

DNA and Protein Adduct Formation in the Colon and Blood of Humans after Exposure to a Dietary-relevant Dose of 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine¹

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Abstract

Epidemiology studies have indicated that certain dietary components, including well-cooked meat, are risk determinants for colon cancer. Cooked meat can contain significant quantities of heterocyclic aromatic amines (HCAs), which have been established as carcinogens in laboratory animals. 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) is usually the most mass-abundant HCA, with concentrations up to 480 ppb. We used accelerator mass spectrometry to establish whether DNA and protein adducts can be detected in humans exposed to a quantity of PhIP comparable with levels of exposure that occur in the diet. Five human volunteers were administered a dietary-relevant dose of [¹⁴C]PhIP (70–84 μg) 48–72 h before surgery for removal of colon tumors. Blood samples were collected at various time points, and albumin, hemoglobin, and WBC DNA were extracted for analysis by accelerator mass spectrometry. Tissue samples were collected during surgery and used to assess either tissue available doses of [¹⁴C]PhIP or adduct levels. The results of this study show: (a) PhIP is activated to a form that will bind to albumin, hemoglobin, and WBC DNA in peripheral blood. WBC DNA adducts were unstable and declined substantially over 24 h; (b) PhIP is bioavailable to the colon, with levels in normal tissue in the range 42–122 pg PhIP/g tissue; and (c) PhIP binds to both protein and DNA in the colon. DNA adduct levels in the normal tissue were 35–135 adducts/10¹² nucleotides, which was

significantly lower than tumor tissue. The results of this study demonstrate that PhIP is bioavailable to the human colon following defined dietary-relevant doses and forms DNA and protein adducts.

Introduction

It has been estimated that there will be 94,700 new cases of colon cancer in the United States in 1999 (1). Although the causes remain largely unknown, dietary factors have been strongly implicated in the etiology of this disease, and some epidemiological evidence suggests an increased risk associated with the consumption of well-done cooked meat (2–4). The identification of mutagenic and carcinogenic HCAs³ in cooked meat has raised the possibility that these compounds may play a role in the development of cancer in humans (reviewed in Ref. 5).

Dietary exposure to HCAs is considered to be a potential human cancer risk because these compounds are produced in relatively high concentrations during the cooking of red meats, poultry, and fish under normal household conditions (reviewed in Ref. 6), with PhIP levels up to 480 ppb reported for very well done grilled/barbecued chicken (7). At present, 19 HCAs have been identified in cooked foods, 10 of which have been tested for carcinogenicity and have been found to induce tumors in rodents at multiple sites (reviewed in Ref. 8). Of all of the HCAs, PhIP (Fig. 1) is considered to be a significant colon cancer risk factor because it is usually the most mass-abundant in cooked meat (9), is a mutagen in bacteria and mammalian cell genotoxicity assays (10), and causes colon tumors in rats (11).

The mutagenicity and, presumably, the carcinogenicity of PhIP is considered to initially involve the oxidation of the exocyclic amino group to its corresponding *N*-hydroxylated derivative HNOH-PhIP by cytochrome P450 enzymes (12). In humans, this is catalyzed primarily by CYP1A2 (13). Subsequent conjugation of the exocyclic *N*-hydroxyl group to acetate or sulfate (14), which can be catalyzed by human *N*-acetyltransferases (15) and sulfotransferases (16), is believed to result in the formation of the ultimate genotoxic species, which is capable of binding to DNA, resulting in covalent adducts (14). DNA adduct formation by chemical carcinogens is considered important because it demonstrates the active dose of a chemical reaching its target site and is thought to be the initiating event in chemical carcinogenesis (reviewed in Ref. 17). Protein adducts may also provide a useful measure of the active carcin-

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³ The abbreviations used are: HCA, heterocyclic amine; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; HNOH-PhIP, 2-*N*-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; CYP1A2, cytochrome P-450 1A2; Hb, hemoglobin; AMS, accelerator mass spectrometry; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; NAT, *N*-acetyltransferase.

ogen dose in tissues. Consequently, the measurement of adduct levels may potentially provide a measure of cancer risk following exposure to PhIP (18). However, it is not known whether bioactive metabolites are formed in humans at the low doses encountered in the diet or if macromolecular adducts are consistently formed in tissues at these levels. Likewise, PhIP adducts with albumin, Hb, and WBC DNA in the peripheral blood need to be investigated as biomarkers for identifying individuals at risk for cancer, either because of exposure to high levels of PhIP or due to interindividual differences in metabolism (18, 19).

The measurement of carcinogen adducts in human samples can be problematic, because it requires extremely sensitive techniques capable of accurately measuring subfemtomol (10^{-15} mol) levels of carcinogen-bound macromolecules. AMS is a highly sensitive technique capable of measuring attomol (10^{-18} mol) levels of a radiolabeled compound (20, 21). We have used AMS to study the bioavailability and macromolecular adduct formation following very low, environmentally relevant exposures of radiolabeled carcinogens (Refs. 22–26 for examples). This allows more accurate assessment of biomarkers of exposure and metabolism, and perhaps, better assessment of the human cancer risk due to low-level chemical exposures. In the present study, we have used AMS to ascertain whether PhIP is a human colon cancer risk factor by determining bioavailability to the colon in humans and formation of adducts with DNA and protein in this tissue following a dietary-relevant dose. Furthermore, we have investigated adduct formation with albumin, Hb, and WBC DNA in peripheral blood as potential biomarkers for use in molecular epidemiology studies.

Materials and Methods

Chemicals. [2- 14 C]PhIP (see Fig. 1 for position of radiolabel) was obtained from Toronto Research Chemicals (North York, Ontario, Canada). [14 C]PhIP used in this study was repurified by HPLC. After repurification, the chemical purity was >99%, and the radiopurity was >95%. Qiagen tip-500 anion exchange columns were obtained from Qiagen (Valencia, CA). Proteinase K, RNase A, and RNase T₁ were obtained from Sigma Chemical Co. (St. Louis, MO). Affi-Gel Blue Gel and columns, protein assay reagent, and BSA standard were purchased from Bio-Rad Laboratories (Hercules, CA). Slide-A-Lyzer dialysis cassettes were obtained from Pierce Chemical Co. (Rockford, IL). Centriplus concentrators were purchased from Amicon, Inc. (Beverly, MA). Nucleon BACC2 kits were obtained from Amersham Life Science (Buckinghamshire, United Kingdom). All other chemical reagents were of analytical grade.

Human Protocol. The protocol for this study was approved by the human subjects review boards at Lawrence Livermore National Laboratory and at the University of Arkansas Medical School Hospital and the J. L. McClellan Memorial Veterans Administration Medical Center in Little Rock, AR. Human volunteers were recruited at University of Arkansas Medical School Hospital and the J. L. McClellan Memorial Veterans Administration Medical Center. All human volunteers participating in the study were undergoing surgery to remove colon carcinomas and gave informed consent prior to enrollment.

[14 C]PhIP was packaged in gelatin capsules containing lactose filler and then administered p.o. to volunteers, who were not required to fast prior to capsule administration. Subjects 1 and 2 received a dose of 70 μ g of [14 C]PhIP per person (specific activity, 56.0 mCi/mmol), and subjects 3–5 received a dose of 84 μ g [14 C]PhIP (specific activity, 41.8 mCi/mmol). The differences in the amounts and the specific activities of

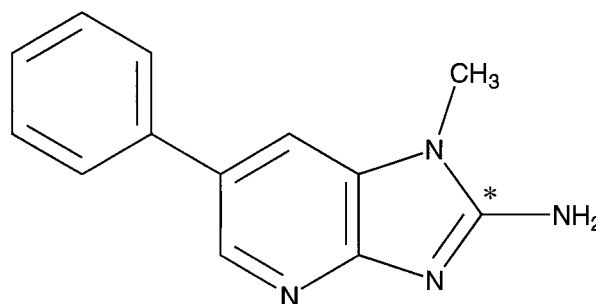


Fig. 1. Structure of PhIP and the position of the carbon-14 isotope label (*).

[14 C]PhIP given to the subjects are a result of preparing the capsules on two different occasions from two batches of [14 C]PhIP. For reference, the dose of PhIP the subjects in this study received is approximately equivalent to eating 175 g of very well-done chicken (7), and the radioactive dose was <1% of an individual's yearly exposure to background sources of radiation.

At various times up to 24 h after dosing, blood samples (30 ml) were collected and separated into plasma, RBCs, and buffy coat (consisting of WBCs and platelets) by centrifugation. Forty-eight to 72 h after dosing, the volunteers underwent surgery in which tissue samples not required for diagnosis or staging were collected and frozen. This dosing time was chosen so that blood sample collection would be completed prior to surgery and administration of associated medications, which could affect plasma kinetics. Control tissue samples were collected from subjects who were not dosed with [14 C]PhIP.

Albumin Extraction. Plasma was dialyzed for 48 h against two changes of 0.1 M KCl, 0.05 M Tris (pH 7; buffer A). To avoid any cross-contamination with carbon-14, each sample was dialyzed in a separate 250-ml disposable beaker. Albumin was extracted from dialyzed plasma using Affi-Gel Blue Gel columns packed with 5 ml gel/ml plasma to be purified. Columns were equilibrated with buffer A, the plasma was added to the columns, and then washed with two column volumes of buffer A. The albumin was eluted with 10 ml of 1.5 M KCl, 0.05 M Tris (pH 7.0), and samples were concentrated using centriplus concentrators. The albumin concentrations of the samples were determined using Bio-Rad protein assay reagent and BSA standards. Samples were then prepared for analysis by AMS.

Hb Extraction. RBCs were lysed with an equal volume of distilled water and centrifuged at $3300 \times g$ for 15 min. Samples were then dialyzed for 48 h against two changes of 0.25 M sodium phosphate buffer (pH 7.25). To avoid cross-contamination of carbon-14 between samples, each sample was dialyzed in a separate disposable beaker. After dialysis, the lysate was added dropwise to 15 ml of ethanol with vortex mixing to precipitate Hb. The Hb was then sequentially washed with ethanol:ether (80:20 v/v), ethanol:ether (25:75 v/v), and ether. The Hb samples were air dried and analyzed by AMS. This procedure does not separate heme from globin; hence, binding detected using AMS may have occurred to either of these two components of Hb.

Extraction of WBC DNA. DNA was extracted from the buffy coat samples using a Nucleon BACC2 kit, and the protocol was supplied by the manufacturers. Briefly, the buffy coat samples were resuspended in 1 ml of Reagent A and centrifuged at

Table 1 Human subject sex, age, and body weight

Subject number	Sex	Age	Body weight (kg)	Height (cm)
1	Male	79	75	180
2	Male	72	130	158
3	Male	80	83	178
4	Male	44	95	175
5	Male	74	70	172

1300 × *g* for 5 min, and the supernatant was discarded. Two ml of Reagent B were added to the pellets, and samples were incubated at 37°C for 1–2 h until they were completely dissolved. The samples were transferred to a 15-ml polypropylene centrifuge tube; 0.75 μg of RNaseT₁ was added and then incubated at 37°C for 30 min. Five hundred μl of 5 M sodium perchlorate were then added to the samples and mixed, followed by the addition of 2 ml of chloroform. Samples were mixed again; 300 μl of Nucleon resin were added to the samples and then centrifuged at 1300 × *g* for 3 min. The upper aqueous phase was transferred to a centrifuge tube, and the DNA was precipitated with two volumes of ice-cold ethanol. Pellets were washed once with 70% (v/v) ethanol and dissolved in distilled water. The concentration of DNA was calculated by measuring the absorbance at 260 nm (*A*₂₆₀ nm), assuming an absorbance value of 1.0 is equal to a DNA concentration of 50 μg/ml. DNA purity was determined from the *A*₂₆₀ nm:*A*₂₈₀ nm ratio. All DNA used in this study had a ratio of 1.7–1.8.

DNA and Protein Extraction from Tissue. DNA was extracted from colon tissue using Qiagen DNA extraction columns, as described previously (23). The quantity of DNA and purity were calculated as described above. DNA with a *A*₂₆₀ nm:*A*₂₈₀ nm ratio of 1.7–1.8 was used in this study.

To extract protein from the colon tissue samples, 1–2-g aliquots of tissue were homogenized and added to tubes containing 5 ml of lysis buffer [4 M urea, 1% (v/v) Triton X-100, 10 mM EDTA, 100 mM NaCl, 10 mM Tris-HCl (pH 8.0), and 10 mM DTT]. Samples were placed in a 37°C shaking water bath overnight. To remove any incompletely lysed tissue, samples were centrifuged at 3000 × *g* for 15 min. Five hundred μl of 70% (v/v) perchloric acid were added to the supernatant to precipitate protein. Samples were vortexed and centrifuged at 3000 × *g* for 15 min. The resulting pellets were then washed with 5% (v/v) perchloric acid, twice with 50% (v/v) methanol, and twice with 1:1 (v/v) ether:ethanol. Pellets were left to air dry overnight. Two ml of 0.1 M potassium hydroxide were added to each sample prior to preparing the samples for AMS analysis.

AMS Analysis. The carbon-14:carbon-13 ratios of tissue, protein, DNA, Hb, and albumin samples were determined by AMS, as described previously (20–26). Before analysis, two mg of tributyrin were added to each DNA sample to provide the carbon content necessary for efficient graphitization. Aliquots of 2–10 mg of wet-weight tissue, tissue protein, albumin, or Hb were graphitized without addition of tributyrin. The carbon-14:carbon-13 ratios were converted to pg PhIP/g of tissue or protein or DNA adduct levels following the subtraction of the carbon-14 contribution from any added tributyrin and control samples.

Statistical Analysis. Data represent the mean ± the greatest of the SD or the AMS measurement error. Each sample was independently prepared and measured up to six times, depending upon sample size. Mean values were compared using the Student's *t* test, available with Microsoft Excel 5.0 (Microsoft,

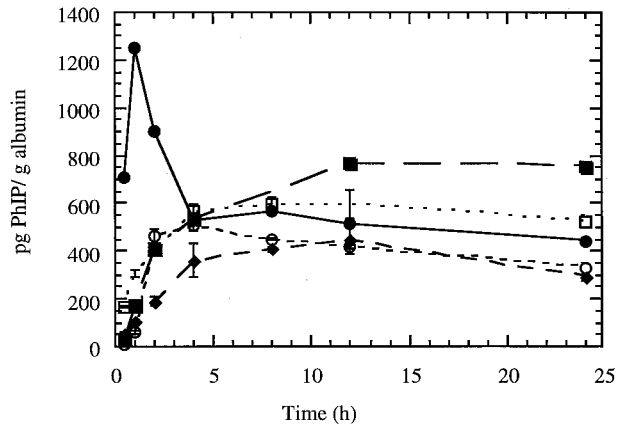


Fig. 2. PhIP-albumin adduct levels in human subjects 1 (●), 2 (■), 3 (◆), 4 (□), and 5 (○). Data represent the mean of three replicate analyses; bars, greatest of the SD or the AMS measurement error.

Redmond, WA), and were considered statistically different with *P* < 0.05.

Results

The ages and body weights of the five human subjects are shown in Table 1. They were between the ages of 44 and 80 and of average weight and height, with the exception of subject 2. Each subject was also taking a variety of medications during the study period (data not shown).

Albumin and Hb Adduct Formation. The levels of covalent [¹⁴C]PhIP binding to albumin and Hb in peripheral blood of the five human subjects for 24 h after exposure to [¹⁴C]PhIP are shown in Figs. 2 and 3. In all cases, PhIP-albumin binding was significantly greater than PhIP-Hb binding (*P* < 0.005), with the mean adduct level per unit dose in the five human subjects at the 24-h time point of 11.52 ± 9.29 and 0.22 ± 0.13 fmol PhIP/g protein per pmol PhIP/kg body-weight for albumin and Hb, respectively.

Comparison of the albumin and Hb adduct profiles over time demonstrated interindividual differences. The albumin adduct levels of human subjects 2, 3, 4, and 5 increased gradually with time and then reached a plateau by 12 h. This trend was also seen for the Hb adduct levels of subjects 2 and 3. However, both albumin and Hb adduct levels in the blood of human subject 1 and the Hb adduct levels of subjects 4 and 5 demonstrated a rapid increase, followed by a rapid decline prior to stabilizing by 12 h.

Adduct Formation with WBC DNA. Adduct levels with WBC DNA extracted from buffy coat samples are shown in Fig. 4. In all cases examined, adduct levels peaked 2–4 h after exposure to [¹⁴C]PhIP, with levels up to 1.4 adducts/10⁹ nucleotides. However, after peak adduct levels were achieved, the adduct levels declined significantly over 24 h.

Tissue, DNA, and Protein Adducts. The level of [¹⁴C]PhIP and its metabolites in tissues removed during surgery, as well as PhIP-protein and -DNA adduct levels in these tissue samples are illustrated in Table 2. Both tumor and normal colon tissue from each subject were available for analysis, in addition to cecum and ileum tissue samples from subject number 1 and ileum from subjects 2 and 4.

[¹⁴C]PhIP and its metabolites were detectable in all tissue samples up to 72 h after exposure. [¹⁴C]PhIP was also meas-

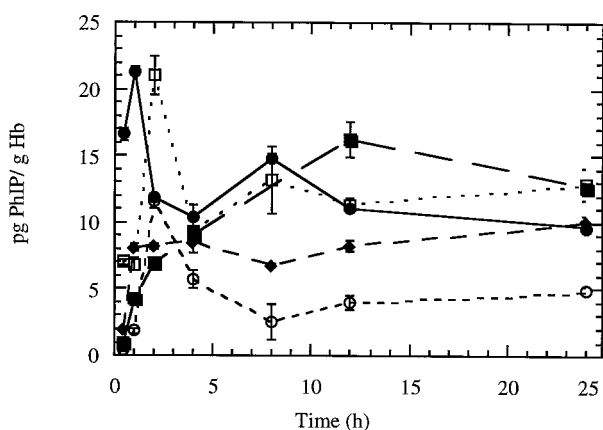


Fig. 3. PhIP-Hb adduct levels in human subjects 1 (●), 2 (■), 3 (◆), 4 (□), and 5 (○). Data represent the mean of three replicate analyses; bars, greatest of the SD or the AMS measurement error.

ured covalently bound to protein and DNA in those samples. Interindividual differences were observed in the amount of PhIP in the tissue and the protein and DNA adduct levels of the five subjects. However, there was no correlation between the levels of PhIP in the tissue and the PhIP protein or DNA adduct levels in that tissue. However, DNA adduct levels in tumor tissue were significantly greater than those from the normal tissue ($P = 0.04$).

Discussion

This is a pilot study using AMS to examine PhIP bioavailability, and adduct formation with DNA and protein in five human volunteers after the administration of a defined dietary-relevant dose of [^{14}C]PhIP. Despite the fact that the subjects were undergoing surgery to remove tumors, the data presented are crucial to assess whether PhIP at dietary levels of exposure may be involved in the initiation of colon cancer in humans. Moreover, this study provides a means to validate biomarkers for use in molecular epidemiology studies.

PhIP adducts with protein and DNA were measured by AMS. Although AMS analyses offer no structural information, plasma samples were extensively dialyzed, Hb, and protein from tissues repeatedly solvent extracted and DNA extensively purified to ensure that only covalently bound PhIP was analyzed. Although the major DNA adduct formed by PhIP in laboratory animals and a minor adduct with albumin formed *in vitro* have been characterized (27–29), investigations to determine the nature of the binding detected in the human samples were beyond the scope of this study and is presently under way.

In a previous human study with the HCA MeIQx, we demonstrated that adducts with albumin provide a useful biomarker of exposure and bioactivation (25). However, studies conducted with PhIP *in vitro* with rat serum albumin and *in vivo* in rats after high-dose exposures have indicated that PhIP-protein adducts in blood are unstable and therefore unlikely to be useful biomarkers (29, 30). However, laboratory animal and *in vitro* models may not accurately predict the human response. Fortunately, the technique of AMS has allowed us to conduct low-dose studies in humans.

Our results indicate that in humans PhIP forms adducts with both albumin and Hb in the peripheral blood within 30 min of exposure. In four of five of the subjects, albumin adduct

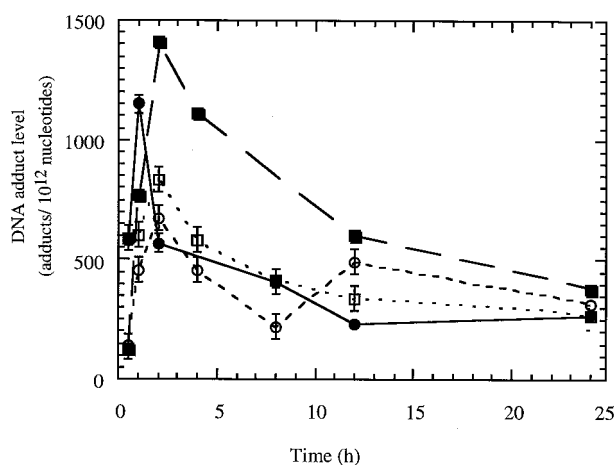


Fig. 4. PhIP-DNA adduct levels in WBCs of human subjects 1 (●), 2 (■), 4 (□), and 5 (○). WBC DNA was not available from subject 3. Data represent one measurement per sample; bars, the AMS measurement error.

levels did not decline but increased steadily, reaching a plateau within 12–24 h. In contrast, albumin adduct levels of subject 1 showed an initial peak of binding, with the adduct level declining by 65% by 24 h. A similar trend was also seen in Hb adduct formation in three of five of the subjects, in which the mean adduct level 24 h after dosing was ~50% of the peak adduct levels. This initial peak of binding may be due to unstable adducts or the presence of noncovalently bound PhIP in high-affinity binding sites. Although the blood samples were collected prior to surgery and administration of anesthetics, it is also possible that it may be the result of the preoperative treatment and medications taken by the subjects. Twenty-four h after exposure, PhIP-albumin adduct levels were ~50-fold higher than Hb adduct levels. These results indicate that albumin adducts provide a more sensitive biomarker of exposure to PhIP than Hb adducts and is similar to data obtained in humans after exposure to [^{14}C]MeIQx (25).

Analysis of DNA extracted from WBCs demonstrated that PhIP also formed high levels of DNA adducts in the blood. Previous studies conducted at high dose in rats demonstrated that PhIP forms DNA adducts in WBCs, although peak adduct levels were measured 2 days after dosing (31). In this human study, the highest DNA adduct levels in WBCs were attained within 4 h of exposure; however, in all of the subjects examined, the adduct levels declined rapidly over 24 h. Although these results suggest that DNA adducts in the blood may not be suitable biomarkers, they indicate that genotoxic metabolites are rapidly formed and enter the circulation. These bioactive metabolites may include HNOH-PhIP and/or the acetate ester of HNOH-PhIP, which have been shown in rats to be transportable in the bloodstream, resulting in DNA adduct formation in extrahepatic sites, such as the colon (32).

Analysis of tissue samples removed during surgery demonstrated that [^{14}C]PhIP and its metabolites could be detected in colon, cecum, and ileum tissue up to 72 h after exposure. Although the number of samples is small, there was no significant difference measured in the tissue available doses either between colon, cecum, and ileum or between tissue from normal colon and colon tumor. Interindividual variation was seen in the levels of bioavailability in the colon samples. However, in some cases, replicate analysis of up to six separate aliquots

Table 2 Tissue-available dose of PhIP and its metabolites and PhIP-DNA and -protein adduct levels after oral administration of a single dose of [¹⁴C]PhIP

Human subject no.	Organ	Normal or tumor tissue	Tissue (pg PhIP/g tissue)	Protein (pg PhIP/g protein)	DNA (Adducts/10 ¹² nucleotides)
1	Colon	Normal	42.0 ± 7.9 (3) ^a	67.4 ± 1.4 (2)	44.5 ± 1.9 (2)
	Colon	Tumor	40.5 ± 0.6 (1)	NA ^b	NA
	Ileum	Normal	61.0 ± 9.8 (3)	NA	103.1 ± 0.6 (2)
	Cecum	Normal	19.3 ± 0.4 (1)	52.8 ± 0.6 (1)	54.3 ± 2.6 (2)
	Cecum	Tumor	47.2 ± 0.5 (1)	67.5 ± 1.1 (1)	82.8 ± 29.6 (2)
2	Colon	Normal	114.6 ± 1.0 (1)	108.2 ± 1.6 (1)	69.6 ± 3.6 (3)
	Colon	Tumor	122.2 ± 1.3 (1)	NA	130.8 ± 3.9 (1)
	Ileum	Normal	311.3 ± 3.0 (1)	440.5 ± 4.6 (1)	194.3 ± 37.7 (3)
3	Colon	Normal	82.4 ± 1.0 (1)	98.3 ± 1.1 (1)	135.0 ± 16.1 (3)
	Colon	Tumor	82.9 ± 72.9 (4)	167.1 ± 1.8 (1)	308.4 ± 11.0 (1)
4	Colon	Normal	121.8 ± 124.9 (6)	183.1 ± 49.0 (3)	34.9 ± 24.9 (2)
	Colon	Tumor	56.2 ± 15.3 (6)	82.2 ± 42.7 (3)	90.3 ± 66.3 (3)
	Ileum	Normal	92.2 ± 64.5 (6)	94.5 ± 8.5 (3)	83.4 ± 11.6 (3)
5	Colon	Normal	58.1 ± 19.0 (3)	66.5 ± 0.8 (1)	75.1 ± 16.2 (1)
	Colon	Tumor	96.6 ± 4.1 (3)	102.5 ± 1.3 (1)	174.1 ± 16.5 (1)

^a Numbers in parentheses, the number of replicate samples measured.

^b NA, not analyzed because of insufficient sample.

of whole tissue revealed large variation due to unequal distribution of PhIP within the specimens.

The main aim of this study was to determine whether PhIP-DNA adducts could be detected in human colon tissue following defined dietary-relevant PhIP exposures. Previous work by Friesen *et al.* (33), in which human tissue DNA was analyzed by gas chromatography/electron capture mass spectrometry and ³²P-postlabeling, demonstrated that PhIP-DNA adducts are formed in human colon, although levels were only detectable in two of six samples examined. Consistent with this finding, AMS analysis of DNA extracted from the colon tissue samples demonstrated the presence of covalently bound [¹⁴C]PhIP up to 72 h after exposure. The mean DNA adduct level per unit dose at the time of surgery in the normal colon tissue was 1.13 ± 0.67 fmol PhIP/g DNA per pmol dose/kg body weight, which was significantly lower than the tumor tissue ($P = 0.05$). These data indicate that the tumor tissue may either have a greater capacity for metabolic activation of PhIP, decreased capacity for detoxification of bioactive metabolites, or decreased DNA repair capacity. Although it is difficult to determine the consequence of PhIP-DNA adduct formation in the colon, we have demonstrated that in humans, bioactive PhIP metabolites reach the DNA in the colon, a target site for PhIP carcinogenesis in rats (11). However, adduct levels in the colon were not significantly different from the cecum and ileum samples analyzed. Similarly, high PhIP-DNA adduct levels were measured in these organs in rats dosed with PhIP (30, 31). Although the cecum and small intestine have not been reported as targets for PhIP-induced carcinogenicity in long-term bioassays in normal rats, chronic PhIP administration resulted in the induction of tumors in these organs in Nagase albuminemic mutant strain rats devoid of albumin (34).

Consistent with the formation of DNA adducts, protein adducts were also detected in the tissue samples. Protein adducts are normally used as a surrogate marker of DNA adduct formation, because protein adducts are not subject to repair and protein is more plentiful than DNA. However, it is not known whether protein and DNA adducts of PhIP are formed via common metabolic pathways. Extraction of DNA and protein from separate aliquots of tissue and comparison of DNA and protein adduct levels did not show a correlation, as might be expected if they were formed via common reactive metabolites. However, PhIP was not homogeneously distributed in the tis-

suess, and the samples were collected many hours after exposure. In this time, the cell turnover and DNA repair may have significantly reduced the adduct levels detected, making interpretation difficult.

The data collected in this study show interindividual variation in PhIP bioavailability in the tissue, as well as protein and DNA adduct formation in the tissue and blood after exposure to the dietary carcinogen PhIP. Such variation did not appear related to differences in body weight but may be due to the health status of the subjects, medications, or linked to metabolic polymorphisms in enzymes thought to be involved in the metabolic activation of PhIP.

Studies have indicated considerable interindividual variability in the CYP1A2-mediated metabolic activation step in humans (35), with apparent fast, intermediate, and slow phenotypes (36). Although genetic polymorphisms affecting enzyme activity have not been reported, CYP1A2 can be induced by cigarette smoking (37) and meat cooked at high temperature (38) and may be modulated by cytochrome P450 1A1 and glutathione *S*-transferase M1 genotypes (39). Polymorphisms affecting enzyme activity have also been identified for phenol sulfotransferases (40), NAT1 and NAT2 (reviewed in Ref. 41). Therefore, as a result of PhIP exposure, people with rapid phenotypes should form higher PhIP-adduct levels and might be at greater risk of developing cancer than people with slow phenotypes (4). This hypothesis is supported by epidemiological evidence that shows that people with fast CYP1A2 and fast NAT2 phenotypes who are exposed to dietary heterocyclic amines are at greater risk of colon cancer (4). Phenotyping of CYP1A2 and NAT2 was performed on the five subjects in this study (data not shown). Thus far, the CYP1A2 and NAT2 phenotypes do not appear to relate to the PhIP-DNA or protein adduct levels in either the blood or colon, although presently the sample population is too small to perform any meaningful correlations.

In summary, this study has shown for the first time that a well-defined dietary-relevant dose of [¹⁴C]PhIP is bioavailable to the colon tissue and forms DNA and protein adducts in the colon and blood. The extremely low level of detection of carbon-14-labeled compounds afforded by AMS makes such human work possible and is leading us to a better understanding of the risks posed by exposure to environmental carcinogens.

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