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PhIP metabolites in human urine after consumption of well-cooked chicken

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Abstract

We devised an assay to quantify the metabolites of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in human urine following a single exposure to well-cooked meat. Our method uses LC/MS/MS to detect four metabolites and four deuterated internal standard peaks in a single chromatographic run. N^2-OH–PhIP–N^2-glucuronide was the most abundant urinary metabolite excreted by the 12 individuals who participated in our study. N^2-PhIP glucuronide was the second most abundant metabolite for 8 of the 12 volunteers. The stability of PhIP metabolism over time was studied in three of the volunteers who repeated the assay eight times over a 2.5 year-period. PhIP metabolite excretion varied in each subject over time, although the rate of excretion was more constant. Our results suggest that quantifying PhIP metabolites should make future studies of individual susceptibility and dietary interventions possible.
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Keywords: Food analysis; Metabolism; 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; Heterocyclic aromatic amine

1. Introduction

Potent genotoxic carcinogens of the heterocyclic amine (HA) class of compounds are produced in meat during cooking at high temperatures. The demonstrated mutagenicity of these compounds in bacteria [1], cells in culture [2,3] and mice [4], support the many studies of carcinogenicity in mice [5] and rats [6,7]. Mechanistic data show that, even at low doses, HAs form DNA adducts in rodents [8,9] and humans [10]. Of the 14 mutagens identified from cooked meat, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) is the most mass abundant [11].

Humans are exposed to PhIP through the consumption of various cooked muscle meats, notably beef, pork and chicken [12–15]. The amount of PhIP that an individual is exposed to is related to food preparation methods [16–18], and the frequency of consumption. The presence of PhIP in restaurant and home-cooked meats has been documented, suggesting that humans may be exposed to PhIP in the range of 0.1–200 ng/g by consuming common foods [19–21]. These consumption levels may result in possible exposure doses in the milligram range for an individual.

The impact of heterocyclic amine exposure on human health is not clear, and its contribution to human cancer is a current subject of debate. Several epidemiological studies reported a positive correlation between the consumption of well-done meat and cancer risk [22–24]. In 1998, Zheng et al. [25] described a significant dose-dependence between meat preparation and breast cancer risk; women who preferred well-done hamburger, steak and bacon had a 4.6-fold greater risk of breast cancer than did women who preferred meats cooked “rare” or “medium”. A recent case–control study of women in Shanghai, China showed a positive association of breast cancer risk and red-meat intake, especially well-done meat, which was more pronounced among women with a high body mass index [26]. Several studies reported an increased risk of colorectal adenomas and lung cancer with well-done and/or fried meat consumption [27–29]. African American males, who are at increased risk for prostate cancer, consume 2 to 3 times more PhIP than age-matched white males [30]. Two recent studies investigated the effect of *N*-acetyltransferase polymorphisms and cooked meat consumption on prostate cancer risk. Hein et al. [31] found that a particular subset of NAT2 acetylator genotypes were at increased risk for prostate cancer. In

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contrast, the study of Barrett et al. [32] provided no support for the hypothesis that fast NAT2 acetylators are at increased risk of colon cancer, even if exposed to high levels of HAs from well-cooked meats. Another study, performed in New Zealand, reported equivocal associations for well-done meat and prostate cancer [33]. Negative associations with cooked meat consumption have been reported with breast, colon, and rectal cancer [34–37].

PhIP is a procarcinogen that must be metabolically activated in order to damage DNA [38,39]. During Phase I metabolism PhIP is oxidized to the hydroxylated intermediates 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine (*N*-OH–PhIP) or 2-amino-1-methyl-6-(4′-hydroxy) phenylimidazo[4,5-*b*]pyridine (4′-OH–PhIP). Phase II metabolizing enzymes, primarily the acetyltransferases or sulfotransferases, then further convert *N*-OH–PhIP to a biologically active form that has been shown to bind DNA and cellular proteins [40–43]. Detoxification primarily involves glucuronidation. *N*-hydroxy–PhIP can form stable glucuronide conjugates at the N^2 and *N3* positions that can be excreted or transported to extrahepatic tissue for further metabolism [44,45]. 4′-Hydroxy–PhIP can be conjugated by sulfation and glucuronidation to polar compounds that are readily excreted [46,47]. In addition, the parent compound can be directly glucuronidated at the N^2 and *N3* positions. These glucuronides are not reactive and this reaction is believed to be a detoxification pathway [45,48]. Fig. 1 describes the formation of the four major human PhIP metabolites.

Human PhIP metabolism has been most intensively studied using hepatic microsomes or cells in culture. A recent study comparing PhIP metabolism in human and rat hepatocytes showed that the major human biotransformation pathway of PhIP was cytochrome P4501A2 (CYP1A2)-mediated N-oxidation followed by glucuronidation at N^2 and *N3* positions of PhIP [49]. In contrast, rat hepatocytes transformed PhIP to 4′-OH–PhIP as the primary product. Glucuronide and sulfate conjugates of 4′-OH PhIP were detected in human hepatocytes, but as relatively minor products [49]. Extrahepatic metabolism of PhIP has been demonstrated in breast, prostate, and colon. Studies have shown that human mammary cells have the capacity to metabolize the parent compound PhIP as well as the hydroxylated intermediates [50–52]. PhIP is glucuronidated by UGT1A1 in the human colon carcinoma cell line Caco2 [53] and human prostate cells have also been shown to metabolize PhIP ([54,55], Kulp, personal observations). The metabolic pathways and the metabolites produced during

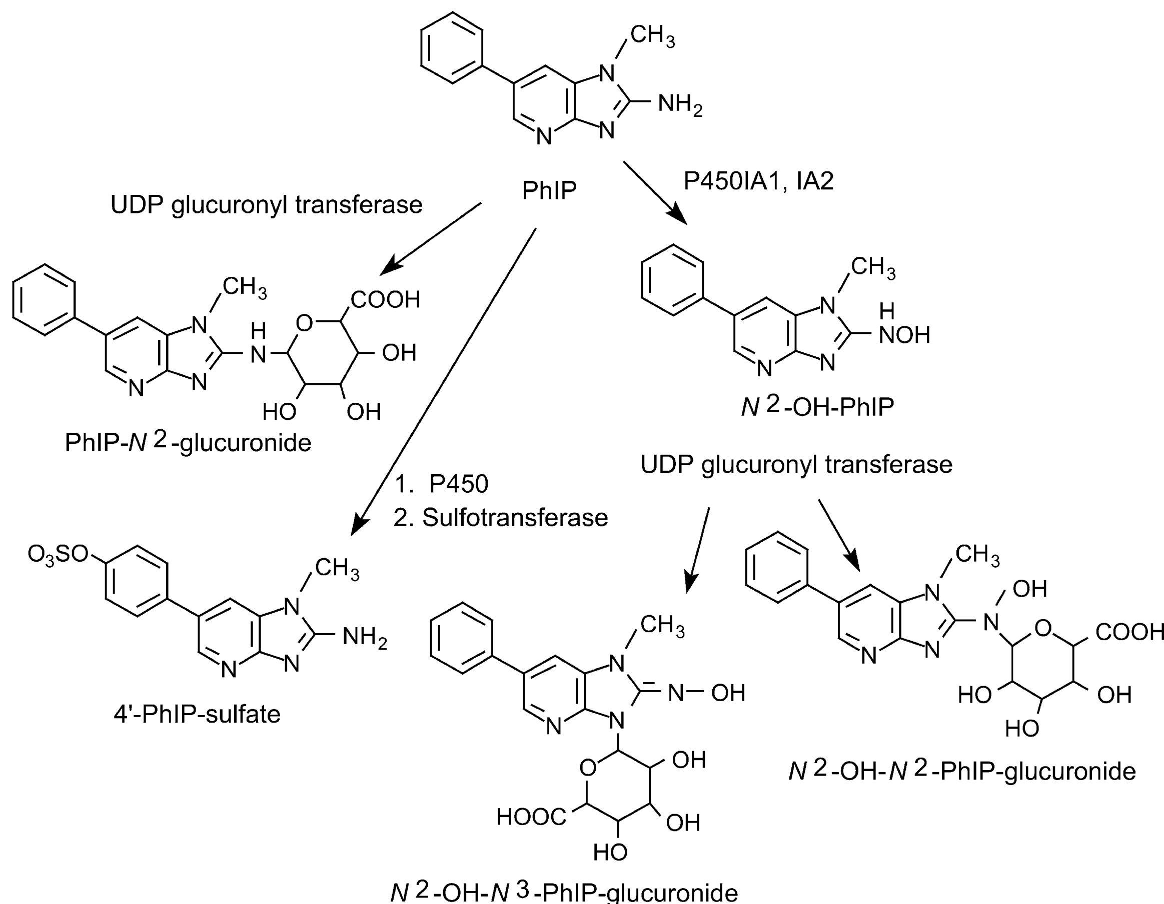

Fig. 1. Formation pathway for the major metabolites of PhIP found in human urine.

PhIP bioactivation in these target organs have not been fully determined.

Other studies of human PhIP metabolic pathways have been done in healthy volunteers by quantifying urinary metabolites. Pioneering work examined the relationship of urinary excretion of the unmetabolized parent compound and the dose received in well-done hamburgers [56,57]. PhIP and PhIP conjugates have been quantified in human urine using acid- or alkali-hydrolysis. These investigations demonstrate PhIP bioavailability, time course of excretion and the correlation between meat consumption and urinary metabolites, but do not give information about specific metabolic pathways [58–62]. Identification of human PhIP metabolites was determined in studies that investigated PhIP metabolism following administration of [^{14}C]-labeled PhIP to patients undergoing cancer surgery [63–65]. In these studies, body fluids and tissues were examined using accelerator mass spectrometry to investigate PhIP metabolic pathways. In 2002, Stillwell et al. [66] correlated the excretion of N^2–(β-1-glucosiduronyl)-2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, measured as the deaminated product 2-OH PhIP, to CYP1A2 and NAT2 activity in 66 healthy subjects.

There have been four major PhIP metabolites identified in human urine: N^2-OH–PhIP–N^2-glucuronide, PhIP–N^2-glucuronide, PhIP–4′-sulfate, and N^2-OH–PhIP–*N3*-glucuronide [64]. Recently, we described a solid-phase extraction LC/MS/MS method for quantifying these four metabolites in human urine, following a meal of well-cooked chicken. We applied this method to characterize PhIP metabolism in healthy individuals receiving a known dose of naturally-produced PhIP [67,68]. We have also extended that method to examine the interactions of potentially preventive foods [69]. In the current study, we describe PhIP metabolism of 12 male volunteers, three of whom collected urine at 4 month intervals during a more than 2 years time span.

2. Material and methods

2.1. Study design

The study protocol was reviewed and approved by the Institutional Review Board for Human Research at Lawrence Livermore National Laboratory. Informed consent was obtained from each subject prior to beginning the study. The individuals participating were recruited from the local workforce, were all male, in good health, non-smokers, and of normal weight.

2.2. Meat preparation and controlled dietary period

Meat preparation conditions have been described previously [67]. Briefly, boneless, skinless chicken breasts were cut into approximately 2.5 cm pieces and fried in a non-stick coated pan, for 35–40 min. A representative chicken sample was removed for heterocyclic amine analysis. HA analysis was performed according to previously published methods [19]. The study subjects were provided with 150 g chicken with other non-meat foods and beverages. Total PhIP dose depended on the exact cooking time and was different for each batch of chicken cooked. The PhIP content in the various batches ranged from 61 to 131 ppb, providing doses of 9.2–19.6 μg PhIP in 150 g of cooked chicken. The PhIP dose was known for each subject. Two of the subjects repeated the assay eight times over the course of 2.5 years. A third subject repeated the assay seven times in the same time period.

Subjects were asked to abstain from meat consumption for 24 h prior to eating the well-done chicken breast. There were no other dietary restrictions. Control urine was collected before eating the chicken and for 24 h after in 6 h increments. Samples were coded, the volume recorded and stored frozen at −20 °C until analysis.

2.3. Extraction of PhIP metabolites

Urine samples (5 ml) were spiked with internal standard of urine (100 μl) from a rat dosed with pentadeutero-PhIP [70] (1 mg per day) generating the four different PhIP metabolites we detect in human urines. Samples were first applied to a pre-conditioned 60 mg Strata™ X SPE column (Phenomonex, Torrance, CA). Metabolites were eluted with 5 ml methanol. The elution aliquot was evaporated to dryness under nitrogen and the metabolites were re-dissolved in 2.5 ml 0.01 M HCl. Proteins and high molecular weight contaminants were removed by filtering the solution through a Centricon® YM-3 centrifugal filter (Millipore Corp., Bedford, MA). The filtrate was applied to a pre-conditioned benzenesulfonic acid column (SCX, 500 mg, Varian Sample Preparation Products, Harbor City, CA) and the column washed with 3 ml of 10% (v/v) methanol/0.01 M HCl. The metabolites were eluted onto a coupled C18 column (Bakerbond spe®, 1000 mg, J.T. Baker, Phillipsburg, NJ) with 60 ml of 0.05 M ammonium acetate, pH 8. The C18 column was washed with 3 ml of 5% (v/v) methanol/H_2O and eluted from the C18 column with 5 ml of 60% (v/v) methanol/H_2O. The metabolites were dried under nitrogen and 1 ml urine equivalent was injected into the LC/MS/MS in a volume of 20 μl.

Chromatography was done on an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) equipped with a YMC ODS-A column (3.0 mm × 250 mm). Metabolites were eluted at a flow rate of 200 μl/min using a mobile phase of A (water:methanol:acetic acid, 97:2:1) and 25% B (methanol:water:acetic acid, 95:4:1) with a linear gradient to 100% B at 20 min and held for 5 min.

Analytes were detected with a mass spectrometer (model LCQ, Finnigan, San Jose, CA) in the MS/MS positive ion mode using an electrospray interface. A capillary temperature of 240 °C, a source voltage of 4.5 kV, a sheath gas of 70

units and 5% auxiliary gas were used. An ion trap injection time of 1000 ms and one microscan were used.

Alternating scans were used to isolate $[M + H]^+$ ions at mass 417, 401, and 321 for natural PhIP metabolites, and 422, 406, and 326, for the pentadeutero-labeled internal standard metabolites. Collision energy was 25%. Daughter ions were detected at appropriate masses: 241 $[M+H\text{–glucuronic acid}]^+$ and 225 $[M + H\text{–glucuronic acid–OH}]^+$ from 417 for the *N*-hydroxy-N^2 and *N3* glucuronide, respectively, 225 $[M + H\text{–glucuronic acid}]^+$ from 401 for the PhIP N^2 glucuronide, 241 $[M+H\text{–}SO_3]^+$ from 321 for PhIP–4′-sulfate. Ion fragments detected for the deuterated internal standards were 5 mass units greater than the natural PhIP metabolite fragments.

2.4. Sample analysis and statistics

The overall recovery of the metabolites was determined by spiking each urine sample with known amounts of deuterium-labeled metabolites obtained from the rat urine. Final metabolite amounts were adjusted for losses based on the recovery of the internal standards. Each urine sample was analyzed at least twice. Total metabolite concentrations excreted in each time period were calculated by multiplying by the urine volume. Peak areas were converted to masses based on a response factor for PhIP and then normalized to percent of the original PhIP dose consumed in the chicken. Excretion rate was calculated by summing each of the four metabolites and calculating the percent of the total metabolites that were excreted in each time period. Spearman rank-correlation tests were used to determine the association between the excretion level of the N^2-OH–PhIP–N^2-glucuronide excreted and the ingested dose of PhIP. Thirty-three data pairs were used in the analysis; data from all 12 subjects as well as each individual subject's repetitions of the assay. Subjects were divided into "fast" and "slow" excretion groups based on a comparison of the amount of metabolite excreted in the 0–6 and 6–12 h time intervals. Subjects that excreted more metabolite in the 0–6 h interval were considered "fast", those that excreted more in the 6–12 h time interval were considered "slow". Average metabolites excreted by the two groups were compared using the Student's *t*-test.

3. Results

3.1. Human PhIP metabolite excretion after a meal of well-cooked chicken

Our method using LC/MS/MS detects peaks for the four identified human PhIP metabolites as well as four deuterated internal standard peaks in a single chromatographic run. Fig. 2 shows a set of mass chromatograms for a typical sample of the equivalent of 1 ml of urine injected. For increased sensitivity, the data acquisition was made over three segments, isolating mass 321 for 14 min, masses 417, 401, and 422 for 7 min, and mass 417 only for the final 5.5 min. Since other ion peaks are often present in the chromatograms that do not represent one of the four identified PhIP metabolites (Fig. 2), expected peak retention times and peak widths are compared to reference samples to confirm the identity of the PhIP metabolites. N^2-OH–PhIP–N^2-glucuronide and its deuterium-labeled analog are detected as broader HPLC peaks that fragment into two daughter ions. The sum of these two peak areas is used for quantitation (Fig. 2). The N^2-OH–PhIP–*N3*-glucuronide is separated in time from the N^2-OH–PhIP–N^2-glucuronide and fragments to mass 225 only (Fig. 2). HPLC column lifetime is a problem with these samples. We slurry-pack our own columns with 10 μm particle size resin and replace the column after 24 injections to obtain the best results for routine samples.

Control urine samples were collected before the well-done chicken was consumed, during the period that the volunteers abstained from eating cooked meat. No PhIP metabolite peaks were seen in the control samples from the 12 individuals. Total urine excreted after chicken consumption was collected for 24 h in 6 h increments. Metabolite values shown are corrected for the total volume of urine. Fig. 3 shows the percentage of the ingested PhIP dose recovered in the urine as PhIP metabolites for the 12 subjects. Recovered doses varied nine-fold despite the fact that all urine was collected and amounts were normalized to account for differences in PhIP dose. The total amounts of each of the four individual metabolites excreted during the 24 h collection period are also shown in Fig. 3 as variably shaded regions of the bars. N^2-OH–PhIP–N^2 glucuronide was the most abundant urinary metabolite in all individuals, comprising 44 (Subject K) to 80% (Subject F) of the total metabolite excreted. N^2-PhIP glucuronide was the second most abundant metabolite for 8 of the 12 volunteers and these two metabolites together account for 77–95% of the total metabolite excretion for these individuals. In three individuals (B, L, and M) N^2-OH–PhIP–*N3*-glucuronide was the second most abundant metabolite and in Subject H PhIP–4′-sulfate was second most abundant, comprising almost 30% of the total metabolite excreted.

Fig. 4 shows the rate of excretion of the PhIP metabolites in time periods of 0–6, 6–12, 12–18 and 18–24 h. Subject L did not provide a sample for the 12–18 h period. Subjects F and G provided sample for the 18–24 h time period, but no metabolites were detected in these samples. In all of the subjects, the majority of the metabolites were excreted in the first 12 h (61–92%). The individuals showed variation in the time of metabolite excretion. Six of the subjects (A, E, G, K, L and M) excreted more than 45% of the total metabolite in the 6–12 h time period. The other six individuals excreted 34–50% of the total metabolite in the 0–6 h time period. Seventy-six to 100% of the dose was excreted in the first 18 h after consuming the cooked chicken meal.

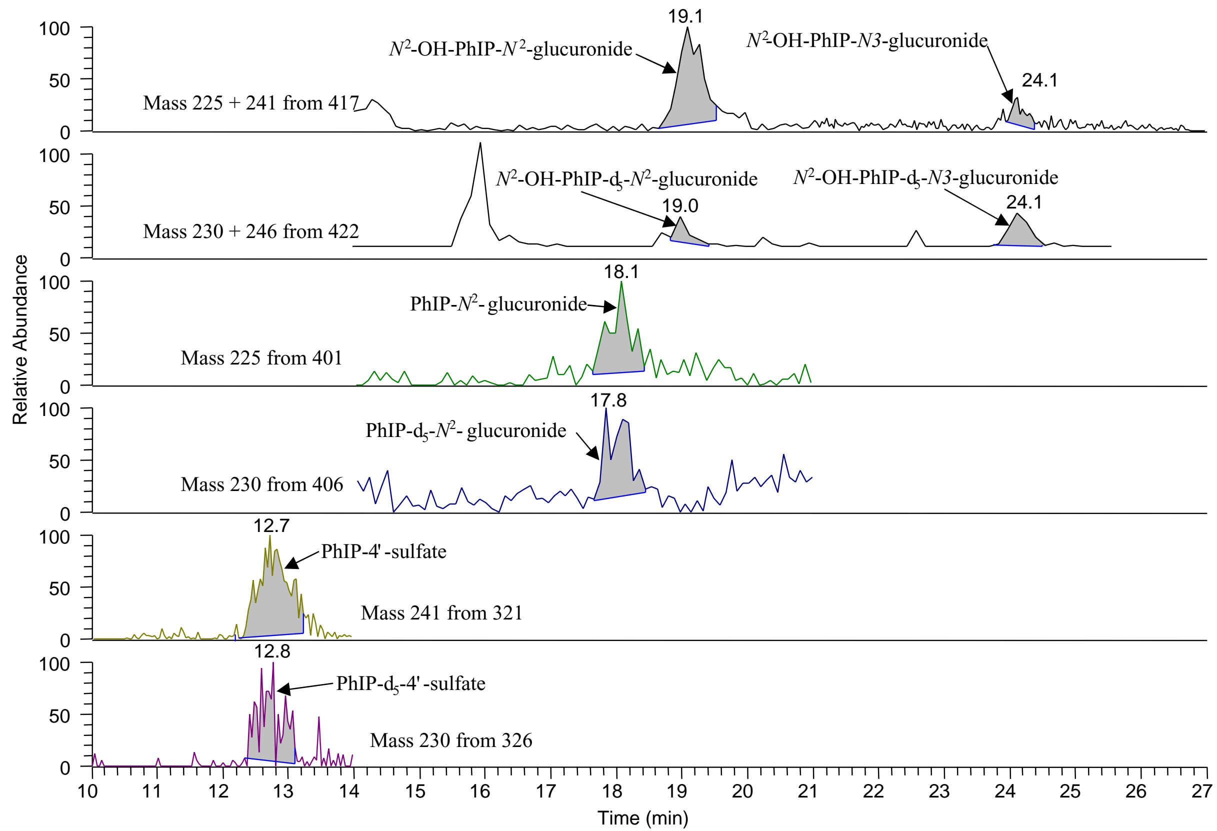

Fig. 2. Ion plots of PhIP metabolites and the pentadeutero-PhIP (PhIP-d_5) metabolite internal standards from the injection of the equivalent 1 ml of urine. See Section 2 for LC/MS/MS conditions.

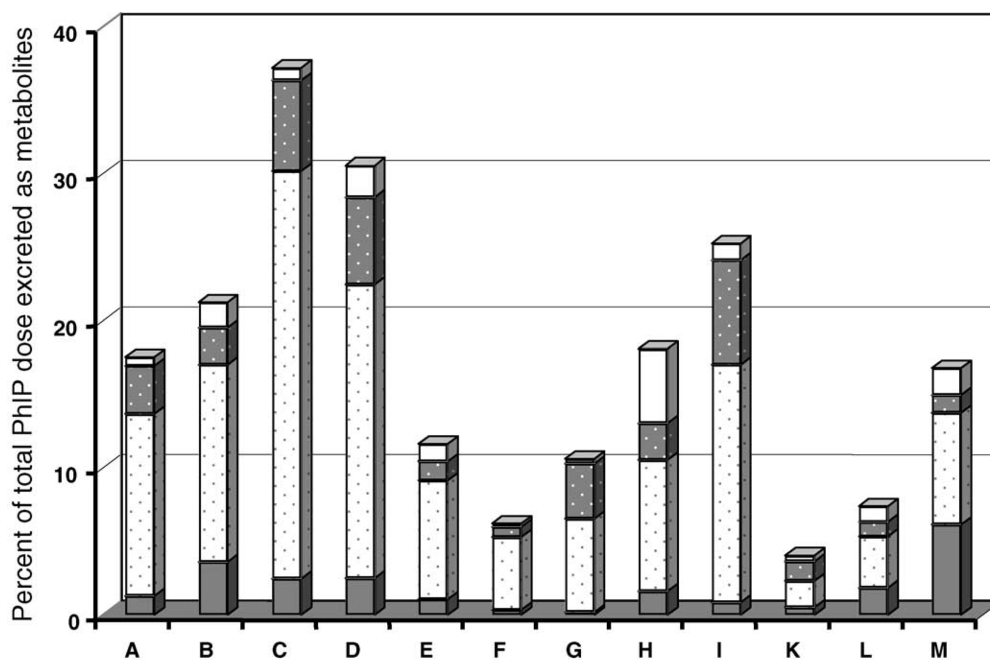

Fig. 3. Total 24 h excretion of urinary PhIP metabolites for 12 subjects. Total excretion of each metabolite during the 24 h time period was calculated and expressed as percent of the PhIP dose ingested. ( ) N^2-OH–PhIP–$N3$-glucuronide; ( ) N^2-OH–PhIP–N^2-glucuronide; ( ) PhIP–N^2-glucuronide; ( ) PhIP–4′-sulfate.

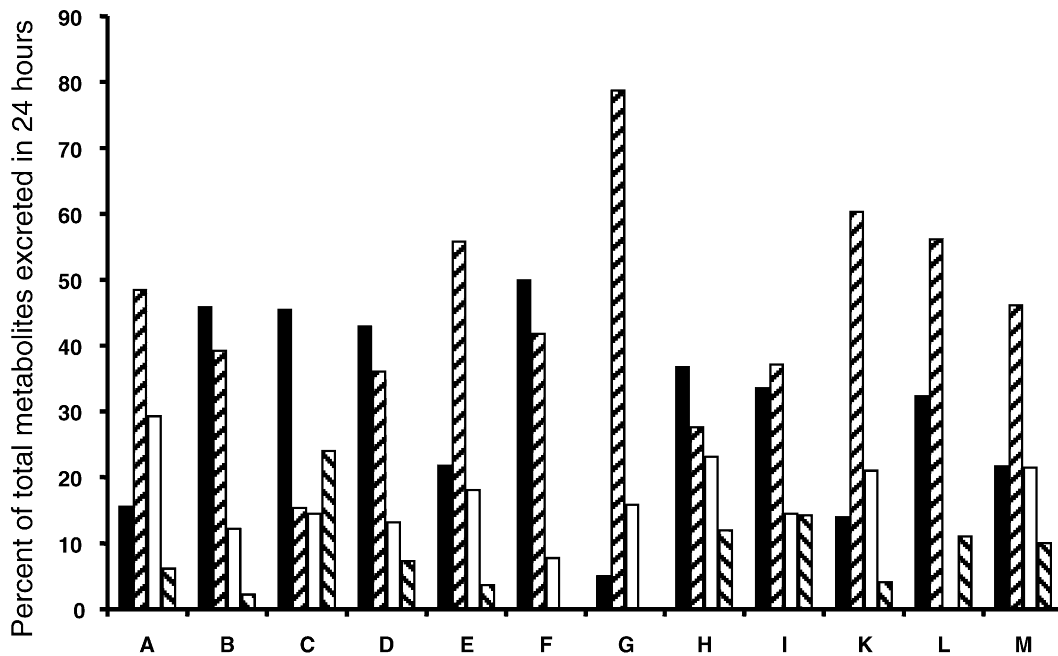

Fig. 4. Rate of excretion of four PhIP metabolites for 12 subjects. Total urinary metabolites recovered during the 24 h after dosing were quantified. Data represent the percentage of the total metabolites excreted during the designated time period. Time increments shown are: (■) 0–6; (▨) 6–12; (□) 12–18; (▧) 18–24 h.

3.2. Correlation of metabolites excreted to PhIP dose ingested

A weak association was observed (Fig. 5) between the amount of PhIP ingested and the total amount of N^2-OH–PhIP–N^2 glucuronide excreted in the 24 h urine ($r_s = 0.29$, $P < 0.1$). We also compared the total amount of PhIP ingested to the sum of all of the metabolites excreted, but this did not improve the correlation.

3.3. Comparing "fast" and "slow" excretion groups

The subjects in the study were divided into "fast" and "slow" excretion groups based on the amount of metabolites

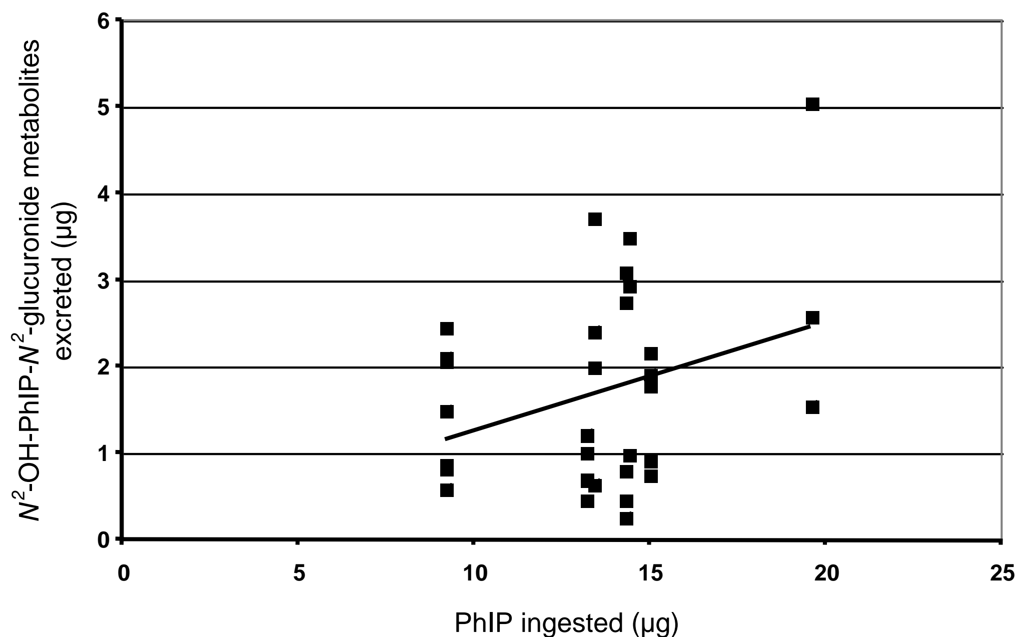

Fig. 5. Linear regression analysis of total N^2-OH–PhIP–N^2-glucuronide excreted 0–24 h after chicken consumption as a function of the amount of the PhIP consumed for each individual.

Table 1
Average metabolite excretion of “fast” and “slow” excretion groups

	N^2-OH–PhIP–*N3*-glucuronide	N^2-OH–PhIP–N^2-glucuronide	PhIP–N^2-glucuronide	PhIP–4′-sulfate	Total
Fast	3.2 ± 3.2	20.6 ± 15.1	5.5 ± 5.1	2.5 ± 1.9	31.8 ± 19.6
Slow	1.6 ± 1.4	10.4 ± 6.9[a]	2.7 ± 1.9	0.9 ± 0.6[a]	15.6 ± 8.2[a]

Data are means ± standard deviation.
[a] Fast significantly different than slow ($P < 0.05$).

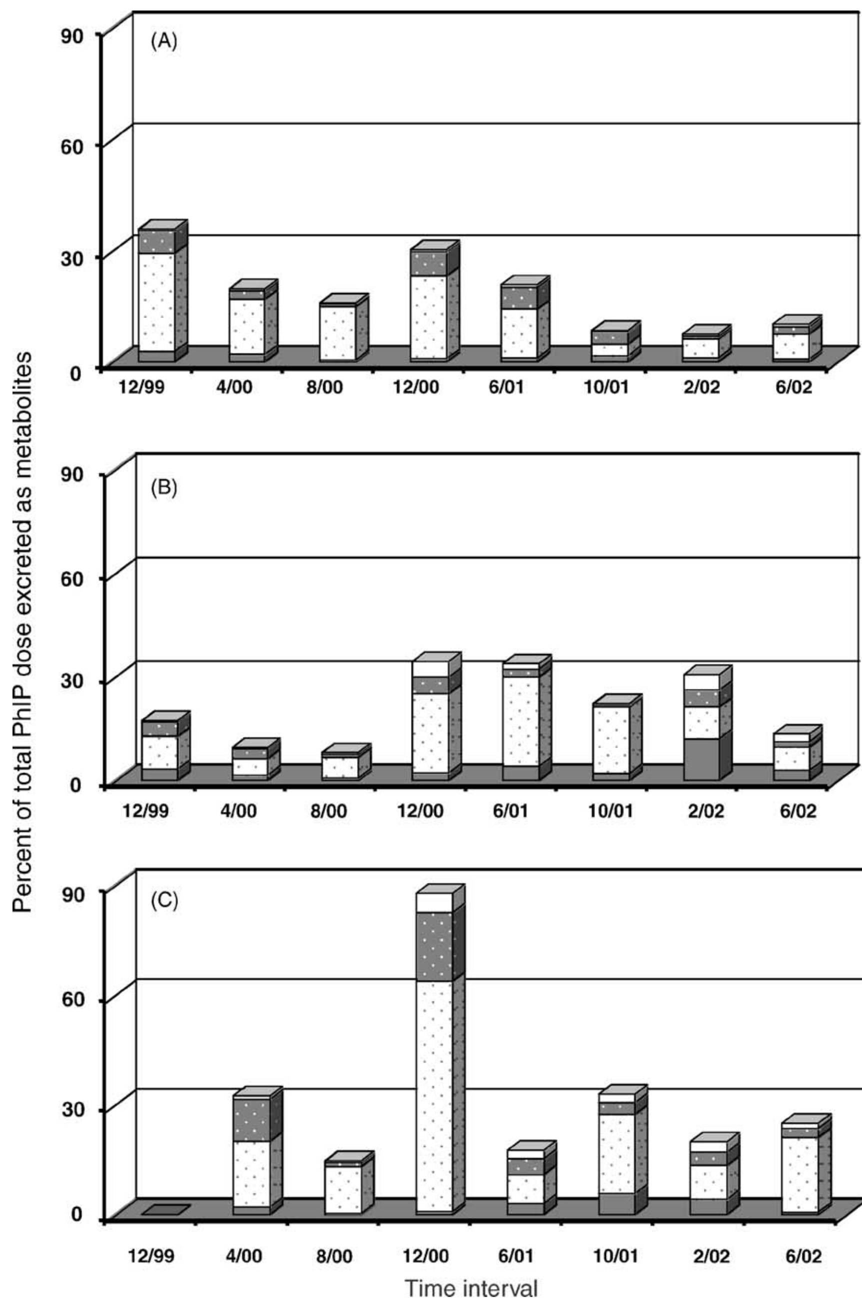

Fig. 6. Total 24 h excretion of urinary PhIP metabolites for three subjects at several different times. Total excretion of each metabolite during the 24 h time period was calculated and expressed as percent of the PhIP dose ingested. (A) Subject A; (B) Subject B; (C) Subject C. (■) N^2-OH–PhIP–*N3*-glucuronide; (⁛) N^2-OH–PhIP–N^2-glucuronide; (■) PhIP–N^2-glucuronide; (□) PhIP–4′-sulfate.

excreted in the 0–6 h time period. Subjects were considered "fast" excretors if the ratio of the metabolites excreted in the 0–6 h time interval to the 6–12 h time interval was greater than or equal to 1. "Slow" excretors were defined as a ratio less than 1. The average metabolite excretion for each group is presented in Table 1. Subjects considered "fast" excreted significantly more N^2-OH–PhIP–N^2 glucuronide, 4′-PhIP sulfate and total metabolites than the subjects considered "slow" ($P < 0.05$).

3.4. Human PhIP metabolism in three individuals over time

To determine individual changes in PhIP metabolism over time, we measured PhIP metabolite excretion in 3 subjects repeatedly over a 2.5 year-period (Figs. 6 and 7). The assay was repeated at approximately 4 month intervals. Subject C did not participate in the assay in December 1999. As seen in Fig. 6, the amount of PhIP metabolites excreted, expressed

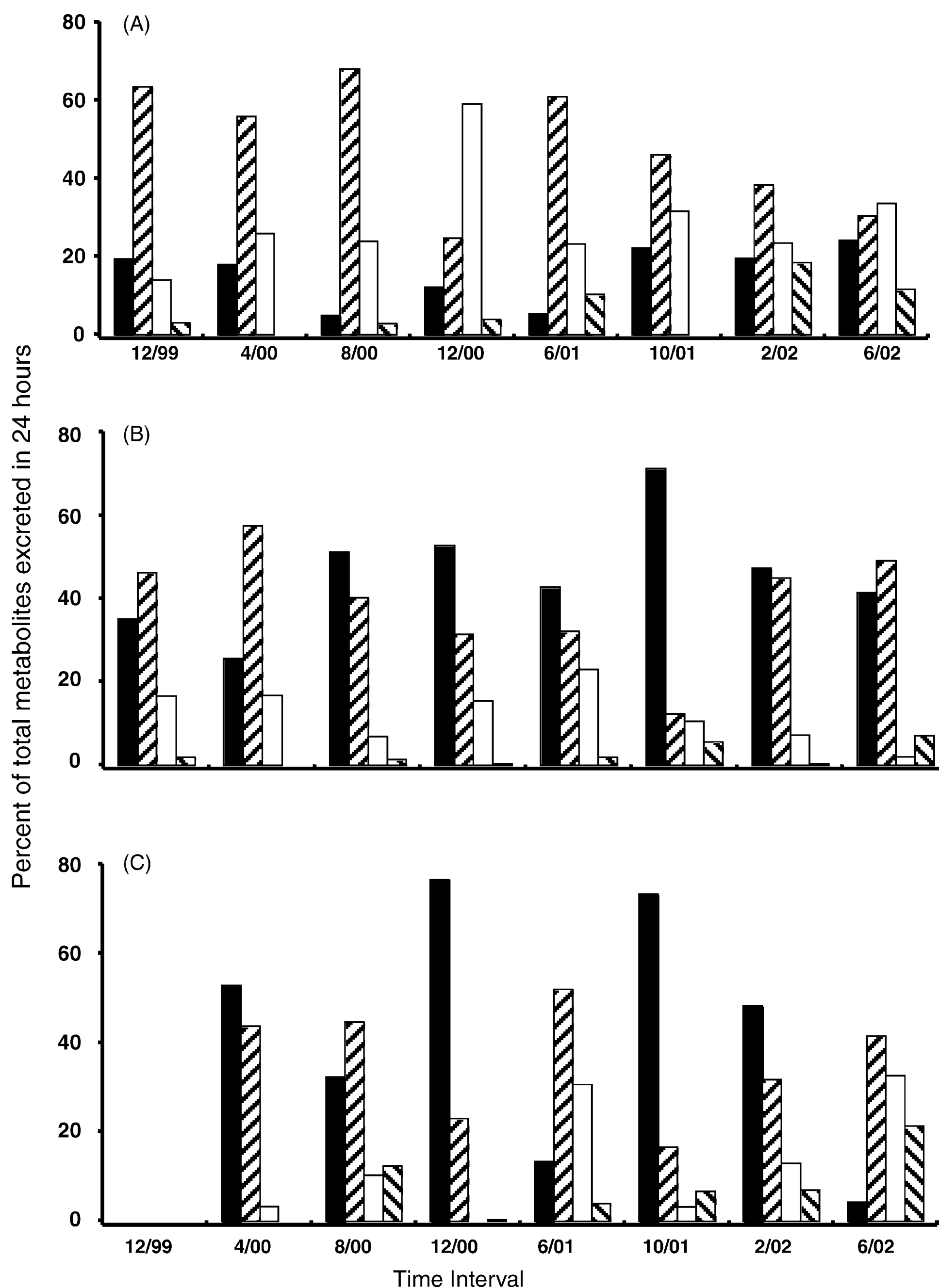

Fig. 7. Rate of excretion of four PhIP metabolites for 12 subjects. Total urinary metabolites recovered during the 24 h after dosing were quantified. Data represent the percentage of the total metabolites excreted during the designated time period. Time increments shown are: (■) 0–6; (▨) 6–12; (□) 12–18; (▧) 18–24 h.

as percent of the PhIP dose ingested, is not constant in these individuals over time. Considerable variation exists not only in the amount of each individual metabolite excreted (shown as variably shaded regions within the bar) but in the total amount of the PhIP dose excreted as well. In contrast, the rate of metabolite excreted is more constant (Fig. 7). For Subject A, the larger fraction of the metabolites excreted was always in the later time intervals; 6–12 h or 12–18 h. Subject B, on the other hand, tended to excrete metabolites more quickly; in five of the eight trials the largest fraction of the metabolites were excreted in the 0–6 h time interval. Subject C, similarly to Subject B, excreted the largest fraction of the metabolites in the 0–6 time interval in four out of seven trials. Both B and C excreted almost all of the metabolites in first 12 h after consuming chicken (an average of 80% for both subjects over all time intervals), whereas Subject A excreted an average of 64% of the metabolites in the first 12 h.

4. Discussion

This study reports the variation in PhIP metabolism among twelve healthy human subjects who have been fed a single meal containing well-cooked chicken. Both the amounts of chicken consumed by our volunteers and the PhIP levels were comparable to consumption levels possible in households or restaurants [71].

In the present study, we found that the amount of metabolites excreted in the 0–24 h urine represented $17 \pm 10\%$ of the ingested PhIP. In a previous study of normal females, we reported a similar average of 21.5% of the PhIP dose recovered in the urine [67]. Strickland et al. [60] reported that 16.6% of the ingested PhIP could be quantified in the 0–12 h acid-hydrolyzed urine of their population and Stillwell et al. [66] reported the recovery of N^2-OH–PhIP–N^2-glucuronide (measured as 2-OH–PhIP) as an average of 24.6%. These studies all confirm that PhIP present in the meat matrix is not completely bioavailable. In an earlier study of hospitalized elderly cancer patients given PhIP in a gelatin capsule, 90% of the ingested dose was recovered in the urine for two of the three subjects [64]. This indicates that PhIP provided in capsule form is more bioavailable than PhIP ingested in meat. We are currently investigating the bioaccessibility of PhIP from cooked meat using an in vitro digestion model. In that study we show that release of PhIP from the meat matrix was dependent upon pancreatic enzyme concentration and meat doneness [72]. We are also investigating the impact that other foods in the GI tract may have on PhIP bioaccessibility. Other factors such as transport across the intestinal cell monolayer and individual differences in metabolic pathway capacities may also ultimately affect how much of the ingested PhIP dose is recoverable in the urine.

The kinetics of PhIP metabolite excretion in our study are similar to those seen previously for humans [56,64]. Our results demonstrate that excretion times vary among the volunteers but that most of the dose (76–100%) is excreted in the first 18 h. This suggests that these metabolites are suitable for investigating individual variation in rates and ratios of PhIP metabolism. Further, these metabolite measurements may be used as biomarkers of recent exposure, but are not suitable for long-term exposure estimates.

The detection of individual metabolites also confirms our earlier findings [64]. The ratio of the individual metabolites varied among our 12 individuals, although N^2-OH–PhIP–N^2-glucuronide was always the most abundant. In our previous study of female volunteers, we also found N^2-OH–PhIP–N^2-glucuronide in the greatest amounts, although in the female volunteers PhIP–N^2-glucuronide was consistently the second most abundant. In the current study, we found that N^2-OH–PhIP–*N3*-glucuronide and PhIP–4′-sulfate, which were minor metabolites in the female population, contributed substantially to the total metabolite excretion. Other studies have investigated the effect of gender difference on PhIP dose–response relationships and excretion of 2-OH–PhIP and found no significant association [60,66]. However, neither of these studies identified and compared the excretion of the specific PhIP metabolites that are noticeably different in our studies.

In 1997, Reistad et al. [57] found that 4′-OH–PhIP could be detected in cooked meat as well as in human urine. Although the same result has not been shown for cooked chicken, it is possible that the 4′-PhIP-sulfate detected in the urine may be formed from 4′-OH–PhIP found in the cooked meat, rather than a metabolite of the ingested parent compound.

Our results demonstrate only a weak association between metabolites excreted and PhIP dose ingested. Other studies of more individuals have reported much stronger correlations [60,66]. Analyzing the average metabolite excretion of the "fast" versus "slow" individuals demonstrated that volunteers who excreted metabolites more quickly excreted significantly more N^2-OH–PhIP–N^2-glucuronide, PhIP-4′-sulfate and total metabolites than the individuals who excreted more slowly. It is possible that individuals that excrete more slowly excrete less metabolite because (1) less compound is being absorbed or it is being absorbed more slowly, (2) more of the compound is being sequestered in the tissues, or (3) the compound is being processed by other, unidentified pathways.

However, due to the small size of our study population, it is impossible to attribute meaning or significance to any of these intriguing results. More work will need to be done in much larger populations to verify trends in gender differences or differences in excretion rate.

We repeatedly analyzed PhIP metabolism in the same three individuals over time to determine the consistency of metabolite excretion. We found that both the percent of the dose excreted in the urine as well as the amounts of each of the metabolites was highly variable in each individual. Although the rate of metabolite excretion appeared to be more constant over time (one of the individuals was

consistently slow, the other two were more often fast), the percentages of metabolites that were measured in each time interval also varied widely. Given the numerous reports of diet and lifestyle affecting metabolizing enzyme activity it is not surprising that there are metabolic variations in individuals eating a normal diet over time. Although these differences may make correlating PhIP metabolite excretion with genotype more difficult, it does suggest that it is possible to devise dietary intervention strategies to reduce the impact of PhIP exposure. Of the metabolites we detected, two appear to be part of the activation pathway for PhIP, N^2-OH–PhIP–N^2-glucuronide and N^2-OH–PhIP–*N3*-glucuronide [67]. It is likely that interventions that reduce the *N*-hydroxylation of PhIP or increase the direct glucuronidation of PhIP are desirable. We are currently investigating the effect of potentially chemopreventive foods on PhIP metabolism in small populations.

Altering the metabolism of PhIP to prevent formation of biologically active species may reduce individual susceptibility and prevent the occurrence of cancers in target tissues. Our results suggest that quantifying PhIP metabolites should make studies of individual susceptibility and dietary interventions possible in the future.

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