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Comparability and repeatability of methods for estimating the dietary intake of the heterocyclic amine contaminant 2-amino-1-methyl-6-phenylimidazo[4,5*b*]pyridine (PhIP)

Nicole C. Deziel[a,c,*], **Timothy J. Buckley**[a,b], **Rashmi Sinha**[c], **Salahaddhin Abubaker**[a], **Elizabeth A. Platz**[d], and **Paul T. Strickland**[a]

[a]Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe St., Baltimore, MD

[b]Department of Environmental Health Sciences, Ohio State College of Public Health, 1841 Neil Ave., Columbus, OH

[c]Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd., Bethesda, MD

[d]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe St., Baltimore, MD

Abstract

Inconsistent risk estimates for dietary heterocyclic amine (HCA) exposure and cancers may be due to differences in exposure assessment methods and the associated measurement error. We evaluated repeatability and comparability of intake estimates of the HCA 2-amino-1-methyl-6-phenylimidazo[4,5*b*]pyridine (PhIP) among two food frequency questionnaire (FFQ) collections, three diary collections, and three measurements of urinary PhIP and its metabolites in thirty-six non-smokers in Baltimore, MD during 2004–2005. Collections spanned ~9 months. Method repeatability was characterized with intraclass correlation coefficients (ICCs). Comparability among methods was assessed with Spearman correlation coefficients. Within-subject variability in PhIP intake was comparably high across all methods (ICCs of 0.20, 0.30, and 0.15 for FFQ, diary, and creatinine-adjusted urinary PhIP, respectively). Mean diary-based PhIP intake and mean urinary PhIP concentration were strongly correlated when restricting the analysis to participants with at least one non-zero diary-based estimate of PhIP intake (n=15, r=0.75, *P*=0.001), but not in the full study population (n=36, r=0.18, *P*=0.28). Mean PhIP intake from the FFQ was not associated with that either based on the diary or urinary PhIP separately, but was modestly correlated with a metric that combined the diary- and biomarker-based approaches (r=0.30, *P*=0.08). The high within-subject variability will result in significantly attenuated associations if a single measure is used to estimate exposure within an epidemiologic study. Improved HCA assessment tools, such as a combination of methods or validated biomarkers that capture long term exposure, are needed.

[*]Corresponding Author: Nicole Cardello Deziel, Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd. EPS 8111, Bethesda, MD 20892, USA. dezielnc@mail.nih.gov; Fax: 301-402-1819.

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Keywords

heterocyclic amines; biomonitoring; meat; food frequency questionnaire; PhIP

Introduction

Epidemiologic evidence linking dietary intake of heterocyclic amines (HCAs) with cancers of the colon (Augustsson et al., 1999; Butler et al., 2003; Gunter et al., 2005; Nowell et al., 2002; Shin et al., 2007; Sinha et al., 2001; Wu et al., 2010), breast (Delfino et al., 2000; Sinha et al., 2000), pancreas (Anderson et al., 2005; Li et al., 2007), and prostate (Cross et al., 2005) is inconsistent, possibly due to differences in exposure assessment methods and the associated measurement error (Alaejos et al., 2008; Butler et al., 2003; Cantwell et al., 2004; Cross et al., 2011; Norat and Riboli, 2001; Turesky and Le, 2011). The standard method for assessing dietary HCA intake in epidemiologic studies of cancer combines meat intake information from food frequency questionnaires (FFQs) with a database of mean concentrations of HCAs measured in cooked meats (Kazerouni et al., 2001; Keating et al., 2007; Sinha et al., 2005). Strengths of this method include low participant burden and the ability to provide an estimate of usual intake of HCAs, typically over the previous year. Possible limitations include imperfect recall of usual intake, recall bias (in retrospective studies), and differing accuracy of reporting of meat intake information by gender, social desirability (the tendency to respond to questions in a manner to avoid embarrassment or present oneself favorably), or body mass index (Scagliusi et al., 2009). In addition, the FFQ may poorly represent individual-level exposure because HCA concentrations in meats vary with many aspects of cooking that may not be included in the FFQ or HCA database, such as pre-cooking in the microwave, frequency of flipping meat, concentrations of precursors, or use of marinades (Sinha and Rothman, 1997; Skog, 2002; Salmon, 2000). Despite the importance of exposure classification in epidemiologic evaluation of PhIP and reliance of the FFQ for this purpose, little is known of its reliability and comparability with other methods.

Diet diaries offer another strategy for classifying exposure. An advantage of diet diaries is that they don't rely on recall, but accuracy of recording may differ by the same individual characteristics as FFQs (Scagliusi et al., 2009). Furthermore, the number of collection days required to obtain accurate estimates of usual intake increases with increasing intra-individual variability in intake of the target food items. Increasing the number of days of diary collection decreases compliance (Willett, 1998). Like FFQs, diaries must be paired with measured values of mean concentrations of HCAs to obtain individual-level estimates of intake. As with FFQs, there has been little previous work examining the comparability of diary-based estimated HCA intake with other methods.

Biomarkers reflect the body burden from exposure without the measurement error introduced from self-reported dietary intake (Cantwell et al., 2004). HCA biomarkers likely have high specificity for the dietary route, because diet is the major source of HCA exposure (Sugimura et al., 2004). Urinary concentration of one of the most mass-abundant HCAs, 2-amino-1-methyl-6-phenylimidazo[4,5*b*]pyridine (PhIP) and its metabolites, is sensitive to increases in dietary PhIP as demonstrated in controlled feeding studies (Friesen et al., 2001; Kulp et al., 2000; Reistad et al., 1997; Stillwell et al., 1997; Strickland et al., 2002). Urinary PhIP and its metabolites have been detected at background levels in populations on unrestricted diets (Kidd et al., 1999; Ushiyama et al., 1991). One of the limitations of urinary PhIP for estimating long-term exposure indicative of cancer risk is its short half-life (less than 12 hr) (Boobis et al., 1994; Turteltaub et al., 1999). Although multiple days of

urine samples can be used to estimate usual exposure, like diaries, this can present issues of participant burden and compliance. While longer-term HCA biomarkers (e.g. DNA, hemoglobin, albumin adducts, hair levels) are more likely to capture the more relevant exposure period in epidemiologic studies of cancer, these methods are not yet adequately sensitive, reliable, or feasible for such studies (Magagnotti et al., 2000; Turesky and Le, 2011).

Recognizing the importance of HCA exposure classification for evaluating linkages with cancer and the limited availability of evaluative data, we conducted a multi-day, multi-method study comparing repeated measures from an FFQ, diary, and urinary biomarker, using PhIP as a representative compound.

Materials and Methods

Study design

Details on the study design have been presented previously (Deziel et al., 2011). In brief, 36 participants (24 women, 12 men) were included in this study. They were recruited between October 2004 and October 2005 from controls in a case-control study of colorectal adenoma at the Johns Hopkins University and included residents of urban, suburban, and rural areas in Maryland, Virginia, and Pennsylvania. Smokers, people who lived with smokers, and people with potential for substantial exposure to smoke during occupational or recreational activities were excluded to minimize potential for non-dietary exposure to HCAs. Smoking status was based on self-report and verified with urinary cotinine.

Data and sample collection

At entry (baseline) and approximately nine months later (follow-up), participants completed a meat-specific FFQ that included photographs and questions about meat intake, cooking method, and degree of doneness (Sinha et al., 2005). At one, five, and nine months after baseline, participants completed 1-day diet diaries. Participants recorded in the diaries all food items eaten that day as well as cooking method and degree of doneness for meats consumed (Cantwell et al., 2004). At the end of the same day that the diary was completed, participants collected urine samples that included the last urine void of the evening through and including the first morning void. Due to availability of overnight courier services and laboratory personnel, diary completion and urine collection were restricted to days other than Friday or Saturday. Participants stored the urine specimens in insulated shippers with frozen ice packs and returned them via overnight courier with the completed diaries on the day of the first morning void. When received, the urine samples were aliquotted, and stored at −80°C. After all collections were complete, the samples were transferred to a −20°C freezer until analysis (total maximum storage time 2.5 yr).

Assessment of meat and PhIP intake from FFQs and diaries

Daily intake of meat (g/day) was assessed from the meat-specific FFQs and diet diaries. The FFQ responses were entered into a database with validation checks. The diet diaries were coded and analyzed using Nutrition Data System for Research© v. 2006 (Schakel et al., 1988). The daily number of servings of each type of meat (hamburger, steak, bacon, sausage, pork chop, chicken) from each FFQ was multiplied by the number of grams of that type of meat per serving and summed to obtain the total number of grams of meat consumed daily. For the diet diaries, the number of servings of meat consumed, including main dishes and side dishes, was multiplied by the grams per serving and summed.

Daily intake of PhIP (ng/day) was estimated using the meat-specific FFQs and diet diaries coupled with the NCI Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease (CHARRED) v.1.7 (Sinha, 2002). Daily intake of each type of meat cooked via a particular method and to a particular degree of doneness from the FFQs or diet diaries was multiplied by the corresponding measured concentration of PhIP in the CHARRED database. To obtain total PhIP intake (ng/day), the daily intakes of PhIP for each combination of meat type, cooking method, and degree of doneness were summed. If a type of meat reported on a diary was not available in the CHARRED database, it was coded as the most similar meat according to the CHARRED conversion manual (National Cancer Institute, 2006). Because data on concentrations of fish were not available in CHARRED and therefore we could have underestimated PhIP intake in fish eaters, analyses were conducted with and without participants who consumed fish that was broiled, grilled, or fried, cooking methods known to form PhIP (Sugimura et al., 2004).

Assessment of urinary PhIP

Urine samples were analyzed for total PhIP (parent compound and deconjugated metabolites) by high performance liquid chromatography with laser-induced fluorescence detection (HPLC-LIF). In developing the method, urine samples were spiked with PhIP standard (Toronto Research Chemicals, North York, Ontario) to confirm retention time. The identity of the PhIP peak was confirmed by pooling peaks from multiple HPLC runs and using synchronous fluorescence spectroscopy to compare the fluorescence spectrum of the pooled sample to the PhIP standard (Strickland et al., 2001). The HPLC-fluorescence method was previously found to be in good agreement with gas chromatography-mass spectrometry (Strickland et al., 2001).

Total PhIP was measured to improve method sensitivity and to obtain our best estimate of intake. Prior to analysis, samples were acid-hydrolyzed to deconjugate PhIP from glucuronic acid or sulfate and processed with solid phase extraction and immunoaffinity chromatography as previously described (Strickland et al., 2001). Immunoaffinity columns were filled with 0.4 mL CNBr-activated Sepharose 4B (Sigma, St. Louis, MO) coupled with monoclonal antibody 4F5 that recognizes PhIP (Kidd et al., 1999). Five μL of the immunopurified sample were injected into an HPLC system (flow rate of 5μL/min) consisting of a Rheodyne injector with a 5 μL loop, Agilent 1100 series pumps, a Picometrics Zetalif laser-induced detector (P/N1701-201) with a Series 56 helium-cadmium Omnichrome laser (325 nm). Separation was achieved with a Zorbax SB C18 (5μm) 150 mm × 0.5 mm column. The sample was eluted using a linear gradient of acetylnitrile (ACN) and water (25% to 30% ACN over 20 minutes) followed by column cleaning steps. Area under the peak was determined by manually integrating the peaks (retention time 13.6 to 13.9 min) using ChemStation Software Rev. A.08.04 (Agilent Technologies, Germany).

To determine the method limit of detection (LOD), urine specimens containing little or no measurable PhIP were spiked with a PhIP standard solution (Toronto Research Chemicals, Inc.) to achieve final concentrations of 2 pg/mL (n=6), 4 pg/mL (n=10), and 8 pg/mL (n=10). These spiked concentrations were selected to be in the range of one to five times the expected LOD.

The LOD was calculated by multiplying the standard deviation of the spiked samples by the Student's t-value corresponding to 99% confidence and n-1 degrees of freedom. The limit of detection (LOD) was determined to be 3 pg/mL based on the standard deviation at 2 pg/mL concentration. The coefficient of variation of the entire method (extraction and analysis) was 17% at 4 pg/mL and 20% at 8 pg/mL.

Assessment of urinary creatinine

All urine specimens were analyzed for creatinine concentration by Hagerstown Medical Laboratories (Hagerstown, MD). 500 μL aliquots were evaluated using an automated colorimetric process based on a modified Jaffé reaction using a Dade Behring analyzer (Dade Behring, Newark, DE) (Larsen, 1972). The LOD was 5 mg/dL.

Statistical analysis

Both parametric and non-parametric techniques were used in this study. PhIP intake variables were natural log-transformed for all parametric analyses. To avoid losing data during natural logarithm transformation due to a relatively high prevalence of intake values of zero or below the LOD, values of zero for PhIP intake based on the diary or FFQ were assigned a value of 0.1 ng based on half the reported LOD for measuring PhIP (0.2 ng/g) in 1 gram of food (Kazerouni et al., 2001). Urinary PhIP concentrations below the detection limit were substituted with LOD/2. For non-parametric analyses, values of zero PhIP intake based on the diary and FFQ were retained as zeros, and LOD/2 was substituted for urinary PhIP values below the LOD.

The repeatability of the HCA intake estimates from the three methods (FFQ, diary, and urinary PhIP) was determined by calculating the intraclass correlation coefficient (ICC) with within-subject (σ^2_{WS}) and between-subject (σ^2_{BS}) variance components obtained from mixed-effects models with natural logarithm-transformed PhIP intake as the outcome variable and no fixed effects included. A uniform correlation structure was used to account for the repeated collections on the same subject. To examine the impact of imperfect reliability of the measurement of PhIP intake on the assessment of risk in a hypothetical case-control study, a hypothetical observed odds ratio (OR_{obs}) was calculated assuming a single sample was used to estimate PhIP intake and assuming a true odds ratio (OR_{tr}) of 2.0 (equation 1) (Alaejos et al., 2008; Rappaport and Kupper, 2008).

$$OR_{obs}=\exp[\ln OR_{tr}/(1+\sigma^2_{WS}/\sigma^2_{BS})] \quad (1)$$

The relationships among PhIP intake estimates from the three methods (FFQ, diary, and urinary PhIP) were evaluated using non-parametric techniques. Spearman correlation coefficients were computed among meat or PhIP intake estimated using the mean intake from the FFQs, diet diaries and creatinine-adjusted and unadjusted urinary PhIP. Due to the high prevalence of zero values for PhIP intake from the diary, Spearman correlation coefficients were assessed for all participants as well as for a subset of those who had a diary-based PhIP intake estimate greater than zero for at least one time point. We used Spearman correlation coefficients to evaluate the relationship between the longer-term FFQ-based mean PhIP intake and a metric that combined the two shorter-term methods, urinary PhIP and diary-based PhIP intake. Because the two shoter-term measures have different units (pg/mL and ng/day, respectively), we standardized the variables [(variable-mean)/ standard deviation] and added them together before assessing the correlation with the FFQ. We also examined whether there were observable differences in PhIP intake based on urinary PhIP concentrations or diaries across tertiles of FFQ subgroups using a non-parametric trend test for comparison. All analyses were conducted with Stata 11 (Statacorp; College Station, TX ©2007).

Results

PhIP intakes estimated from each of the three methods are summarized in Table 1. The median (of the individual mean values) PhIP intake for the FFQ and diary methods were 32 (range: 0 to 368 ng/day) and 0 ng/day (range: 0 to 630 ng/day), respectively. The diary was relatively insensitive to detecting PhIP intake (20%) relative to the FFQ (96%). The median creatinine unadjusted and adjusted urinary PhIP concentration (of individual mean values) from the three collection periods were 3.42 pg/mL (range: <LOD to 35.6 pg/mL) and 7.23 ng/g (range: <LOD to 37.4 ng/g), respectively. PhIP was detectable in 56% of the urine samples.

The within-subject variability in intake estimates for all three methods far exceeded the between-subject variability, yielding ICCs less than 0.5 (Table 2). The FFQ was administered twice 9 months apart and, of the three methods, was the most reliable for estimating PhIP intake, though modest (ICC=0.30, 95% confidence interval [−0.02, 0.57]); Spearman correlation between two FFQs=0.50, P=0.02). The urinary biomarker, which was measured three times over 9 months, was the least reliable method of PhIP intake (ICC=0.01, 95% confidence interval [−0.16, 0.23]). However, the reliability improved substantially with creatinine-correction (ICC=0.15, 95% confidence interval [-0.04, 0.38]). These low ICCs indicate that use of a single collection of any of these metrics in a case-control study would substantially attenuate the observed OR (Table 2). For example, using a single FFQ to estimate PhIP intake in a study population in which the true OR is 2.0, the observed OR would be 1.2.

Examining the associations across all three methods, the mean diary PhIP intake and the mean urinary PhIP concentration achieved significance only when restricting the analysis to those with at least one non-zero intake estimate from the diary (n=15, r=0.75, P=0.001; Figure 1B). No association was observed when all participants were included (n=36, r=0.18, P=0.28; Figure 1Ab). Fifteen of the 21 participants (71%) with diary-based mean PhIP intake of zero had measurable urinary PhIP at one or more time points (Figure 1a). Mean PhIP intake estimated from the FFQ was not correlated with mean urinary PhIP levels (r=0.20, P=0.23) nor or mean PhIP intake estimated using the diary (r=0.17, P=0.32), but was modestly correlated with a metric that combined urinary and diary-based PhIP (r=0.30, P=0.08) (Table 3). Meat consumption (diary and FFQ reported) was not associated with mean urinary PhIP concentrations. We also looked for associations across methods by aggregating the individual-level data into tertiles with no positive trend data evident (Table 4). Analysis with and without participants who consumed fish that was broiled, grilled, or fried did not yield appreciable differences in results, so all fish eaters were retained in the analyses. Correlations were of similar magnitude and statistical significance with creatinine-adjusted PhIP, and therefore only correlations with unadjusted PhIP are presented.

Discussion

We used a repeated measures design to evaluate the repeatability and comparability of three different methods for assessing dietary exposure to HCA: FFQ, diary, and the urinary biomarker PhIP. We observed relatively high within-subject variability in the repeated collections for all methods and relatively weak agreement among the different methods. The current study raises important methodological considerations for future studies.

We observed PhIP intake estimates that were lower compared to those of controls in previous case-control studies that used the same methods. For example, estimated PhIP intake from the baseline (median=26 ng/day; mean=60 ng/day) and follow-up (median=33

ng/day; mean=75 ng/day) FFQs were lower than intake reported in controls in North Carolina (mean=86.7 ng/day, n=611) (Butler et al., 2003); Maryland (mean=78.1 ng/day, n=165) (Cantwell et al., 2004); Los Angeles, Seattle, Detroit, and Iowa (median=73.8 ng/day) (Cross et al., 2006); and California (median=92 ng/day) (Delfino et al., 2000). The lower intakes observed in the current study may be due to differences in season of ascertainment (Subar et al., 1994) or the exclusion of Fridays and Saturdays from our collection days, since meat intake tends to be higher on the weekends (Haines et al., 2003).

Similar to the FFQ-based estimates, the mean estimated PhIP intake from the diary in our study (85 ng/day) was lower than the previously reported mean intake based on the diary (264 ng/day) (Cantwell et al., 2004). In addition to our exclusion of Fridays and Saturdays, this difference may also be due in part to the larger number of diaries collected by Cantwell et al. (12 days) relative to the current study (3 days). Because of the large within-subject variability, the true long-term PhIP intake is likely not well-represented by 3 days of diary collections.

The concentrations of urinary PhIP in our study population were within the range of previously reported results. The mean urinary PhIP concentration in our study (5.5 pg/mL) was 80% lower than the mean observed in pre-feed levels in a controlled feeding study (30.4 pg/mL) in which participants were asked to refrain from eating charbroiled, smoked, or fried meat, suggesting our study group had low well-done meat consumption (Strickland et al., 2001; Strickland et al., 2002). However, the geometric mean (4.1 ng/g), range (<LOD to 10.4 ng/g), and percent of samples above the LOD (56%) of creatinine-adjusted urinary PhIP concentrations in our study were somewhat higher than those reported for Caucasian participants on unrestricted diets in a prior study in Los Angeles (geometric mean=1.18 ng/g, range=<LOD to 14.7 ng/g, and %>LOD=21%) (Kidd et al., 1999). The median (0.43 ng) and range (0.080-23 ng) of the mass of PhIP excreted in our approximately 8 to 12-hr samples were similar to the median (0.31 ng) and range (0.12–2.0 ng) reported in 24-hr urine samples in ten healthy volunteers in Japan who were on unrestricted diets (Ushiyama et al., 1991).

We observed high within-subject variability in the PhIP intake estimates across all methods (ICC of 0.20, 0.30, and 0.15 for FFQ, diary, and creatinine-adjusted biomarker, respectively), which will substantially attenuate observed ORs if a single measurement is used in an epidemiologic study. The ICC of 0.30 for PhIP intake based on the FFQ was similar to the ICC of 0.36 reported for two dietary interviews asking about fried meat intake over the prior year, administered 4 to 7 years apart (Knekt et al., 1994). We previously assessed the repeatability of the urinary biomarker 1-hydroxypyrene glucuronide (1-OHPG), an indicator of exposure to the dietary carcinogens polycyclic aromatic hydrocarbons (PAHs), in the same study population (Deziel et al., 2011). The repeatability was higher for 1-OHPG concentration (ICC=0.37) than that of PhIP (ICC=0.01), possibly because PAHs are found in a wider variety of foods. In contrast to the current study, we previously observed that creatinine-adjustment decreased the repeatability of the 1-OHPG concentration (ICC=0.15 for creatinine-adjusted 1-OHPG). These contradictory findings add to the discussion about the utility of and approach for creatinine adjustment (Barr et al., 2005). The large within-subject variability in consumption of PhIP-containing foods highlights the challenges in dietary exposure assessments and provides justification for the use of a repeated measure approach or development and use of longer-term biomarkers, such as protein adducts or hair PhIP (Bessette et al., 2009; Kobayashi et al., 2007).

We did not observe an association between any of the three exposure methods examined separately when the full study population was considered. A previous study observed a

modest correlation between FFQ and diary-based estimates of PhIP intake (Pearson r=0.22, 95% confidence interval: 0.07–0.36) (Cantwell et al., 2004). Two studies were identified that compared an FFQ against biomarker measurements. In a study of 129 men, Kidd et al. observed that the odds of having detectable urinary PhIP was not different in individuals with higher frequencies of intake of various meats compared to those with lower frequency of intake (Kidd et al., 1999). This study only used a single urine sample to compare with the biomarker, and the FFQ did not ask participants to report on meat cooking method or degree of doneness. Kobayashi et al. observed a Spearman correlation coefficient of 0.47 (P=0.036) between PhIP intake based on an FFQ and PhIP measured in hair among 20 volunteers (Kobayashi et al., 2007).

We did observe a modest association between mean PhIP intake based on the FFQ and a metric that combined PhIP intake based on the diary and urinary PhIP measurements. If the biomarker-based and survey-based methods to assessing PhIP intake are indeed capturing different time periods or sources of exposure, using HCA biomarkers in combination with survey-based methods could potentially reduce some of the uncertainty in HCA intake estimates in epidemiologic studies (Freedman et al., 2010). For example, Peters et al. used urine mutagenicity in combination with an FFQ and observed a stronger relationship with cancer risk than when using either method alone (Peters et al., 2003).

We did not observe correlations between meat intake and urinary PhIP, though we had observed an association between meat intake and urinary 1-OHPG concentration in our previous analysis of PAHs (Deziel et al., 2011). This may be because PhIP formation is more specific to meat preparation methods that involve direct and/or efficient transfer of heat, such as broiling, grilling, or frying at high temperatures and/or long cooking times (Skog 2002). We expected a positive correlation between diary-based PhIP and urinary PhIP measurements in the full study population because these two methods reflected a similar time period. Furthermore, we observed such a correlation for the PAH benzo[a]pyrene in our previous analysis in this population (Deziel et al., 2011). We observed several participants with diary-based PhIP intake of 0 ng/g, but detectable urinary PhIP concentrations at concurrent collections. There are many plausible explanations for the lack of correlation. Firstly, the diary and database may not capture all foods contributing to exposure. While we focused only on meats, there have been some reports in the literature that certain non-meat foods, such as smoked cheeses (Naccari et al., 2009), beer (Manabe et al., 1993b), and wine (Manabe et al., 1993b), contain HCA, though their contribution to total exposure is likely minimal.

Secondly, in addition to only including meat-based foods, the FFQ, diary, and database did not include information on factors related to cooking preparation that can influence the concentrations of PhIP in food. For example, increasing the frequency of flipping meat during cooking can reduce the formation of PhIP (Salmon et al., 2000). Use of marinades may increase or decrease the formation of PhIP, depending on the components of the marinade, contributing to increased variability in HCA concentrations in foods (Busquets et al., 2006).

Thirdly, it is possible that the biomarker is capturing non-dietary exposures. PhIP has been isolated in non-dietary environmental samples, such as tobacco smoke (Manabe et al., 1991), cooking smoke (Thiebaud et al., 1995), and diesel exhaust (Manabe et al., 1993a). The relative contribution of these other sources to total exposure is likely minimal. Furthermore, our selection criteria aimed to exclude people with potentially high exposure to these other sources.

Finally, inter-individual differences in toxicokinetics may account for some of the variability in the relationship between PhIP intake estimated from the diaries relative to PhIP urine levels. For example, in two controlled feeding studies, participants consuming the same preparation of cooked meat had urinary PhIP concentrations that varied 2.7-fold (Strickland et al., 2002) and 4.2-fold (Reistad et al., 1997). Additionally, since urinary PhIP is actually reflecting the balance of activation and detoxification pathways, the levels of excretion of PhIP conjugates may be affected by other dietary constituents, such as the amount of fruits and vegetables consumed (Murray et al., 2001; Walters et al., 2004).

Strengths of this study included the multiple methods included and the repeated measures design. However, we were limited by a relatively small sample size, the infrequent consumption of PhIP containing foods, and the low detection rates of urinary PhIP. Our study could have been strengthened by incorporating a more robust analytical technique, such as liquid chromatography tandem mass spectrometry, potentially yielding increased detection rates in our urine samples and an improved ability to compare methods. Also, additional diary and urine collections may be needed to make a fair comparison, given the episodic nature of PhIP consumption. We recognize the collection and analysis of duplicate diet samples as another strategy for directly measuring PhIP dietary exposure, however, this approach was not feasible in the context of the current investigation due to cost and participant burden. Although duplicate diet has some of its own limitations (in addition to cost and burden, there is potential for bias in foods provided for the duplicate diet), it is considered the “gold standard” for dietary exposure and therefore would be a useful method to include in future studies.

Our objective was to compare commonly used approaches for estimating PhIP intake in epidemiologic studies. Among the methods tested, there is no clear gold standard, making the poor associations observed across the three methods difficult to interpret. In general, this lack of association may be an indication of the uncertainty with which these measures are made and the need for additional research to improve assessment methods. Alternatively, the lack of association may be a reflection of the different time windows of exposure being captured by each method, rather than a flaw in any single method. Even the timing of the concurrently collected urinary PhIP concentrations and the diary-based PhIP intake may not be optimal. The very short half-life of PhIP (less than 12 hr) suggests that foods consumed early in the day of collection may not be captured by the PhIP measured in the last evening void.

This study provides insight into the interpretation of previous epidemiologic findings, suggesting that reported risk estimates, using a single FFQ to estimate exposure, could be substantially attenuated due to relatively large errors in exposure classification. The current study highlights concerns about the repeatability of current methods for assessing the episodically consumed HCA PhIP. Incorporation of repeated measures or a combination of methods in future studies could address this issue. In addition, development of a well-validated, widely applicable, longer-term biomarker for assessing dietary exposure would be useful.

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References

Alaejos MS, Gonzalez V, Afonso AM. Exposure to heterocyclic aromatic amines from the consumption of cooked red meat and its effect on human cancer risk: a review. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2008; 25:2–24. [PubMed: 17952757]

Anderson KE, Kadlubar FF, Kulldorff M, Harnack L, Gross M, Lang NP, Barber C, Rothman N, Sinha R. Dietary intake of heterocyclic amines and benzo(a)pyrene: associations with pancreatic cancer. Cancer Epidemiol Biomarkers Prev. 2005; 14:2261–2265. [PubMed: 16172241]

Augustsson K, Skog K, Jagerstad M, Dickman PW, Steineck G. Dietary heterocyclic amines and cancer of the colon, rectum, bladder, and kidney: a population-based study. Lancet. 1999; 353:703–707. [PubMed: 10073512]

Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect. 2005; 113:192–200. [PubMed: 15687057]

Bessette EE, Yasa I, Dunbar D, Wilkens LR, Le ML, Turesky RJ. Biomonitoring of carcinogenic heterocyclic aromatic amines in hair: a validation study. Chem Res Toxicol. 2009; 22:1454–1463. [PubMed: 19588936]

Boobis AR, Lynch AM, Murray S, de la Torre R, Solans A, Farre M, Segura J, Gooderham NJ, Davies DS. CYP1A2-catalyzed conversion of dietary heterocyclic amines to their proximate carcinogens is their major route of metabolism in humans. Cancer Res. 1994; 54:89–94. [PubMed: 8261468]

Busquets R, Puignou L, Galceran MT, Skog K. Effect of red wine marinades on the formation of heterocyclic amines in fried chicken breast. J Agric Food Chem. 2006; 54:8376–8384. [PubMed: 17032054]

Butler LM, Sinha R, Millikan RC, Martin CF, Newman B, Gammon MD, Ammerman AS, Sandler RS. Heterocyclic amines, meat intake, and association with colon cancer in a population-based study. Am J Epidemiol. 2003; 157:434–445. [PubMed: 12615608]

Cantwell M, Mittl B, Curtin J, Carroll R, Potischman N, Caporaso N, Sinha R. Relative validity of a food frequency questionnaire with a meat-cooking and heterocyclic amine module. Cancer Epidemiol Biomarkers Prev. 2004; 13:293–298. [PubMed: 14973110]

Cross AJ, Major JM, Sinha R. Urinary biomarkers of meat consumption. Cancer Epidemiol Biomarkers Prev. 2011; 20:1107–1111. [PubMed: 21527577]

Cross AJ, Peters U, Kirsh VA, Andriole GL, Reding D, Hayes RB, Sinha R. A prospective study of meat and meat mutagens and prostate cancer risk. Cancer Res. 2005; 65:11779–11784. [PubMed: 16357191]

Cross AJ, Ward MH, Schenk M, Kulldorff M, Cozen W, Davis S, Colt JS, Hartge P, Cerhan JR, Sinha R. Meat and meat-mutagen intake and risk of non-Hodgkin lymphoma: results from a NCI-SEER case-control study. Carcinogenesis. 2006; 27:293–297. [PubMed: 16113054]

DeBruin LS, Martos PA, Josephy PD. Detection of PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) in the milk of healthy women. Chem Res Toxicol. 2001; 14:1523–1528. [PubMed: 11712910]

Delfino RJ, Sinha R, Smith C, West J, White E, Lin HJ, Liao SY, Gim JS, Ma HL, Butler J, Anton-Culver H. Breast cancer, heterocyclic aromatic amines from meat and N-acetyltransferase 2 genotype. Carcinogenesis. 2000; 21:607–615. [PubMed: 10753193]

Deziel NC, Strickland PT, Platz EA, Abubaker S, Buckley TJ. Comparison of standard methods for assessing dietary intake of benzo[a]pyrene. Cancer Epidemiol Biomarkers Prev. 2011; 20:962–970. [PubMed: 21430297]

Freedman LS, Kipnis V, Schatzkin A, Tasevska N, Potischman N. Can we use biomarkers in combination with self-reports to strengthen the analysis of nutritional epidemiologic studies? Epidemiol Perspect Innov. 2010; 7:2–9. [PubMed: 20180978]

Friesen MD, Rothman N, Strickland PT. Concentration of 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) in urine and alkali-hydrolyzed urine after consumption of charbroiled beef. Cancer Lett. 2001; 173:43–51. [PubMed: 11578808]

Gunter MJ, Probst-Hensch NM, Cortessis VK, Kulldorff M, Haile RW, Sinha R. Meat intake, cooking-related mutagens and risk of colorectal adenoma in a sigmoidoscopy-based case-control study. Carcinogenesis. 2005; 26:637–642. [PubMed: 15579480]

Haines PS, Hama MY, Guilkey DK, Popkin BM. Weekend eating in the United States is linked with greater energy, fat, and alcohol intake. Obes Res. 2003; 11:945–949. [PubMed: 12917498]

Kazerouni N, Sinha R, Hsu CH, Greenberg A, Rothman N. Analysis of 200 food items for benzo[a]pyrene and estimation of its intake in an epidemiologic study. Food Chem Toxicol. 2001; 39:423–436. [PubMed: 11313108]

Keating GA, Bogen KT, Chan JM. Development of a meat frequency questionnaire for use in diet and cancer studies. J Am Diet Assoc. 2007; 107:1356–1362. [PubMed: 17659903]

Kidd LC, Stillwell WG, Yu MC, Wishnok JS, Skipper PL, Ross RK, Henderson BE, Tannenbaum SR. Urinary excretion of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in White, African-American, and Asian-American men in Los Angeles County. Cancer Epidemiol Biomarkers Prev. 1999; 8:439–445. [PubMed: 10350440]

Knekt P, Steineck G, Jarvinen R, Hakulinen T, Aromaa A. Intake of fried meat and risk of cancer: a follow-up study in Finland. Int J Cancer. 1994; 59:756–760. [PubMed: 7989114]

Kobayashi M, Hanaoka T, Tsugane S. Validity of a self-administered food frequency questionnaire in the assessment of heterocyclic amine intake using 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) levels in hair. Mutat Res. 2007; 630:14–19. [PubMed: 17392018]

Kulp KS, Knize MG, Malfatti MA, Salmon CP, Felton JS. Identification of urine metabolites of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine following consumption of a single cooked chicken meal in humans. Carcinogenesis. 2000; 21:2065–2072. [PubMed: 11062169]

Larsen K. Creatinine assay in the presence of protein with LKB 8600 Reaction Rate Analyser. Clin Chim Acta. 1972; 38:475–476. [PubMed: 5026368]

Li D, Day RS, Bondy ML, Sinha R, Nguyen NT, Evans DB, Abbruzzese JL, Hassan MM. Dietary mutagen exposure and risk of pancreatic cancer. Cancer Epidemiol Biomarkers Prev. 2007; 16:655–661. [PubMed: 17416754]

Magagnotti C, Orsi F, Bagnati R, Celli N, Rotilio D, Fanelli R, Airoldi L. Effect of diet on serum albumin and hemoglobin adducts of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in humans. Int J Cancer. 2000; 88:1–6. [PubMed: 10962432]

Manabe S, Kurihara N, Wada O, Izumikawa S, Asakuno K, Morita M. Detection of a carcinogen, 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine, in airborne particles and diesel-exhaust particles. Environ Pollut. 1993a; 80:281–286. [PubMed: 15091848]

Manabe S, Suzuki H, Wada O, Ueki A. Detection of the carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in beer and wine. Carcinogenesis. 1993b; 14:899–901. [PubMed: 8504483]

Manabe S, Tohyama K, Wada O, Aramaki T. Detection of a carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), in cigarette smoke condensate. Carcinogenesis. 1991; 12:1945–1947. [PubMed: 1934275]

Murray S, Lake BG, Gray S, Edwards AJ, Springall C, Bowey EA, Williamson G, Boobis AR, Gooderham NJ. Effect of cruciferous vegetable consumption on heterocyclic aromatic amine metabolism in man. Carcinogenesis. 2001; 22:1413–1420. [PubMed: 11532863]

Naccari C, Galceran MT, Moyano E, Cristani M, Siracusa L, Trombetta D. Presence of heterocyclic aromatic amines (HAS) in smoked "Provola" cheese from Calabria (Italy). Food Chem Toxicol. 2009; 47:321–327. [PubMed: 19056456]

National Cancer Institute. Cooking Method Conversions. 2006. CHARRED: Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease.

Norat T, Riboli E. Meat consumption and colorectal cancer: a review of epidemiologic evidence. Nutr Rev. 2001; 59:37–47. [PubMed: 11310774]

Nowell S, Coles B, Sinha R, MacLeod S, Luke RD, Stotts C, Kadlubar FF, Ambrosone CB, Lang NP. Analysis of total meat intake and exposure to individual heterocyclic amines in a case-control study of colorectal cancer: contribution of metabolic variation to risk. Mutat Res. 2002; 506–507:175–185.

Peters U, DeMarini DM, Sinha R, Brooks LR, Warren SH, Chatterjee N, Rothman N. Urinary mutagenicity and colorectal adenoma risk. Cancer Epidemiol Biomarkers Prev. 2003; 12:1253–1256. [PubMed: 14652290]

Rappaport, S.; Kupper, L. Exposure Measurement Errors; Quantitative Exposure Assessment; 2008.

Reistad R, Rossland OJ, Latva-Kala KJ, Rasmussen T, Vikse R, Becher G, Alexander J. Heterocyclic aromatic amines in human urine following a fried meat meal. Food Chem Toxicol. 1997; 35:945–955. [PubMed: 9463528]

Salmon CP, Knize MG, Panteleakos FN, Wu RW, Nelson DO, Felton JS. Minimization of heterocyclic amines and thermal inactivation of Escherichia coli in fried ground beef. J Natl Cancer Inst. 2000; 92:1773–1778. [PubMed: 11058620]

Scagliusi FB, Ferriolli E, Pfrimer K, Laureano C, Cunha CS, Gualano B, Lourenco BH, Lancha AH Jr. Characteristics of women who frequently under report their energy intake: a doubly labelled water study. Eur J Clin Nutr. 2009; 63:1192–1199. [PubMed: 19603055]

Schakel SF, Sievert YA, Buzzard IM. Sources of data for developing and maintaining a nutrient database. J Am Diet Assoc. 1988; 88:1268–1271. [PubMed: 3171020]

Shin A, Shrubsole MJ, Ness RM, Wu H, Sinha R, Smalley WE, Shyr Y, Zheng W. Meat and meat-mutagen intake, doneness preference and the risk of colorectal polyps: the Tennessee Colorectal Polyp Study. Int J Cancer. 2007; 121:136–142. [PubMed: 17354224]

Sinha R. An epidemiologic approach to studying heterocyclic amines. Mutat Res. 2002; 506–507:197–204.

Sinha R, Cross A, Curtin J, Zimmerman T, McNutt S, Risch A, Holden J. Development of a food frequency questionnaire module and databases for compounds in cooked and processed meats. Mol Nutr Food Res. 2005; 49:648–655. [PubMed: 15986387]

Sinha R, Gustafson DR, Kulldorff M, Wen WQ, Cerhan JR, Zheng W. 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, a carcinogen in high-temperature-cooked meat, and breast cancer risk. J Natl Cancer Inst. 2000; 92:1352–1354. [PubMed: 10944558]

Sinha R, Kulldorff M, Chow WH, Denobile J, Rothman N. Dietary intake of heterocyclic amines, meat-derived mutagenic activity, and risk of colorectal adenomas. Cancer Epidemiol Biomarkers Prev. 2001; 10:559–562. [PubMed: 11352869]

Sinha R, Rothman N. Exposure assessment of heterocyclic amines (HCAs) in epidemiologic studies. Mutat Res. 1997; 376:195–202. [PubMed: 9202756]

Skog K. Problems associated with the determination of heterocyclic amines in cooked foods and human exposure. Food Chem Toxicol. 2002; 40:1197–1203. [PubMed: 12067584]

Stillwell WG, Kidd LC, Wishnok JS, Tannenbaum SR, Sinha R. Urinary excretion of unmetabolized and phase II conjugates of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline in humans: relationship to cytochrome P4501A2 and N-acetyltransferase activity. Cancer Res. 1997; 57:3457–3464. [PubMed: 9270013]

Strickland PT, Qian Z, Friesen M, Rothman N, Sinha R. Measurement of 2-amino-1-methyl-6-phenylimidazo (4,5-b)pyridine (PhIP) in acid-hydrolyzed urine by high performance liquid chromatography with fluorescence detection. Biomarkers. 2001; 6:313–325.

Strickland PT, Qian Z, Friesen MD, Rothman N, Sinha R. Metabolites of 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) in human urine after consumption of charbroiled or fried beef. Mutat Res. 2002; 506–507:163–173.

Subar AF, Frey CM, Harlan LC, Kahle L. Differences in reported food frequency by season of questionnaire administration: the 1987 National Health Interview Survey. Epidemiology. 1994; 5:226–233. [PubMed: 8172998]

Sugimura T, Wakabayashi K, Nakagama H, Nagao M. Heterocyclic amines: Mutagens/carcinogens produced during cooking of meat and fish. Cancer Sci. 2004; 95:290–299. [PubMed: 15072585]

Thiebaud HP, Knize MG, Kuzmicky PA, Hsieh DP, Felton JS. Airborne mutagens produced by frying beef, pork and a soy-based food. Food Chem Toxicol. 1995; 33:821–828. [PubMed: 7590526]

Turesky RJ, Le Marchand L. Metabolism and Biomarkers of Heterocyclic Aromatic Amines in Molecular Epidemiology Studies: Lessons Learned from Aromatic Amines. Chem Res Toxicol. 2011; 24:1169–1214. [PubMed: 21688801]

Turteltaub KW, Dingley KH, Curtis KD, Malfatti MA, Turesky RJ, Garner RC, Felton JS, Lang NP. Macromolecular adduct formation and metabolism of heterocyclic amines in humans and rodents at low doses. Cancer Lett. 1999; 143:149–155. [PubMed: 10503895]

Ushiyama H, Wakabayashi K, Hirose M, Itoh H, Sugimura T, Nagao M. Presence of carcinogenic heterocyclic amines in urine of healthy volunteers eating normal diet, but not of inpatients receiving parenteral alimentation. Carcinogenesis. 1991; 12:1417–1422. [PubMed: 1907222]

Walters DG, Young PJ, Agus C, Knize MG, Boobis AR, Gooderham NJ, Lake BG. Cruciferous vegetable consumption alters the metabolism of the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in humans. Carcinogenesis. 2004; 25:1659–1669. [PubMed: 15073045]

Willett, W. Nutritional Epidemiology. Oxford University Press; 1998.

Wu K, Sinha R, Holmes MD, Giovannucci E, Willett W, Cho E. Meat mutagens and breast cancer in postmenopausal women--a cohort analysis. Cancer Epidemiol Biomarkers Prev. 2010; 19:1301–1310. [PubMed: 20447922]

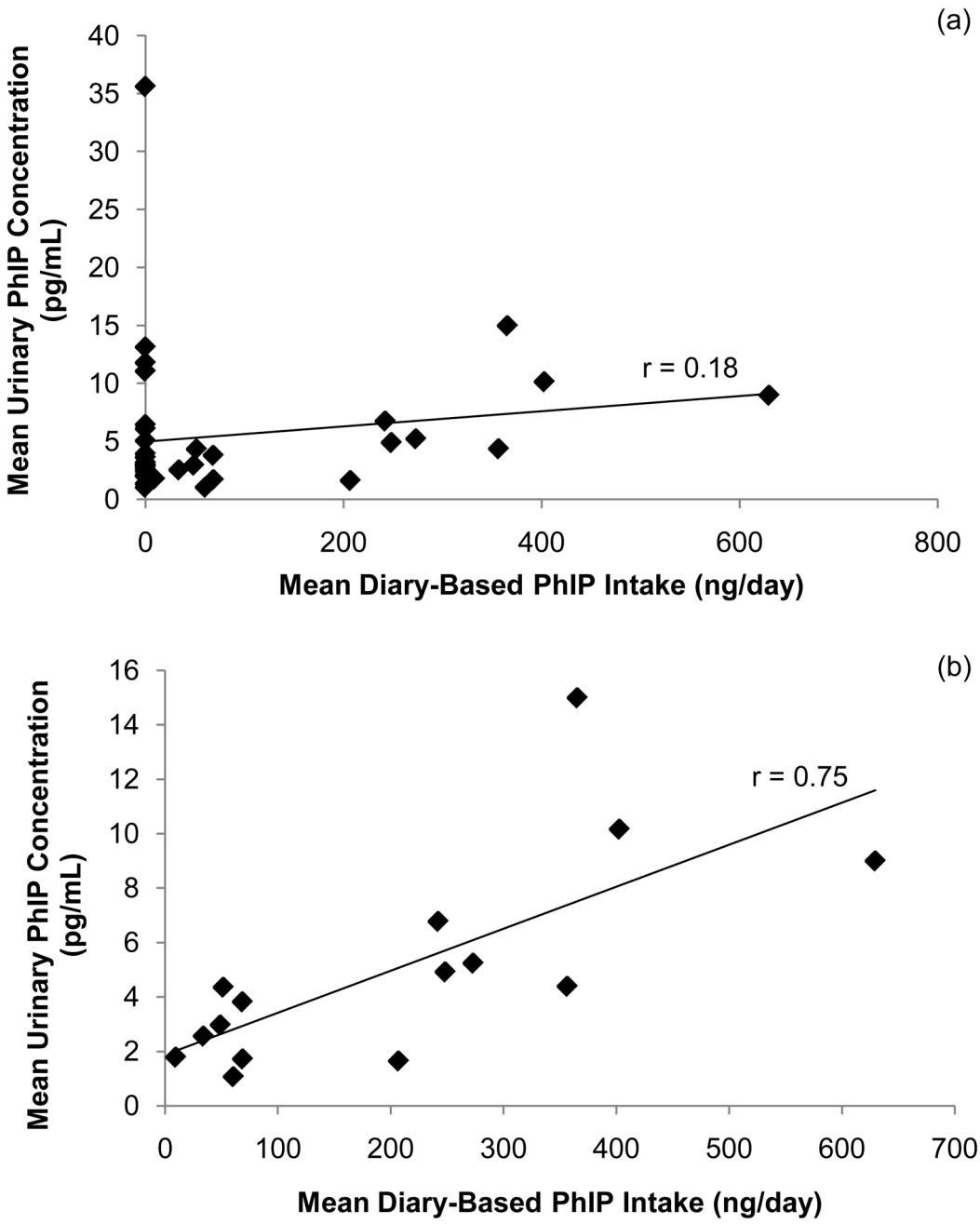

Figure 1.
Correlation between diary-based PhIP intake and urinary PhIP concentration in all participants (n=36, r=0.18, p=0.28) (a) and those with mean PhIP intake greater than zero (n=15, r=0.75, p=0.001) (b).

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Table 1

Estimated mean PhIP intake among 36 participants.

Intake Metric	# of Collections	% >0 or LOD[a]	Minimum	Median	IQR	Maximum
PhIP Intake from Baseline FFQ (ng/day)	1	97	0	26	(12 , 90)	229
PhIP Intake from Follow-up FFQ (ng/day)	1	94	0	33	(13, 91)	508
Mean PhIP Intake from FFQ[a] (ng/day)	2	96	0	32	(15, 101)	368
Mean PhIP Intake from Diaries[a] (ng/day)	3	20	0	0	(0, 69)	630
Mean Urinary PhIP[a,b] (pg/ml)	3	56	1.06	3.42	(2.19, 6.36)	35.6
Mean Creatinine Adjusted Urinary PhIP[a] (ng/g)	3	56	1.12	7.23	(2.46, 17.0)	37.4

[a]Percentage of all individual collections that were either greater than zero or the limit of detection (LOD).

[b]We took the mean of the repeated measures for each intake metric and calculated the distribution of the means.

FFQ, food frequency questionnaire; IQR, inter-quartile range; LOD, limit of detection; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5*b*]pyridine

Table 2

Intraclass correlation coefficients (ICC) and associated potential attenuation of odds ratios in a hypothetical case-control study.

Intake Metric[a]	ICC[d] (95% CI)	OR_{obs} if OR_{tr} = 2.0 [e]
FFQ (ng/d)[b]	0.30 (−0.02, 0.57)	1.23
Diary (ng/d)[b]	0.20 (0.00, 0.42)	1.15
Urinary PhIP Concentration (pg/mL)[c]	0.01 (−0.16, 0.23)	1.01
Creatinine Adjusted Urinary PhIP Concentration (ng/g)[c]	0.15 (−0.04, 0.38)	1.11

LOD, limit of detection; OR_{obs}, observed odds ratio; OR_{tr}, true odds ratio; σ^2_{BS}, between-subject variance; σ^2_{WS}, within-subject variance

[a] All variables natural log-transformed.

[b] Values of zero were assigned 0.1 , corresponding to 1 gram of food at 1/2 the LOD for PhIP detection in food (0.2 ng/g).

[c] Values below the detection limit were assigned 1/2LOD

[d] $ICC=\sigma^2_{BS}/(\sigma^2_{BS} + \sigma^2_{WS})$

[e] $OR_{obs}=\exp[\ln OR_{tr}/(1+ \sigma^2_{WS}/\sigma^2_{BS})]$

Table 3

Spearman correlation coefficients (r) between mean FFQ-based PhIP intake, mean urinary PhIP, mean diary-based PhIP intake, and urine and diary-based methods combined (n=36).

	FFQ PhiP	
	r	***P*-value**
Urinary PhIP	0.20	0.23
Diary-RD	0.17	0.32
Combined Metric[a]	0.30	0.08

[a] Combined metric = $[(\text{Individual Estimate} - \text{Overall Mean})/\text{Overall Standard Deviation}]_{\text{diary}}$ + $[(\text{Individual Estimate} - \text{Overall Mean})/\text{Standard Deviation}]_{\text{urine}}$

Table 4

Median urinary PhIP by tertiles of PhIP intake (low, medium, high) based on FFQs.

	Low (median=13 ng/d)	Medium (median=32 ng/d)	High (median=129 ng/d)	p-value (trend)[a]
Median of Mean Urinary PhIP (pg/ml)	2.75	4.34	4.79	0.11
Median of Mean Creatinine Adjusted Urinary PhIP(ng/g)	4.30	7.50	8.17	0.18
Diary-based PhIP Intake (ng/day)	0	4.72	0	0.63

[a] non-parametric test for trend across ordered groups.