HHS Public Access

Author manuscript

Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2015 October 24.

Published in final edited form as:

Cancer Epidemiol Biomarkers Prev. 2011 May ; 20(5): 962–970. doi:10.1158/1055-9965.EPI-10-1344.

Comparison of Standard Methods for Assessing Dietary Intake of Benzo[*a*]pyrene

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Abstract

Background—Inconsistent presence and strength of associations between dietary benzo[*a*]pyrene (BaP) exposure and cancers may be due to differences in exposure assessment methods. Thus, we determined correlations of usual meat and BaP intake among three methods: food frequency questionnaires (FFQ), diet diaries, and a biomarker.

Methods—Thirty-six nonsmokers were recruited in Baltimore, MD during 2004–2005. Meat and BaP intake estimated from baseline and follow-up FFQs combined with a BaP residue database (FFQ-RD), mean meat and BaP intake estimated from three diet diaries coupled with the residue database (Diary-RD), and mean of three urinary 1-hydroxypyrene glucuronide (1-OHPG) measurements were compared using Spearman correlations. Collections spanned approximately nine months.

Results—BaP intakes from meat from the baseline [median = 6.4, interquartile range (IQR) = 13.9 ng/d] and follow-up FFQ-RD (median = 7.3, IQR = 35.7 ng/d) were higher than the Diary-RD (median = 1.1, IQR = 7.4 ng/d). Mean 1-OHPG concentration was weakly correlated with mean meat intake ($r = 0.33$, $P = 0.05$) and BaP intake from meat ($r = 0.27$, $P = 0.11$) from the Diary-RD. Mean BaP intake estimated from the Diary-RD was positively correlated with the follow-up ($r = 0.35$, $P = 0.04$) but not baseline ($r = 0.20$, $P = 0.24$) FFQ; the converse was true for meat intake.

Conclusions—Diary-RD estimates were supported by biomarker measurements, but considerable unexplained variability remained. Limited correlation among the dietary BaP exposure assessment methods could be due to differences in timeframes covered by the assessments, interpersonal variability in metabolism, deficiencies in the residue database, or nondietary exposures to BaP.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Impact—Limited correlation in estimated BaP intake among standard methods may contribute to inconsistent epidemiology of BaP and cancer.

Introduction

Polycyclic aromatic hydrocarbons (PAH) constitute a class of chemicals including known and probable human carcinogens (1). While the epidemiologic evidence for associations with cancer has primarily been occupational, PAHs are also found in the diet, mainly in well-cooked meats and some cooked or contaminated grains (1). Epidemiologic studies investigating links between dietary exposure to PAHs and colon and colorectal (2–7), pancreatic (8, 9), prostate (10, 11), breast (12, 13), and lung cancers (14, 15) have yielded inconsistencies in the presence and strength of associations. These inconsistencies among studies may be partially due to differences in the exposure assessment methods or variability in the extent of measurement error among the exposure assessment methods.

The general method for estimating dietary PAH exposure in epidemiologic studies is to combine usual meat intake information from food frequency questionnaires (FFQ) with existing residue databases (RD) containing mean concentrations of PAHs measured in cooked meats (FFQ-RD; refs. 16–18). Often, benzo[*a*]pyrene (BaP) is used as a representative of the class of PAHs due to its carcinogenic potency, prevalence, and correlation with other PAHs (16, 19). The FFQ-RD method is believed to offer improved accuracy compared with previously used surrogates of dietary PAH exposure, such as intake of meat or well-done meat. However, limitations remain, including inaccurate reporting of usual intake and inadequacies (i.e., completeness and accuracy) of the RD (17). These limitations can potentially lead to measurement error in the exposure, thereby limiting the power of studies to detect associations (20, 21).

Diet diaries are collected in real time; they capture the food consumed during the time of the record without depending on memory. They are open-ended, and their sources of error are generally independent of those associated with the FFQ. Because a single day rarely represents usual exposure, multiple days of diaries are required to have an accurate estimate of usual intake. The number of days required depends on the intraindividual variability in intake of the food items of interest and the level of precision desired. However, the level of compliance decreases as the number of days of diaries collected increases (22).

Biological monitoring offers a direct measure of PAH exposure not subject to limitations of self-report or the RDs (23, 24). Urinary excretion of 1-hydroxypyrene glucuronide (1-OHPG), a metabolite of the PAH pyrene, increases with intake of grilled/charbroiled meat (cooked over a coal or gas outdoor grill; refs. 25–27). Urinary 1-OHPG has been shown to correlate with other urinary PAH metabolites as well as with BaP exposure (27–29), including PAHs from the diet (26). However, 2 important limitations for its application in assessing long-term dietary exposure are its short half-life (6–35 hours; ref. 27), and the lack of specificity for dietary exposure.

Despite its importance, little previous work has examined comparability in estimated intake of meat-derived BaP among these standard methods; the few studies that have been done

show poor correlation (30, 31). Thus, the objective of this study was to assess the correlations among FFQ, diary, and biomarker-based estimates of exposure to BaP from cooked meat to inform the interpretation of findings from published epidemiologic studies, as well as the development and refinement of PAH exposure assessment methods and the design of future epidemiologic studies on diet-derived PAHs and cancer risk.

Materials and Methods

Study population

Between October 2004 and October 2005, a total 54 participants were recruited as a convenience sample from controls in an ongoing case–control study of colorectal adenomas conducted at Johns Hopkins. Only controls were selected to reduce the possibility of major dietary changes during the study period due to diagnosis of a polyp. To minimize exposure to PAHs via nondietary routes, the following exclusion criteria were applied: current smoking; living with a smoker; frequenting smoky establishments, such as restaurants or bars; and working in an occupation with potentially high PAH exposure. All potentially eligible participants provided informed consent. Participants entered the study in a staggered fashion over a 13-month period and were followed for 9 to 13 months. This study was approved by the Institutional Review Board at the Johns Hopkins Bloomberg School of Public Health.

Data and sample collection

At entry (baseline) and again approximately 9 months later (follow-up/time 3; Table 1), participants were asked to complete a meat-specific FFQ developed by the National Cancer Institute (NCI), which included photographs and questions addressing meat intake, preparation methods, and degree of doneness (17, 32). At baseline, participants also completed a 60-item Block FFQ (33). Diet diaries with instructions to record all food items consumed as well as meat preparation method and degree of doneness for meats consumed (34) were sent to participants 1 month after baseline (time 1), at month 5 (time 2) and at month 9 (time 3; Table 1). Participants were asked to complete each diary on a day other than a Friday or Saturday (for availability of overnight courier services and laboratory personnel), and on the same day, to collect the last urine void of the evening through and including the first morning void. Participants collected the urine specimens, stored them 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, urine samples were thawed, the volume measured, aliquotted, and stored at −80°C until analysis.

Assessment of intake of meat and BaP from cooked meat and grains

The daily intake of meat and cooked meat-derived BaP were 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 (35). 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. A similar method was used to estimate meat intake 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.

The daily intake of BaP from cooked meat was estimated using the meat-specific FFQs and diet diaries coupled with the NCI CHARRED v. 1.7 RD (16, 36). The meat-specific FFQ was designed to match the categories of meat included in CHARRED. The diaries contained write-in responses from participants. Therefore, if a type of meat reported on a diary was not available in CHARRED, it was coded as the most similar meat according to the CHARRED conversion manual (37). Fish was not considered because CHARRED does not include BaP concentrations in fish. The daily number of grams of each type of meat cooked via a particular method and to a particular degree of doneness from the FFQs or the diet diaries was multiplied by the corresponding concentration of BaP (ng/g meat) to obtain the daily intake of BaP (ng/d) for that food item. To obtain the total intake of BaP from cooked meat, the daily intakes of BaP for each combination of meat type, cooking method, and degree of doneness were summed.

In addition to BaP from cooked meats, the daily intake of BaP from grains was estimated from the Block FFQ and the diet diaries, each coupled with data from Kazerouni and colleagues (16). To obtain the daily intake of BaP from grains (in ng/d) from the Block FFQ, the reported number of grain servings consumed per day was multiplied by 50 grams per serving (38) and by the typical concentration of BaP in grains of 0.1 ng/g (16). To obtain daily intake of BaP from grains from the diaries, daily intake (in grams) of each of 6 grain items (rice, bread, pasta, cereal, oatmeal, and popcorn) was multiplied by the BaP concentration (in ng/g) for that grain item from Kazerouni and colleagues (16). Those items were selected because they had the highest concentration of BaP out of all the grain products measured.

Assessment of a urinary biomarker of BaP

Each urine sample was analyzed for 1-OHPG concentration using high-performance liquid chromatography (HPLC) with fluorescence detection. After undergoing solid phase extraction and immunoaffinity purification (39), the purified sample was evaporated to 2 mL under vacuum and then analyzed with HPLC and laser-induced fluorescence (LIF). Five microliters of the sample were injected into an HPLC system consisting of a Rheodyne injector with a 5 μm loop, Agilent 1100 series pumps, Series 56 helium-cadmium Omnichrome laser, and a Picometrics Zetalif LIF detector (P/N1701-201) set to 325 nm. Separation was achieved with a Zorbax SB C18 5 μm, 150 × 0.5 mm column. The sample was eluted using a 20-minute linear gradient with acetylnitrile (ACN) and water (35%–55% ACN) followed by a cleaning regimen (55%–70% ACN over 5 minutes, and 70% ACN for 10 minutes). This system was operated at a flow rate of 5 mL/min. Area under the peak at the retention time of 14 minutes was determined by manually integrating the peaks using ChemStation Software Rev. A.08.04 (Agilent Technologies). Recovery of the entire method, based on the ratio of the slopes of the calibration curves for 1-OHPG spiked in urine and water, was 49%.

The method limit of detection (LOD) was determined to be 0.03 ng/mL from the repeated analysis (n = 5 each) of urine spiked at 0.025 and 0.05 ng/mL. The LOD was calculated as

the product of the Student's *t*-value corresponding to 99% confidence and $n-1$ degrees of freedom and the standard deviation of the measured concentration from the lower spiked samples. The small percentage of samples with measurements below the LOD were assigned the LOD divided by the square root of 2 (40). The between-batch coefficient of variation for 41 replicate positive control samples included with each batch of 10 to 20 urine samples analyzed was 19%. The within-batch coefficient variation for 20 replicates positive control samples (0.4 ng/mL) was 14%.

All urine specimens were analyzed for creatinine concentration from a 500 μL aliquot using the modified Jaffé reaction and a Dade Behring analyzer (Dade Behring; ref. 41).

Confirmation that the participants were not exposed to tobacco smoke at each time point was done by measuring urinary cotinine using a rapid semiquantitative (1–10, 10–30, 30–100, 100–200, 200–500, 500–2,000, and >2,000 ng/ mL) colorometric immunochromatographic assay (Accutest NicAlert, Jant Pharmacal Corporation; refs. 42 and 43). Participants with at least 1 urinary cotinine measure more than 30 ng/mL were excluded from all analyses.

Statistical analysis

For each participant, we calculated intake of meat and BaP from cooked meat from the baseline and follow-up FFQs; intake of BaP from grains from the single Block FFQ; mean intakes of meat and BaP from meat estimated from the 3 diet diaries; and mean 1-OHPG and creatinine-adjusted 1-OHPG concentrations from the 3 urine collections. Medians and interquartile ranges (IQR) were determined for these intakes and mean intakes because their distributions were not normal based on histograms, QQ plots, and the Shapiro–Wilk test. Differences in meat or in BaP intake estimates from each FFQ and the diet diaries were tested using the Wilcoxon sign-rank test. Correlations among the meat or BaP intake estimated using each FFQ and the mean intake from the diet diaries and creatinine-adjusted and unadjusted urinary 1-OHPG were assessed using the Spearman correlation coefficient. The intraclass correlation coefficients for the 3 assessments of urinary 1-OHPG were calculated with and without adjustment for urinary creatinine. Data were analyzed using Intercooled Stata v.11.0 (Statacorp; College Station).

Results

Study population

Of the 54 nonsmokers enrolled, 39 (72%) completed the data and sample collection at all time points. Of those 39, 3 individuals had cotinine levels more than 30 ng/mL and were excluded. Of the 36 participants included, 61% were never smokers while 39% were former smokers; 67% were female; 100% were white; and 64% were employed. The median age of participants was 56 years (range: 27–85 years), and the median body mass index was 24.7 kg/m^2 (range: 16.6–37.8 kg/m^2; Table 2).

Intake of meat and BaP from meat and grains

Mean intake of meat estimated from the 3 diaries (median = 128.5, IQR = 110.8 g/d) was higher than from the baseline (median = 49.7, IQR = 31.8 g/d; $P < 0.001$) and follow-up (median = 33.8, IQR = 41.3 g/d; $P < 0.001$) FFQs (Table 3). Mean intakes of BaP from meat estimated from the baseline (median = 6.4; IQR = 13.9 ng/d) and follow-up FFQ-RDs (median = 7.3, IQR = 35.7 ng/d) were nonstatistically significantly higher than from the Diary-RD (median = 1.4, IQR = 14.3 ng/d; Table 3). Median intake of BaP from grains estimated from the single Block FFQ (21.3, IQR = 11.0 ng/d) was similar to BaP intake from grains averaged over the 3 diaries (median = 18.9, IQR = 26.8 ng/d); both of which were higher than the BaP intake estimates derived from cooked meats.

Urinary biomarker 1-OHPG

A total of 84% of urine samples were above the LOD for 1-OHPG. The median (IQR) concentration of the mean 1-OHPG of the 3 time points was 0.10 (0.21) ng/mL, and the median (IQR) creatinine-adjusted concentration was 0.17 (0.20) ng/mg creatinine (Table 3). The intraclass correlation coefficient for the unadjusted 1-OHPG was 0.37 and 0.19 for the creatinine-adjusted 1-OHPG. Because creatinine adjustment decreased the repeatability of the 1-OHPG measurements, the unadjusted 1-OHPG measurements were used in the rest of the analyses.

Correlation among BaP exposure methods

Mean intake of meat from the diaries was statistically significantly correlated with meat intake from the baseline FFQ ($r = 0.39$, $P = 0.02$), but not the follow-up FFQ ($r = 0.09$, $P = 0.62$; Fig. 1). Conversely, cooked meat-derived BaP intake from the diaries was statistically significantly correlated with cooked meat-derived BaP intake from the follow-up FFQ (r=0.35,P=0.04), but not the baseline FFQ ($r = 0.20$, $P = 0.24$; Fig. 2). Intakes of meat and cooked-meat–derived BaP from the diaries were not positively, significantly correlated with 1-OHPG at any of the individual 3 time points (Table 4). However, when averaged over the 3 time points, mean intake of meat from the 3 diaries was statistically significantly correlated with mean 1-OHPG concentrations(r=0.33,P=0.05). Mean intake of cooked-meat–derived from the 3 diaries was weakly correlated with mean 1-OHPG, but not at the level of statistical significance ($r = 0.27$, $P = 0.11$). No exposure metrics from the FFQ were correlated with1-OHPG (Figs. 1 and 2). Meat intake ($r = 0.34$, $P = 0.04$) and BaP from meat ($r = 0.56$, $P < 0.001$) intake were significantly correlated between the baseline and follow-up FFQs.

Correlations among the methods using BaP intake from meat and grains combined had negligible impact on the Spearman correlations when using cooked-meat–derived BaP alone. For example, BaP intake from the diaries derived from both meat and grains was not statistically significantly correlated with the BaP intake from meat and grains from the baseline FFQ ($r = 0.11$, $P = 0.5$) or mean 1-OHPG ($r = 0.29$, $P = 0.09$). As with BaP intake from cooked meat, BaP intake from meat and grains estimated by the baseline FFQ-RD was not correlated with mean 1-OHPG ($r = 0.19$, $P = 0.28$).

Discussion

Despite the importance of evaluating dietary exposure to PAH for epidemiologic studies on dietary PAHs and cancer risk, little is known about how exposure estimates between standardly used methods correlate. To our knowledge, this study is the first to concurrently measure and compare dietary BaP exposure using FFQs, diet diaries, and the urinary biomarker 1-OHPG using a longitudinal design. Using this approach, we evaluated correlations between the means of repeated measures of meat and BaP intake reflecting short-term exposure (the Diary-RD and 1-OHPG) as well as their correlations with estimates of usual exposure derived by the FFQ.

Estimated meat intake from the FFQ in this study population, which consisted of patients undergoing colonoscopy in 2004–2005, tended to be lower than what has been reported in previous studies. The mean meat intakes reported in 2 previous studies using the same NCI FFQ of 92.6 and 114.4 g/d in control populations were about the same or higher than the 90th percentile of meat intake in the baseline (97.1 g/d) and follow-up (90.4 g/d) FFQs in our study (3, 7). The median intakes of total meat in the baseline (49.7 g/d) and follow-up FFQs (33.8 g/d) in our study were similar to the sum of the median intakes of red meat (19.8 g/d) and chicken (27.4 g/d) in a population of controls using the same FFQ (34; Table 3). Though estimates of meat intake from the FFQ tended to be lower than other studies, the median BaP intake from meat at baseline (6.4 ng/d) and follow-up (7.3 ng/d) in the current study (Table 3) were within the wide range of median BaP intakes reported previously in controls using the same FFQ-RD: 1.8 ng/d (9), 5 ng/d (3), and 37.3 ng/d (8).

The median meat intake based on the diaries in our study (128.5 g/d; Table 3) was higher than what was reported by Cantwell and colleagues (2004), who used the same diaries (36.6 g/d for red meat and 23.8 g/d for chicken; ref. 34). No reports in the literature of dietary BaP intake estimates using the NCI diary and CHARRED RD were identified for comparison.

The urinary biomarker 1-OHPG has been used extensively for evaluating PAH exposure related to air pollution (44), employment (45, 46), smoking (26), and diet (27, 47). Because biomarkers integrate across all routes of exposure, studies with a singular focus such as diet need to account for potentially confounding exposures. In the current study, this was accomplished through a recruitment strategy that excluded smokers, occupational exposures, and high-exposure indoor environments (e.g., smoky bars). For this population, the median of the mean concentrations of 1-OHPG over the 3 time points was 0.10 ng/mL (0.25 pmol/mL). This concentration is a little higher than the nonsmoking cohort (n = 299) described by Gunier and colleagues who reported a median of 0.16 pmol/mL for all nonsmokers (26). Participants in the current study with 1-OHPG concentrations at or above the 95th percentile (1.05 ng/mL or 2.66 pmol/mL) had concentrations consistent with an average smoker or those eating a meal that includes well-done grilled meat (25, 26).

Creatinine is widely used to adjust 1-OHPG and other biomarkers for differences in dilution. However, urinary creatinine correction is not a perfect remedy because its excretion is dependent on many factors such as age, sex, red meat intake, time of day, kidney function, body mass index (48, 49). In our study, creatinine adjustment increased the intraclass

correlation coefficient, reducing the ability of the biomarker to reflect differences in exposure among the participants. It increased the within-subject variability and decreased the between-subject variability.

We expected to observe a positive correlation between the Diary-RD and urinary 1-OHPG, the 2 short-term measures of BaP exposure that were done concurrently. However, BaP intake from the Diary-RD was not statistically significantly positively correlated with 1-OHPG at any of the 3 time points or when comparing the mean of the 3 time points (Table 4, Fig. 2). However, mean meat intake alone as estimated by the diary was correlated with mean 1-OHPG, although the 2 exposure metrics were not correlated at any of the individual time points (Table 4). Diet has long been recognized as the most important source of PAH exposure in nonoccupationally exposed nonsmokers (29, 47, 50), yet dietary intake of BaP as evaluated by the diary explained very little of the variability in 1-OHPG. Though we applied strict exclusion criteria to minimize nondietary exposures to PAHs, some of the unexplained variability in 1-OHPG could be due to exposure from other sources, such as diesel exhaust or fossil fuel combustion (51). Some of the unexplained variability may also be attributable to the fact that 2 different PAHs are being compared. Whereas the survey methods consider BaP, 1-OHPG is a metabolite specifically of the PAH pyrene, although others have shown that pyrene and BaP exposures are highly correlated (52). Additionally, interindividual differences in metabolism could explain some of the lack of correlation between estimated intake and excretion products. Kang and colleagues observed that 10 participants ingesting the same amount of PAH from charbroiled meat had an 8-fold range in 1-OHPG concentrations the following morning, corresponding to a CV of 73% (25). Buckley and colleagues demonstrated a statistically significant difference in 1-hydroxypyrene elimination rates in 5 participants ingesting grilled meat (27).

Though grains were observed to be an important contributor to dietary BaP intake, addition of grain-based BaP intake to cooked meat-based BaP intake did not explain any addition variability in 1-OHPG as compared with cooked meat-based intake alone. Though BaP has been measured in many nonmeat foods, much uncertainty surrounds the RD estimates for these foods (16). The BaP in plant-based foods may come from atmospheric deposition, contaminated water or soil, or the cooking process. All of these sources introduce variability that is difficult to capture within a RD.

The stronger correlation between 1-OHPG and meat intake as compared with the BaP intake suggests that the CHARRED RD may be introducing measurement error. The CHARRED database was developed primarily for assessing exposure to another group of dietary carcinogens, the heterocyclic amines. Therefore, certain foods, known to contain BaP, were not included, such as smoked meats, fish, or sausages. However, reported intake of smoked foods in this study population was quite low. Furthermore, the CHARRED database has some limitations, due to the difficulties in developing such a database. For example, the products used to develop the database were purchased from 2 supermarkets within a 50-mile radius in Maryland (16), thereby eliminating geographical variability. In addition, CHARRED does not take into account several factors that may influence BaP content in meats, such as percentage of fat in the meat, use of marinades, frequency of flipping the

meat during cooking, the thickness of the cut of meat, and whether the meal was prepared at a dine-in or fast-food restaurant or at home (53).

FFQs are the most commonly used tool for assessing dietary exposures in large epidemiologic studies, and they have been criticized for lacking the precision to allow for detection of a diet-disease link (54, 55). Given that we did not observe significant correlations between the short-term biomarker and the short-term Diary-RD collected over the same timeframe, it is not surprising that we did not observe any correlations between 1-OHPG and the FFQ, a measure of usual exposure. Because the follow-up FFQ was administered concurrently with the third diary, thereby covering overlapping periods of time, a stronger correlation among estimates of intake might have been expected between the diary and the follow-up FFQ as compared with the baseline FFQ. However, this was only true for BaP intake and not meat intake. The lack of a consistent and strong correlation among BaP and meat intake from the diaries and the FFQs may be due in part to differences in sampling timeframe. Whereas the FFQ is designed to capture usual consumption, the diary represents 3 1-day assessments that excluded Fridays and Saturdays. Studies of dietary patterns in the U.S. show that people tend to consume more calories on weekends (56–58), and one USDA (United States Department of Agriculture) study from 1977 to 1978 observed increased meat intake on the weekends (56). Collecting more days of diaries might have improved the correlation with the FFQ. However, Cant-well and colleagues compared 12 daily diary measures, including weekend days, collected over a 3-month period, to the FFQ that assessed usual intake for the prior year, to estimate the intake of heterocyclic amines and observed low or modest unadjusted Pearson correlations of 0.22 and 0.43 for 2-amino-1-methyl-6-phenylimida-zo[4,5β]pyridine and 2-amino-3,8-dimethylimidazo-[4,5*f*]-quinoxaline, respectively (34).

The current study provides a comparison of standardly used methods for assessing dietary exposure to BaP. Because no gold standard exists for assessing exposure to meat-derived PAHs, we could not determine whether one of the methods provided a better estimate of dietary intake over another. Nonetheless, the comparison is valuable and important in establishing similarities and differences in estimates provided by the different methods. More methodological work may be needed, such as comparing the FFQ or diaries to measurements of duplicate diet or a longer-term biomarker of exposure in a validation substudy.

In conclusion, the findings from the current study show a lack of correlation in cooked-meat–derived BaP exposure among repeated concurrent, short-term diary-based and biomarker-based metrics. Moreover, neither short-term measure of BaP exposure was consistently correlated with usual BaP intake from the FFQ. In the absence of a gold standard, the exposure misclassification of any given method cannot be ascertained. However, the limited correlation in BaP exposure among all the methods undermines confidence in the estimates derived from the methods and limits the ability to compare across epidemiologic studies when different methods are used. Comparison with measurements of BaP in duplicate diet samples could provide the needed gold standard (59). Further development and validation of longer-term markers would be useful, such as PAH adducts with serum proteins (60). The stronger correlation between meat intake from the

diaries and the 1-OHPG as compared with BaP intake suggests that the RD may not be increasing the accuracy of the estimates of BaP intake. These findings highlight the importance of validating exposure assessment methods and raise concerns about the error introduced by these methods when applied in epidemiologic studies of cancer risk.

Measurement error in dietary exposure assessment methods for meat-derived PAHs can attenuate relative risk estimates in epidemiologic studies of cancer. The current study provides insights as to limitations and agreement among dietary PAH exposure assessment methods that can inform interpretation of existing studies, design of future studies, and development of improved methods of assessment. The current study highlights concerns about the reliability of current methods for evaluating BaP exposure and the need for research to develop well-validated methods for assessing dietary exposure.

Acknowledgments

The authors thank Dr. Rashmi Sinha for generously providing the meat-specific food frequency questionnaires and diet diaries, Julitta Brannock and Kathy Schultz for assistance in recruiting and data collections, Dr. Francesca Dominici for statistical guidance, and Dr. Melissa Friesen for reviewing the manuscript.

Grant Support

This work was supported by Johns Hopkins Center for a Livable Future Student Innovation Grant, National Institute for Occupational Safety and Health Pilot Program, and Maryland Cigarette Restitution Fund at Johns Hopkins.

References

1. International Agency for Research on Cancer. Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. 2010:92.
2. Gunter MJ, Probst-Hensch NM, Cortessis VK, Kulldorff M, Haile RW, Sinha R, et al. Meat intake, cooking-related mutagens and risk of colorectal adenoma in a sigmoidoscopy-based case-control study. Carcinogenesis. 2005; 26:637–642. [PubMed: 15579480]
3. Sinha R, Kulldorff M, Gunter MJ, Strickland P, Rothman N. Dietary benzo[*a*]pyrene intake and risk of colorectal adenoma. Cancer Epidemiol Biomarkers Prev. 2005; 14:2030–2034. [PubMed: 16103456]
4. Sinha R, Peters U, Cross AJ, Kulldorff M, Weissfeld JL, Pinsky PF, et al. Meat, meat cooking methods and preservation, and risk for colorectal adenoma. Cancer Res. 2005; 65:8034–8041. [PubMed: 16140978]
5. Shin A, Shrubsole MJ, Ness RM, Wu H, Sinha R, Smalley WE, et al. 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]
6. Ferrucci LM, Sinha R, Graubard BI, Mayne ST, Ma X, Schatzkin A, et al. Dietary meat intake in relation to colorectal adenoma in asymptomatic women. Am J Gastroenterol. 2009; 104:1231–1240. [PubMed: 19367270]
7. Butler LM, Sinha R, Millikan RC, Martin CF, Newman B, Gammon MD, et al. Heterocyclic amines, meat intake, and association with colon cancer in a population-based study. Am J Epidemiol. 2003; 157:434–445. [PubMed: 12615608]
8. Li D, Day RS, Bondy ML, Sinha R, Nguyen NT, Evans DB, et al. Dietary mutagen exposure and risk of pancreatic cancer. Cancer Epidemiol Biomarkers Prev. 2007; 16:655–661. [PubMed: 17416754]
9. Anderson KE, Kadlubar FF, Kulldorff M, Harnack L, Gross M, Lang NP, et al. Dietary intake of heterocyclic amines and benzo(a)pyrene: associations with pancreatic cancer. Cancer Epidemiol Biomarkers Prev. 2005; 14:2261–2265. [PubMed: 16172241]

10. Cross AJ, Peters U, Kirsh VA, Andriole GL, Reding D, Hayes RB, et al. A prospective study of meat and meat mutagens and prostate cancer risk. Cancer Res. 2005; 65:11779–11784. [PubMed: 16357191]
11. Sinha R, Park Y, Graubard BI, Leitzmann MF, Hollenbeck A, Schatzkin A, et al. Meat and meat-related compounds and risk of prostate cancer in a large prospective cohort study in the United States. Am J Epidemiol. 2009; 170:1165–1177. [PubMed: 19808637]
12. Steck SE, Gaudet MM, Eng SM, Britton JA, Teitelbaum SL, Neugut AI, et al. Cooked meat and risk of breast cancer–lifetime versus recent dietary intake. Epidemiology. 2007; 18:373–382. [PubMed: 17435448]
13. Ferrucci LM, Cross AJ, Graubard BI, Brinton LA, McCarty CA, Ziegler RG, et al. Intake of meat, meat mutagens, and iron and the risk of breast cancer in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Br J Cancer. 2009; 101:178–184. [PubMed: 19513076]
14. Tasevska N, Sinha R, Kipnis V, Subar AF, Leitzmann MF, Hollenbeck AR, et al. A prospective study of meat, cooking methods, meat mutagens, heme iron, and lung cancer risks. Am J Clin Nutr. 2009; 89:1884–1894. [PubMed: 19369370]
15. Lam TK, Cross AJ, Consonni D, Randi G, Bagnardi V, Bertazzi PA, et al. Intakes of red meat, processed meat, and meat mutagens increase lung cancer risk. Cancer Res. 2009; 69:932–939. [PubMed: 19141639]
16. 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]
17. Sinha R, Cross A, Curtin J, Zimmerman T, McNutt S, Risch A, et al. Development of a food frequency questionnaire module and data-bases for compounds in cooked and processed meats. Mol Nutr Food Res. 2005; 49:648–655. [PubMed: 15986387]
18. 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]
19. Butler JP, Post GB, Lioy PJ, Waldman JM, Greenberg A. Assessment of carcinogenic risk from personal exposure to benzo(a)pyrene in the Total Human Environmental Exposure Study (THEES). Air Waste. 1993; 43:970–977. [PubMed: 8369112]
20. Spiegelman D. Approaches to uncertainty in exposure assessment in environmental epidemiology. Annu Rev Public Health. 2010; 31:149–63. [PubMed: 20070202]
21. Armstrong BG. The effects of measurement errors on relative risk regressions. Am J Epidemiol. 1990; 132:1176–1184. [PubMed: 2260550]
22. Willett, WC. Nutritional Epidemiology. Oxford: Oxford University Press; 1998.
23. Groopman JD, Kensler TW. The light at the end of the tunnel for chemical-specific biomarkers: daylight or headlight? Carcinogenesis. 1999; 20:1–11. [PubMed: 9934843]
24. Pirkle JL, Needham LL, Sexton K. Improving exposure assessment by monitoring human tissues for toxic chemicals. J Expo Anal Environ Epidemiol. 1995; 5:405–424. [PubMed: 8814778]
25. Kang DH, Rothman N, Poirier MC, Greenberg A, Hsu CH, Schwartz BS, et al. Interindividual differences in the concentration of 1-hydroxypyrene-glucuronide in urine and polycyclic aromatic hydrocarbon-DNA adducts in peripheral white blood cells after charbroiled beef consumption. Carcinogenesis. 1995; 16:1079–1085. [PubMed: 7767968]
26. Gunier RB, Reynolds P, Hurley SE, Yerabati S, Hertz A, Strickland P, et al. Estimating exposure to polycyclic aromatic hydrocarbons: a comparison of survey, biological monitoring, and geographic information system-based methods. Cancer Epidemiol Biomarkers Prev. 2006; 15:1376–1381. [PubMed: 16835339]
27. Buckley TJ, Lioy PJ. An examination of the time course from human dietary exposure to polycyclic aromatic hydrocarbons to urinary elimination of 1-hydroxypyrene. Br J Ind Med. 1992; 49:113–124. [PubMed: 1536818]
28. Li Z, Sandau CD, Romanoff LC, Caudill SP, Sjodin A, Needham LL, et al. Concentration and profile of 22 urinary polycyclic aromatic hydrocarbon metabolites in the US population. Environ Res. 2008; 107:320–331. [PubMed: 18313659]

29. Buckley TJ, Waldman JM, Dhara R, Greenberg A, Ouyang Z, Lioy PJ, et al. An assessment of a urinary biomarker for total human environmental exposure to benzo[*a*]pyrene. Int Arch Occup Environ Health. 1995; 67:257–266. [PubMed: 7591187]
30. Scherer G, Frank S, Riedel K, Meger-Kossien I, Renner T. Biomonitoring of exposure to polycyclic aromatic hydrocarbons of nonoccupationally exposed persons. Cancer Epidemiol Biomarkers Prev. 2000; 9:373–380. [PubMed: 10794481]
31. Gunter MJ, Divi RL, Kulldorff M, Vermeulen R, Haverkos KJ, Kuo MM, et al. Leukocyte polycyclic aromatic hydrocarbon-DNA adduct formation and colorectal adenoma. Carcinogenesis. 2007; 28:1426–1429. [PubMed: 17277232]
32. Sinha R, Rothman N. Exposure assessment of heterocyclic amines (HCAs) in epidemiologic studies. Mutat Res. 1997; 376:195–202. [PubMed: 9202756]
33. Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L, et al. A data-based approach to diet questionnaire design and testing. Am J Epidemiol. 1986; 124:453–469. [PubMed: 3740045]
34. Cantwell M, Mittl B, Curtin J, Carroll R, Potischman N, Caporaso N, et al. Relative validity of a food frequency questionnaire with a meat-cooking and heterocyclic amine module. Cancer Epidemiol Biomar-kers Prev. 2004; 13:293–298.
35. 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]
36. Sinha R. An epidemiologic approach to studying heterocyclic amines. Mutat Res. 2002; 506–507:197–204.
37. National Cancer Institute [internet]. CHARRED Cooking Conversions. Available from: http://dceg.cancer.gov/neb/tools/charred/cooking
38. U.S. Department of Health and Human Services and U.S. Department of Agriculture. Dietary Guidelines for Americans, 2005. 6th Edition. Washington, DC: U.S. Government Printing Office; 2005 Jan.
39. Strickland PT, Kang D, Bowman ED, Fitzwilliam A, Downing TE, Rothman N, et al. Identification of 1-hydroxypyrene glucuronide as a major pyrene metabolite in human urine by synchronous fluorescence spectroscopy and gas chromatography-mass spectrometry. Carcinogenesis. 1994; 15:483–487. [PubMed: 8118933]
40. Lubin JH, Colt JS, Camann D, Davis S, Cerhan JR, Severson RK, et al. Epidemiologic evaluation of measurement data in the presence of detection limits. Environ Health Perspect. 2004; 112:1691–1696. [PubMed: 15579415]
41. Larsen K. Creatinine assay by a reaction kinetic principle. Clin Chim Acta. 1972; 41:209–217. [PubMed: 4645233]
42. Gariti P, Rosenthal DI, Lindell K, Hansen-Flaschen J, Shrager J, Lipkin C, et al. Validating a dipstick method for detecting recent smoking. Cancer Epidemiol Biomarkers Prev. 2002; 11:1123–1125. [PubMed: 12376520]
43. Jant Pharmacal Corporation [internet]. Encino, CA: Accutest NicAlert Test Strip Package Insert; Available from: http://www.accutest.net/products/pdf/DS47NY150NicAlertUSUrineProductInsert.pdf
44. Strickland P, Kang D. Urinary 1-hydroxypyrene and other PAH metabolites as biomarkers of exposure to environmental PAH in air particulate matter. Toxicol Lett. 1999; 108:191–199. [PubMed: 10511262]
45. Bouchard M, Viau C. Urinary 1-hydroxypyrene as a biomarker of exposure to polycyclic aromatic hydrocarbons: biological monitoring strategies and methodology for determining biological exposure indices for various work environments. Biomarkers. 1999; 4:159–187. [PubMed: 23885861]
46. Kang D, Rothman N, Cho SH, Lim HS, Kwon HJ, Kim SM, et al. Association of exposure to polycyclic aromatic hydrocarbons (estimated from job category) with concentration of 1-hydroxypyrene glucuronide in urine from workers at a steel plant. Occup Environ Med. 1995; 52:593–599. [PubMed: 7550799]
47. Van Rooij JG, Veeger MM, Bodelier-Bade MM, Scheepers PT, Jongeneelen FJ. Smoking and dietary intake of polycyclic aromatic hydrocarbons as sources of interindividual variability in the

baseline excretion of 1-hydroxypyrene in urine. Int Arch Occup Environ Health. 1994; 66:55–65. [PubMed: 7927844]

48. Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL, et al. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect. 2005; 113:192–200. [PubMed: 15687057]
49. Boeniger MF, Lowry LK, Rosenberg J. Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: a review. Am Ind Hyg Assoc J. 1993; 54:615–627. [PubMed: 8237794]
50. Menzie CA, Potocki BB, Santodonato J. Exposure to carcinogenic PAHs in the environment. Environ Sci Technol. 1992; 26:1278–1284.
51. Lai CH, Liou SH, Shih TS, Tsai PJ, Chen HL, Buckley TJ, et al. Urinary 1-hydroxypyrene-glucuronide as a biomarker of exposure to various vehicle exhausts among highway toll-station workers in Taipei, Taiwan. Arch Environ Health. 2004; 59:61–69. [PubMed: 16075899]
52. Greenberg A, Hsu CH, Rothman N, Strickland P. PAH profiles of charbroiled hamburgers: pyrene/B[*a*]P ratios and presence of reactive PAH. Polycyclic Aromat Cmpd. 1993; 3:101–110.
53. Martínez ME, Jacobs ET, Ashbeck EL, Sinha R, Lance P, Alberts DS, et al. Meat intake, preparation methods, mutagens and colorectal adenoma recurrence. Carcinogenesis. 2007; 28:2019–2027. [PubMed: 17690112]
54. Kipnis V, Midthune D, Freedman L, Bingham S, Day NE, Riboli E, et al. Bias in dietary-report instruments and its implications for nutritional epidemiology. Public Health Nutr. 2002; 5:915–923. [PubMed: 12633516]
55. Byers T. Food frequency dietary assessment: how bad is good enough? Am J Epidemiol. 2001; 154:1087–1088. [PubMed: 11744510]
56. Thompson FE, Larkin FA, Brown MB. Weekend-weekday differences in reported dietary intake: The nationwide food consumption survey, 1977–78. Nutrition Research. 1986; 6:647–662.
57. 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]
58. Rhodes DG, Goldman JD, Moshfegh AJ. Do Americans eat more on weekends [abstract]? The FASEB Journal. 2008; 22 875.1.
59. Riederer AM, Hunter RE Jr, Hayden SW, Ryan PB. Pyrethroid and organophosphorus pesticides in composite diet samples from Atlanta, USA adults. Environ Sci Technol. 2010; 44:483–490. [PubMed: 19994894]
60. Kwack SJ, Lee BM. Correlation between DNA or protein adducts and benzo[*a*]pyrene diol epoxide I-triglyceride adduct detected *in vitro* and *in vivo*. Carcinogenesis. 2000; 21:629–632. [PubMed: 10753196]

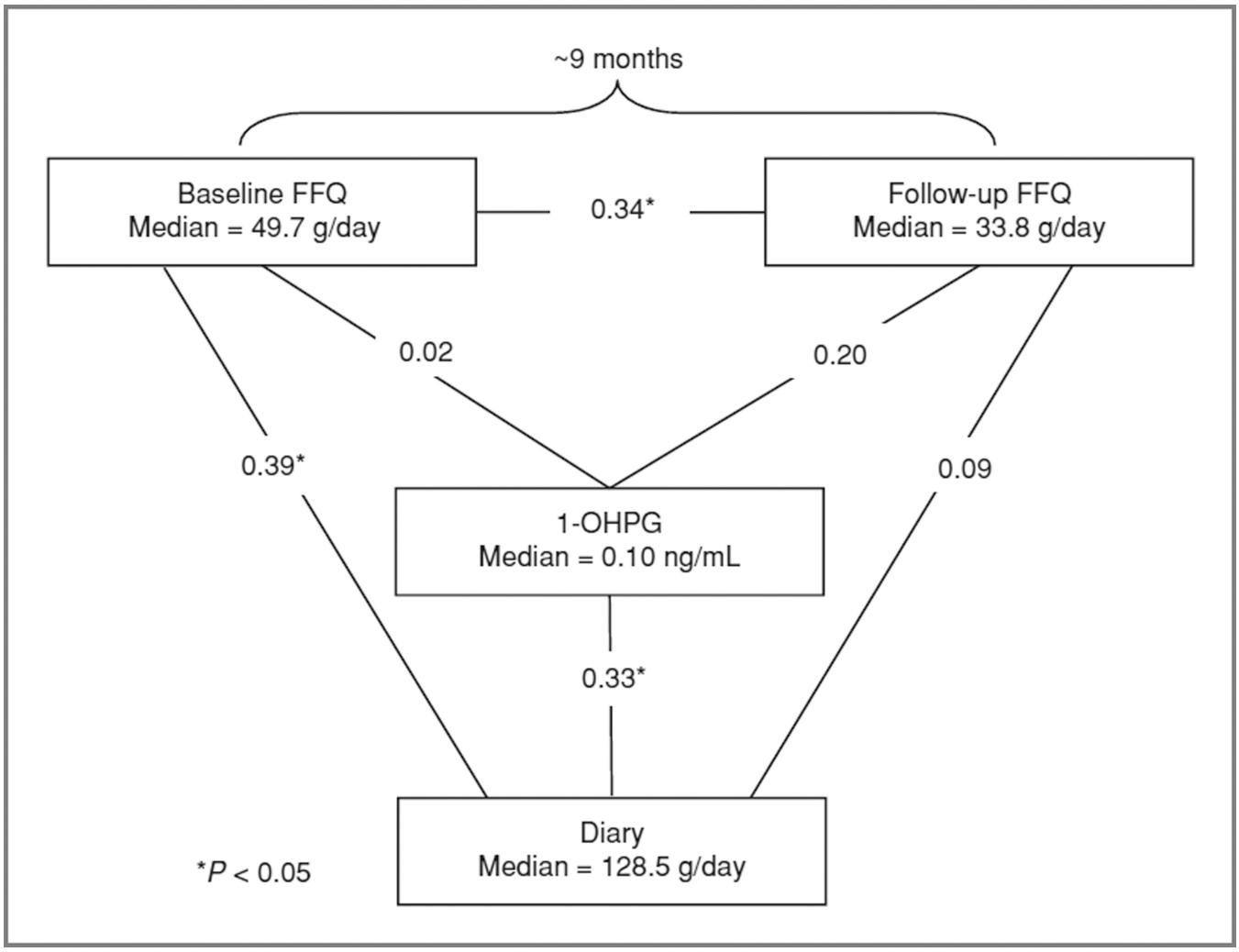

Figure 1.

Spearman correlations among meat intake from Food Frequency Questionnaires (FFQ) and Diaries and measurements of urinary 1-hydroxypyrene (1-OHPG; adapted from Willett 1998). Median 1-OHPG and median meat intake from Diary are calculated for the mean of the repeated measures.

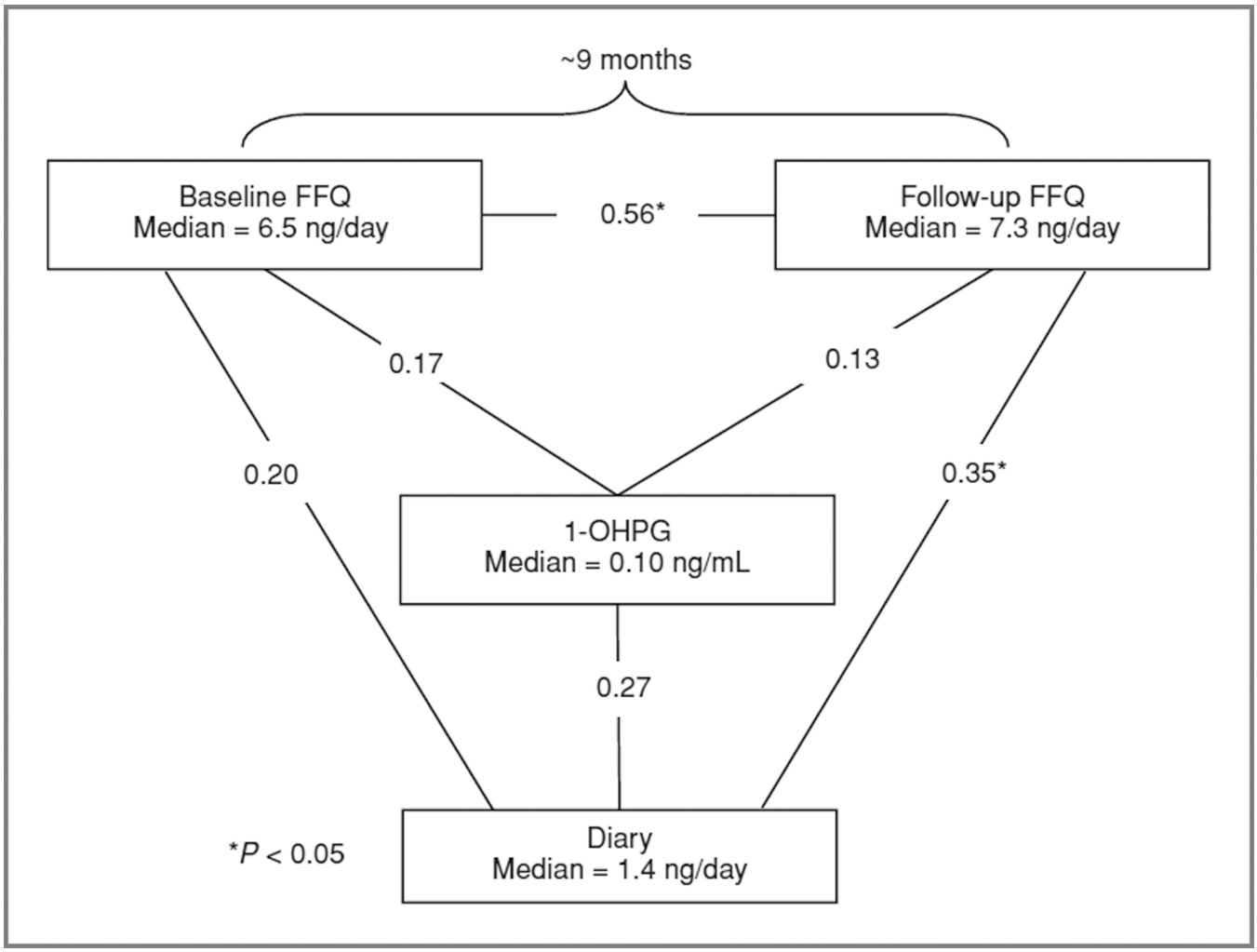

Figure 2.

Spearman correlations among BaP intake from Food Frequency Questionnaires (FFQ) and Diaries and measurements of urinary 1-hydroxypyrene (1-OHPG; adapted from Willett 1998). Median 1-OHPG and median BaP intake from the Diary are calculated for the mean of the repeated measures.

Table 1

Sample and data collection timeline

	Baseline (month 0)	Time 1 (~ month 1)	Time 2 (~month 5)	Time 3 (~month 9)
Meat-specific FFQ	X			X
Diet diary		X	X	X
Urine collection		X	X	X

Table 2

Characteristics of study population (n = 36)

	Frequency (%)
Gender	
Female	24 (67)
Male	12 (33)
Age, years	
≤40	6 (17)
41–50	4 (11)
51–60	11 (31)
>60	15 (41)
Smoking	
Never	22 (61)
Former	14 (39)
Employment	
Employed	23 (64)
Unemployed	2 (6)
Retired	8 (22)
Other	3 (8)
Body mass index, kg/m^2	
<18.5	2 (6)
18.5–24.9	15 (42)
25.0–29.9	11 (31)
≥30	7 (19)

Table 3

Dietary intake of meat and benzo[*a*]pyrene (BaP) from cooked meat as estimated by a food frequency questionnaire, diet diaries; and urinary 1-hydroxypyrene glucuronide (1-OHPG) concentration among 36 participants[a]

	Minimum	Median	Maximum	Interquartile range
Intake estimated from the baseline FFQ				
Meat, g/d	1.9	49.7	129.9	31.8
BaP, ng/d[b]	0.01	6.4	89.7	13.9
Intake estimated from the follow-up (time 3) FFQ				
Meat, g/d	12.9	33.8	303.7	41.3
BaP, ng/d[b]	0.1	7.3	94.2	35.7
Mean intake estimated from 3 diaries, g/d				
Meat, g/d	0.0	128.5	418.0	110.8
BaP, ng/d[b]	0.0	1.4	355.1	14.3
Mean urinary biomarker measurements from three collections				
1-OHPG, ng/mL[c]	<LOD	0.10	1.71	0.14
Adjusted 1-OHPG, ng/g creatinine	<LOD	0.17	1.77	0.24

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

[b] Coupled with a BaP residue database.

[c] The limit of detection (LOD) was 0.03 ng/mL.

Table 4

Spearman correlations between BaP or meat intake from the diaries and 1-hydroxypyrene at 3 time points and overall[a]

	Time 1	Time 2	Time 3	Overall
BaP Intake	0.23	0.21	0.27	0.27
Meat Intake	0.01	0.23	0.10	0.33[b]

[a] The overall correlation is between the mean BaP or meat intake and the mean 1-OHPG, averaged over the 3 time points.

[b] $P < 0.05$