed into a polypropylene tube (15 mL). HAA internal standards were added (50 pg/mL urine), and the solution was made alkaline with 2.5 N Na2CO3 (0.1 mL). The urine samples were extracted twice with ethyl acetate (5 mL). The aqueous and organic phases were separated by centrifugation. The pooled organic phases were acidified with glacial acetic acid (50 μL) and processed by SPE with a Waters Oasis MCX cartridge (30 mg) as reported previously (26).

Isolation of HAA/Conjugates in Urine. Five pooled urine samples derived from the Shanghai Cohort study were measured for urinary HAA using previously published acid or base hydrolysis steps for cleavage of phase II conjugates of the parent amines (26, 31-34) before the tandem solvent-SPE procedure. The acid or base hydrolysis was conducted by addition of 10 N HCl or 10 N NaOH (0.22 mL) to urine (1 mL) and heating at 70°C for 6 h. After cooling, the acid-treated samples were made alkaline with 10 N NaOH (0.32 mL). Both acid-treated and base-treated samples underwent the solvent extraction and SPE-clean up as described previously (26).

Liquid Chromatography-Electrospray Ionization/Tandem MS Measurements of HAAs. The quantification of HAAs in urine samples and cooked meats was conducted on a Finnigan TSQ Quantum Ultra triple quadrupole (TSQ) mass spectrometer (ThermoElectron). The instrument tune parameters used were as follows: capillary temperature of 350°C, source spray voltage of 3.2 kV, sheath gas setting of 65, and tube lens offset of 95. The collision energy was optimized for each HAA and ranged from 26 to 32 eV. The in-source collision-induced dissociation offset was 10 V. Argon, set at 1.5 mTorr, was used as the collision gas. Both Q1 and Q3 were set at a resolution of 0.7 Da. Quantitative analyses were conducted in the positive ion mode using the selected reaction monitoring transitions \([M + H]^+ > [M + H - 15]^+\) (loss of CH4), \([M + H]^+ > [M + H - 18]^+\) (loss of CD3), and \([M + H]^+ > [M + H - 44]^+\) (loss of CD3). For AoC and \([13C6]-A0C\), the selected reaction monitoring transition monitored was \([M + H]^+ > [M + H - 17]^+\), which is attributed to the loss of NH3. A qualifier fragment ion was used for corroboration of the identity of AoC (\([M + H]^+ > [M + H - 44]^+\), which is attributed to loss of NH3 following loss of HCN; ref. 35). Under these collision-induced dissociation conditions, the ratio of the fragment ions \([M + H]^+ > [M + H - 17]^+\) was 0.80: the urinary analyte was identified as AoC if the ion abundance ratio was within ±25% of this value (36). The dwell time for each transition was set at 0.02 s. Individual instrument parameters were optimized by infusion of the HAAs (1 μg/mL) with a syringe pump into the MS source through a mixing tee, at a flow rate of 10 μL/min, with the liquid chromatography solvent (1:1 A:B; A = 0.1% HCO2H containing 10% CH3CN and B = 90% CH3CN99.5% H2O1.0% HCO2H) flowing at 50 μL/min.

The chromatographic separation of the HAAs from urine samples was carried out with a ThermoElectron Aquasil C8 reversed-phase column (1 × 250 mm, 5 μm particle size) and Javelin precolumn. The flow rate was set at 50 μL/min. A linear gradient was used for separation of the analytes, starting at 100% A buffer (0.1% HCO3H containing 5% CH3CN) and ending at 100% B buffer (0.1% HCO3H:4.9% H2O:95% CH3CN) over 30 min. The analysis of HAAs in cooked meats was done.
with the same column, using a linear gradient and starting from 100% A buffer (0.1% HCO$_2$H containing 0.5% CH$_3$CN) and ending at 100% B buffer (0.1% HCO$_2$H:9.9% H$_2$O:90% CH$_3$CN) over 30 min.

The estimates of HAAs in urine were determined with an external calibration curve, using 16.6 pg of internal standards (8 µL, 50 ppt equivalent of HAA/mL urine) and unlabeled HAAs at nine calibrate levels ranging from 0 to 30 pg injected on column or the equivalent of 0 to 90 ppt of HAA in urine. For grilled meat samples, an external calibration curve was established with seven calibrate levels, ranging from 0 to 5 ppb of HAA in cooked meat. The coefficient of determination ($r^2$) of all HAA calibration curves exceeded 0.998.

The identity of Aoc was corroborated in some urine samples, by acquisition of full scan product ion spectra of the protonated molecule [M + H]$^+$ at m/z 184, scanning from m/z 100 to 250 at a scan speed of 150 Da/s, at collision energies of 28 and 38 eV. The same MS acquisition parameters described above were used. Several acquisitions were done with 5 mmol/L ammonium acetate (pH 6.8) containing 5% CH$_3$CN as the A solvent instead of 0.1% HCO$_2$H; the change in pH shifted the retention time ($t_R$) of Aoc later by 5 min.

The tandem solvent-SPE method was validated previously with urine samples from male nonsmokers, who refrained from consumption of grilled meats for >24 h (26). The limit of quantification (LOQ), defined as the background mean signal plus 10 times the SD (37), was estimated at 4 pg/mL for PhIP, 4,8-DiMeIQx, and Aoc and 6 pg/mL for 8-MeIQx: the higher LOQ of this latter HAA is attributed to the lower percentage of HAA in cooked meat. The LOQ of HAAs in cooked meat samples was 0.03 ng/g (11).

**Urinary Creatinine.** Creatinine was measured using a modified method as described previously (38). Urinary HAA concentrations are expressed as ng/g creatinine, to normalize for the varying water content of the spot urine samples, across study subjects.

**Statistical Analysis.** In all statistical analyses, the colorectal cancer/control status of the study subject was considered and controlled for. It is not the aim of the present study to examine whether urinary HAAs differ between colorectal cancer cases and control subjects because the small number of cancer cases ($n = 88$) precludes any statistically meaningful examination of this HAA-cancer association. The statistical power of the present study to detect an odds ratio of 2.0 for colorectal cancer associated with positive versus negative urinary HAAs is <18%. The two-way ANOVA method (39) was used to examine the association between urinary HAA and cigarette smoking status at baseline while controlling for the case-control status of the study subjects. All $P$ values quoted are two sided. $P$ values <0.05 are considered statistically significant.

**Results**

**HAA Content of Shanghai-Style Cooked Meats.** The levels of HAAs (8-MeIQx, 4,8-DiMeIQx, PhIP, and Aoc) in the five samples of pork and chicken prepared using common Shanghai-style frying methods are presented in Table 1. The formation of all four HAAs was low. The highest concentrations of 8-MeIQx (0.3 ng/g) and PhIP (0.5 ng/g) were formed in pork cooked at 280°C for 7 min. The amounts of 4,8-DiMeIQx were low (<0.05 ng/g), and Aoc was below the LOQ (<0.03 ng/g) in all of the meat samples. These estimates of HAAs are consistent with the low values determined previously in stir-fried marinated pork, chicken, beef, and seafood dishes prepared by ethnic Chinese residing in Singapore (40) and in stir-fried meat and chicken, both marinated and nonmarinated, from Singapore Chinese households (41).

**Table 1. HAA formation in pork and chicken dishes cooked by common Shanghai-style cuisine**

<table>
<thead>
<tr>
<th>Meat</th>
<th>Temperature (°C)</th>
<th>Cooking time (min)</th>
<th>8-MeIQx (ng/g cooked meat)</th>
<th>4,8-DiMeIQx (ng/g cooked meat)</th>
<th>PhIP (ng/g cooked meat)</th>
<th>Aoc (ng/g cooked meat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pork loin with bone</td>
<td>200</td>
<td>7</td>
<td>0.15 ± 0.03</td>
<td>&lt;0.03</td>
<td>0.04 ± 0.01</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Pork loin with bone</td>
<td>200</td>
<td>7</td>
<td>0.12 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Pork loin with bone</td>
<td>280</td>
<td>4</td>
<td>0.32 ± 0.03</td>
<td>0.05 ± 0.02</td>
<td>0.48 ± 0.21</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Pork shredded</td>
<td>140</td>
<td>2</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Chicken shredded</td>
<td>135</td>
<td>2</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
</tr>
</tbody>
</table>

NOTE: Each sample was assayed three independent times (mean ± SD). The LOQ of all HAAs was 0.03 ng/g cooked meat.

**Table 2. Characteristics of study subjects**

<table>
<thead>
<tr>
<th>Cancer cases,</th>
<th>Control subjects,</th>
<th>Total, N</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$ (%)</td>
<td>$n$ (%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>88 (51.8)</td>
<td>82 (48.2)</td>
</tr>
<tr>
<td>Age at baseline (y)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>44-50</td>
<td>28 (53.9)</td>
<td>24 (46.2)</td>
</tr>
<tr>
<td>51-55</td>
<td>28 (49.1)</td>
<td>29 (50.9)</td>
</tr>
<tr>
<td>56-66</td>
<td>32 (52.5)</td>
<td>29 (47.5)</td>
</tr>
<tr>
<td>Smoking at baseline (cigarettes/d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>44 (51.2)</td>
<td>42 (48.8)</td>
</tr>
<tr>
<td>1-19</td>
<td>21 (48.8)</td>
<td>22 (51.2)</td>
</tr>
<tr>
<td>20+</td>
<td>23 (56.1)</td>
<td>18 (43.9)</td>
</tr>
</tbody>
</table>
the means for light smokers (1-19 cigarettes per day) and heavy smokers (20+ cigarettes per day) were 7.50 and 11.92 ng/g creatinine, respectively (\( P_{\text{trend}} < 0.001 \)). Cigarette smoking status at baseline was unrelated to either PhIP or 8-MeIQx levels in urine among the study subjects.

**Identification of AaC in Urine of Shanghai Subjects.** Liquid chromatography-electrospray ionization/tandem MS chromatograms of two urine samples, with estimated concentrations of unmetabolized AaC at <4 pg/mL (<LOQ) and 53 pg/mL, respectively, are presented in Fig. 1. The full product ion scan mode, at two different collision energies, was used to corroborate the identity of AaC (Fig. 2). The product ion spectra of the analyte were acquired on an extract obtained from 10 pooled urine samples that were positive for AaC. Under lower collision energy conditions (28 eV), the product ion spectra of the urinary analyte and synthetic AaC display prominent ions attributed to the precursor ion \([M + H]^+\) at \(m/z\) 184 and fragment ions at \(m/z\) 167 and 140, attributed to the loss of NH\(_3\) and by the loss of NH\(_3\) and HCN, respectively. Under elevated collision energy conditions (38 eV), the product ion at \(m/z\) 167 undergoes further fragmentation to form the product ion at \(m/z\) 140 as the base peak. Two other secondary product ions are formed. One ion occurs at \(m/z\) 166 [\(M + H - 18^+\)]; it is attributed to loss of NH\(_3\) followed by the loss of H. The second ion occurs at \(m/z\) 113 [\(M + H - 71^+\)] and is attributed to the cleavage of NH\(_3\) followed by the loss of two HCN groups. The relative abundances of the product ions in the spectra of the analyte and synthetic AaC, under both collision energy conditions, are in excellent agreement and corroborate the identity of the urinary analyte as AaC.

**Measurement of Unmetabolized HAAs Compared with Measurements of HAAs plus Hydrolyzed N-Glucuronide/Sulfamate Phase II Conjugates.** A separate batch (i.e., not taken from the 186 samples described above) of 24 spot urine samples from participants of the Shanghai Cohort study were pooled to form five larger samples of urine (four groups were composed of five subjects and one group was composed of four subjects). The pooled urine samples were assayed without or with hydrolysis (2 N HCl or 2 N NaOH at 70 ºC for 6 h) as reported previously for the hydrolysis of phase II conjugates of the parent HAAs (26, 31-33). The urinary concentrations of 8-MeIQx, PhIP, and AaC arising in these pooled samples, as a function of hydrolysis treatment, are depicted in Fig. 3A to E. The concentrations of 8-MeIQx were below the LOQ in all nonhydrolyzed urine samples, but above the LOQ in two of the acid-treated samples. Both acid treatment and base treatment increased the urinary concentration of PhIP in all urine samples; PhIP was above the LOQ in four of the five acid-treated samples. The concentrations of unmetabolized AaC exceeded the concentrations of 8-MeIQx in all samples and exceeded the amounts of PhIP in four of the five pooled, nonhydrolyzed urine samples. Moreover, the concentrations of AaC were far greater than the concentrations of 8-MeIQx and PhIP in all urine samples that were treated with acid. The measurements of these HAAs in hydrolyzed urine samples of pooled subjects are largely consistent with the data on nonhydrolyzed, individual spot urine samples: AaC is the principal HAA in urine of Shanghai subjects. The ~12-fold increase in urinary AaC concentrations, following acid or base hydrolysis, reveals that this genotoxicant, like 8-MeIQx and PhIP, forms phase II conjugates.

**Discussion**

Although there are certainly distinct features and tastes between the different Chinese cuisines (north versus south, eastern coastal versus inland, etc.), stir frying is the primary mode of cooking throughout most of China, including the City of Shanghai (in the central coastal region) and the provinces of
Guangdong and Fujian (in the southern coastal region), the ancestral homes of virtually all Singapore Chinese. There have been three previous reports on the measurement of HAAs in pork, chicken, and fish dishes prepared by Shanghai-style cooking methods or by Chinese in Singapore (40, 41, 43). The highest concentrations of HAAs were formed in grilled/roasted pork followed by pan-fried chicken; lower to non-detectable levels were reported in stir-fried dishes. PhIP was the most abundant HAA; however, the concentrations were low and PhIP was often present at <1 ng/g for many meat samples. Two of these studies did not examine the formation of AαC (40, 41). In the third study, marinating meats with sugar and/or soy sauce, which is commonly done in Chinese cuisine in Shanghai and Singapore, was reported to increase the concentration of HAAs, by up to severalfold, in a model system of pork simmered at 98°C for 1 h (43). However, even under those conditions, the concentrations of AαC formed were low (<1 ng/g). Our analyses of marinated chicken and pork dishes (Table 1) prepared by Shanghai-style cooking also reveal low levels of HAAs. The low formation of HAAs in these meats was not unexpected; the marinated chicken and pork are cooked for a short period and generally at low temperatures and with frequent stirring. These cooking parameters and the high water content of the marinade are not conducive to the formation of elevated levels of HAAs (44). In contrast to the low levels of HAAs formed in meats prepared by Chinese-style stir frying, the concentrations of some HAAs in western-style, well-done cooked meats can range between 5 and 500 ng/g (6-11).

The low quantities of PhIP and 8-MeIQx formed in the cooked Chinese meat dishes are consistent with the infrequent and low concentrations of these HAAs detected in urine of Shanghai subjects compared with subjects on western-style diets (31-34, 45). Two studies have measured PhIP and 8-MeIQx in urine of male subjects from Los Angeles who consumed an unrestricted diet (31, 32). The comparison of urinary HAA estimates between the Shanghai and Los Angeles studies is not direct because the latter study measured the sum of parent HAA (8-MeIQx or PhIP) and its acid-hydrolyzed conjugates rather than the unchanged HAA alone. Moreover, the Los Angeles study collected an overnight urine specimen (ending with the first morning void) from each subject, whereas the Shanghai cohort specimens were derived from a spot urine sample collected during the day. The geometric mean levels of PhIP and 8-MeIQx in urine of the Los Angeles subjects were 2.48 and 2.47 ng HAA/g creatinine, respectively. Forty percent of the population (n = 131) tested positive for PhIP and 61% of the population (n = 129) tested positive for 8-MeIQx. Levels of AαC were not measured in the Los Angeles Study. On the other hand, the overall mean values for PhIP and 8-MeIQx among Shanghai men in this study were 0.32 and 0.84 ng/g creatinine, with only five (2.9%) and six (3.5%) subjects, respectively, showing detectable levels of urinary PhIP and 8-MeIQx. The low quantities of AαC formed in the Shanghai-style meat staples are consistent with our biomarker findings, suggesting that cigarette smoking is a major source of exposure to AαC in the Chinese population under study.

Our study shows that AαC represents a major HAA exposure in adult men of Shanghai and that cigarette smoking is an important mode of such exposure. Nonetheless, close to 20% of nonsmokers were positive for AαC in urine. AαC is formed through the high temperature pyrolysis reactions of proteins (13). We cannot exclude that flame broiled or barbequed meats or fish, which may contain AαC, contribute to the background levels of AαC (46). However, based on our results for AαC content in Shanghai-style cooked meats, plus other recently published data on Chinese foods (40, 41, 43), it is reasonable to postulate that dietary exposure to AαC in commonly prepared Chinese dishes is negligible. Passive smoke or diesel exhaust may be potential sources of AαC in our nonsmokers (14).

One previous study reported an association between urinary levels of HAAs and cigarette smoking (47). PhIP was found to have the same retention time as one of the major urinary mutagens of smokers of black tobacco fractionated by high-performance liquid chromatography, and 32P-postlabeling analysis of urinary mutagens implicated PhIP as a major DNA-damaging agent (47). In the present study, only 2.9% of subjects were positive for urinary PhIP, and no association with smoking status was found.

The major pathways of metabolism of 8-MeIQx and PhIP in humans and urinary excretion products have been well

![Figure 2](image-url)  
**Figure 2.** Full scan product ion spectra of synthetic AαC and urinary analyte from 10 pooled urine samples. The spectra of the protonated molecule [M + H]⁺ at m/z 184 were acquired at collision energies of 28 and 38 eV (background spectra have been subtracted).

![Figure 3](image-url)  
**Figure 3.** Liquid chromatography/electrospray ionization-tandem MS measurements of 8-MeIQx, PhIP, and AαC in nonhydrolyzed (white columns), base-hydrolyzed (black columns), and acid-hydrolyzed (dotted columns) specimens. A to E, five independent pooled urine samples derived from 24 individual spot urine specimens from participants of the Shanghai Cohort study. Dotted lines, LOQ of PhIP and AαC at 4 pg/mL. Columns, mean of three independent measurements; bars, SD.
characterized; however, the metabolic pathways of ACoC are unknown (48-50). Both 8-MeIQx and PhIP undergo oxidation by cytochrome P450s or undergo direct conjugation by UDP-glucuronosyltransferases or sulfotransferases, to form conjugates at the exocyclic amino groups of both HAAs and at the N3 imidazole atom of PhIP (48, 50). The excretion of unmetabolized 8-MeIQx and PhIP in urine of male subjects in Western Europe and the United States, who ate well-done beef, was reported at, respectively, about 2% to 5% and 0.5% to 2% of the dose within 10 h after consumption of well-done fried beef (27, 33, 45). Comparable percentages of unchanged ACoC were eliminated in urine of subjects during the same time frame, following consumption of very well-done fried beef (26). The confidence interval (85% confidence interval) of unchanged ACoC in a 10-h urine sample from those meat eaters was 19.8 (10.4-29.2) pg/mL. The corresponding figures for ACoC in spot urine of light (1-19 cigarettes per day) and heavy (20+ cigarettes per day) smokers in the present study are 9.3 (5.1-13.6) and 13.8 (9.5-18.1) pg/mL, respectively.

Both PhIP and 8-MeIQx are excreted in urine as a combination of unchanged compound plus phase II conjugates: these conjugates then undergo hydrolysis to the parent amines by either acid or base in vitro (27, 31, 32, 34, 51). For some subjects, hydrolysis increased the urinary HAA content by up to 3- to 10-fold, showing that phase II enzymes are important contributors to the detoxification of 8-MeIQx and PhIP. The acid or base treatment of pooled urine samples from Shanghai subjects also resulted in up to a 12-fold increase in the amount of ACoC (Fig. 3), indicating that phase II conjugates of ACoC are present in urine.

Interindividual variation in the levels of expression of the P450 enzymes involved in HAA metabolism is likely to play a substantial role in the varying urine levels of unchanged 8-MeIQx, PhIP, and ACoC in meat eaters and tobacco smokers. P450 1A2, principally expressed in liver (52), is the major P450 involved in the oxidation of 8-MeIQx, PhIP, and ACoC (51, 53-55). The level of protein expression of P450 1A2 varies by >50-fold in human liver samples (55). Thus, individuals with high P450 1A2 activity may have lower concentrations of unmetabolized HAAs in urine than do individuals with low P450 1A2 activity (54). Tobacco smoking is known to induce hepatic P450 1A2 (56). Therefore, heavy smokers are expected to be rapid metabolizers of HAAs. Despite the induction effect of P450 1A2 by cigarette smoking, we observed in the present study that, among smokers, an increasing number of cigarettes smoked per day was positively related to increasing levels of unmetabolized ACoC in urine.

AcCoC induces tumors in the liver and vascular system of male and female CDF1 mice fed ACoC as part of their diet (800 ppm) for 2 years (1). ACoC also was reported to induce preneoplastic foci in liver of rats (57), although actual tumors did not form during a 2-year feeding study (1). ACoC was found to induce mutations with high frequency in the lacI and lacZ genes in liver and colon of transgenic mice, at potencies comparable with those of IQ, 8-MeIQx, and PhIP (58, 59). More recently, ACoC was reported to induce aberrant crypt foci in the colons of male and female C57BL/6N mice at 5-fold higher levels than did 8-MeIQx (60). The early development of aberrant crypt foci, precursors to carcinoma, suggests that 8-MeIQx and ACoC, especially, are colon carcinogens in C57BL/6N mice (1). However, long-term feeding studies have not been reported on either of these HAAs, to determine their potential carcinogenicities in this mouse strain.

Based on the high abundance of ACoC in tobacco smoke, the high frequency of detection of ACoC in urine samples and the broad spectrum of human phase I and II enzymes expressed in liver and extrahepatic tissues (61) that can bioactivate ACoC, we conclude that this HAA, in particular, may be an important genotoxicant of tobacco-induced cancers in Shanghai Chinese. Molecular epidemiology studies on ACoC exposure and the effect of xenobiotic enzyme polymorphisms associated with ACoC genotoxicity and tobacco smoke–induced tumors of the gastrointestinal tract are warranted.

References


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