

### Short Communication

## Urinary Mutagenicity and Colorectal Adenoma Risk

Ulrike Peters,<sup>1</sup> David M. DeMarini,<sup>2</sup> Rashmi Sinha,<sup>1</sup>  
Lance R. Brooks,<sup>2</sup> Sarah H. Warren,<sup>2</sup>  
Nilanjan Chatterjee,<sup>1</sup> and Nathaniel Rothman<sup>1</sup>

<sup>1</sup>Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Department of Health and Human Services, Bethesda, Maryland and

<sup>2</sup>Environmental Carcinogenesis Division, United States Environmental Protection Agency, Research Triangle Park, North Carolina

#### Abstract

We investigated urinary mutagenicity and colorectal adenoma risk in a clinic-based, case-control study of currently nonsmoking cases ( $n = 143$ ) and controls ( $n = 156$ ). Urinary organics were extracted by C18/methanol from 12-h overnight urine samples, and mutagenicity was determined in *Salmonella* YG1024 +S9 (Ames test). Adenoma risk was 2.4-fold higher in subjects in the highest versus the lowest quintile of urinary mutagenicity (95% confidence interval = 1.1–5.1). Combining urinary mutagenicity with intake of meat-derived mutagenicity (from our earlier analysis) resulted in a 5.6-fold increase in adenoma risk (95% confidence interval = 2.2–13.9, comparing the highest with the lowest quintile). In our study population, diet may have contributed to mutagenic exposure, which was positively associated with colorectal adenoma risk.

#### Introduction

We have shown previously that consumption of red meat cooked at high temperatures, as well as the estimated intake of meat-derived mutagenicity assessed by a food frequency questionnaire that included questions on the level of doneness and pictures of meat module, were associated with an increased risk of colorectal adenomas (1, 2). Such results are compatible with the hypothesis that exposure to HCAs<sup>3</sup> from meats cooked at high temperatures is associated with an increased risk of colorectal adenomas (3). These studies have relied on a questionnaire-based dietary assessment to estimate the exposure levels of the subjects to meat cooked at high-temperature, meat-derived mutagenicity, and HCAs. However, a biological measurement of exposure would be a useful adjunct to questionnaire data and might eventually have value as a direct biomarker of exposure and/or risk. In this regard, we used the Ames test to measure the ability of urine collected from cases and controls to mutate the DNA of *Salmonella* YG1024 +S9 as an integrated

biomarker of the presence of various mutagenic substances in the urine. The association of the biomarker urinary mutagenicity with the consumption of cooked meat, among several other types of exposures, such as smoking, benzidine, chemotherapy, and so forth, has been known for 20 years (4–6). Using our case-control study mentioned above, we report here on urinary mutagenicity and risk for colorectal adenoma among current nonsmokers.

#### Materials and Methods

**Study Population and Design.** This case-control study has been described in detail elsewhere (1, 7). Briefly, the study was performed at the National Naval Medical Center (Bethesda, MD), which serves primarily military officers and their families. Cases had histologically confirmed colorectal adenomas diagnosed by colonoscopy (86%), sigmoidoscopy (13%), or sigmoidoscopy and colonoscopy (1%). Controls had negative sigmoidoscopies and were frequency matched to the cases by age ( $\pm 5$  years) and sex. Cases were enrolled during a return visit after histological confirmation of the adenomas, and controls were enrolled during sigmoidoscopy examination. The participants were residents of the study area, were between the ages of 18 to 74 years, and had never been diagnosed with Crohn's disease, ulcerative colitis, familial polyposis syndrome, or cancer except nonmelanoma cancer of the skin. Approval for the study was given by the institutional review board of the National Cancer Institute, the National Naval Medical Center, and the Human Subjects Research Review Official of the United States Environmental Protection Agency. Informed consent was obtained from all participants.

Two hundred eighty-nine cases and 314 controls were identified as eligible. Of these, 241 cases (84%) and 231 controls (74%) participated. After excluding five participants with implausible dietary data, 239 cases (146 new and 93 recurrent adenomas) and 228 controls remained in the study. Twelve-hour overnight urine samples were collected from all subjects (after 6:00 p.m. until 6:00 a.m., or until the first morning void, whichever was later). Subjects received written and oral information describing urine collection and a collection kit that included a cooler with ice packs to store the urine. Urine samples were kept in the cooler until processed on the same day (92% of samples) or the following day later (8% of samples; no preservative was added to the collected urine). Almost a quarter of the way through the study, additional vials of urine were stored for use in urine mutagenicity analyses. There were 163 cases and 159 controls with suitable amounts of urine available for analysis. Because we were interested in dietary exposures, we eliminated subjects who had smoked any time during the week before collection of urine. In total, we included 143 cases and 156 controls, all of whom were current nonsmokers (Table 1). At a home visit subsequent to enrollment, 12-h overnight urine samples were collected. The time between enrollment and home visit was 2.6 months for cases and 3.3 months for controls. Data on demographics, medical history, diet, and other risk factors were obtained at the same home visit. Intake of

Received 2/28/03; revised 7/18/03; accepted 7/29/03.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**Requests for reprints:** Ulrike Peters, Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, DHHS, 6120 Executive Boulevard, EPS #3024, Rockville, Maryland 20892-7273. E-mail: petersu@mail.nih.gov.

<sup>3</sup> The abbreviations used are: HCA, heterocyclic amine; rev, revertant(s); OR, odds ratio; CI, confidence interval; NSAID, nonsteroidal anti-inflammatory drug.

Table 1 Distribution of demographic and risk factors

Variable <sup>a</sup>	Cases	Controls
<i>n</i>	143	156
Age (yr)	59.8 ± 0.74	59.8 ± 0.71
Female (%)	21.0	43.6
Non-Hispanic white (%)	90.0	87.9
Education, graduate or higher degree (%)	40.9	50.3
Family history of colorectal cancer (%)	17.1	10.9
Body mass index (kg/m <sup>2</sup> )	27.0 ± 0.35	26.4 ± 0.34
Physical activity (h/wk)	7.8 ± 0.59	8.0 ± 0.56
Use of NSAIDs (%)	56.8	67.1
Cigarette smoking (packs/yr)	17.7 ± 2.11	12.4 ± 2.01
Total folate intake (mcg/day)	497 ± 22.5	521 ± 21.51
Total meat (g/day)	102 ± 4.36	94.2 ± 4.17
Red meat (g/day)	47.0 ± 3.69	58.1 ± 3.23
Well- and very-well-done red meat (g/day)	13.3 ± 1.21	8.1 ± 1.16
Meat-derived mutagenicity (1000 rev/day)	5.2 ± 0.37	3.5 ± 0.36
Urinary mutagenicity		
rev/ml	9.1 ± 0.85	6.4 ± 0.81
rev × 1000/overnight (12 h) urine	10.2 ± 1.19	7.7 ± 1.14

<sup>a</sup> All values are adjusted for gender; values are mean ± SE or % if indicated in parentheses.

meat-derived mutagenicity was estimated by a database that we developed for estimating the mutagenicity of variously cooked meats (1, 2). Briefly, this consisted of a food-frequency questionnaire involving the use of photographs of meats cooked to varying degrees and to which subjects could refer to describe the amount and level of doneness of the meats that they consumed.

**Urinary Mutagenicity.** Urinary organics were extracted as described previously (5). Briefly, urine was passed through C18 resin, and the organics were eluted by methanol and then solvent-exchanged into DMSO at 150× for bioassay. The extracts were evaluated once in single plates/dose at 0.15-, 0.3-, 0.75-, 1.5-, 3-, 7.5-, and 15 ml-equivalent/plate in the standard *Salmonella* mutagenicity plate-incorporation assay in the presence of aroclor-induced rat liver S9 mix (2 mg of S9 protein/plate; Ref. 5). We used strain YG1024, which is derived from the frameshift strain TA98 (*hisD3052*,  $\Delta$ *uvrB*, *rfa*, pKM101) and contains acetyltransferase activity, making it particularly sensitive to aromatic amines and heterocyclic amines (5). Mutagenic potencies, expressed as rev/ml, were calculated from the slope of the regression over the linear portion of the dose-response curves. These values were multiplied by the number of milliliters of urine collected during the 12-h period to give the number of rev/12 h. The coefficient of variation (reproducibility of assay) was 45.8% for mutagenicity for these unhydrolyzed urine samples. In addition to the generally used unhydrolyzed urine, we also hydrolyzed portions of the original urine samples as described previously (5) and tested them for mutagenicity. The purpose of the acid hydrolysis was to deconjugate mutagens from glucuronide. However, the coefficient of variation for mutagenicity of these hydrolyzed samples was 82.5%. Because of this high value, data from only unhydrolyzed urine were used in this study. Controls consisted of DMSO (100  $\mu$ l/plate), C18 resin blanks prepared by passing 80 ml of glass-distilled deionized water instead of urine through the columns (15 ml-eq/plate), and 2-aminoanthracene at 0.5  $\mu$ g/plate.

**Data Analysis.** First, we applied the *t* statistic to test whether the means of the urinary mutagenicity values were significantly different between cases with recurrent adenoma and those who

were newly diagnosed. Because we did not find any significant differences, we combined both subgroups for further analysis. Urinary mutagenicity and risk for colorectal adenoma were analyzed as ORs and 95% CIs by unconditional logistic regression. Urinary mutagenicity and meat-derived mutagenicity were coded as quintiles according to the distribution among the controls only. To combine measures of urinary mutagenicity and meat-derived mutagenicity, we standardized both measures, because these measures have different units (rev/ml and rev/day; Fig. 1). We standardized both continuous variables (urinary mutagenicity and meat-derived mutagenicity) according to the mean and SD of the controls of each variable [(variable - mean) ÷ SD], before adding the standardized variables together.

Because urinary mutagenicity is an integrated measure of mutagenic exposure, we did not adjust for meat intake. Instead, we adjusted for the following potential confounders: age, gender, family history of colorectal cancer, family history of cancer, education, ethnicity, use of NSAIDs, past smoking (pack-years), dietary and supplemental intake of vitamins C, A, E,  $\beta$ -carotene, and folate. Trend tests were calculated using continuous variables. Because the variables were log-normally distributed, with most subjects in the low range, we used the Spearman correlation to investigate the association between

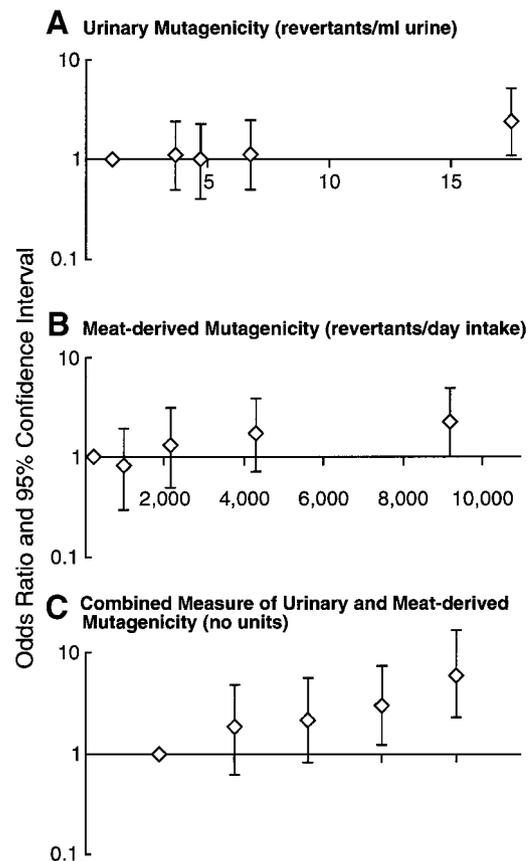


Fig. 1. Association between risk for colorectal adenoma and urinary mutagenicity (A), estimated intake of meat-derived mutagenicity (B), and both mutagenicity measurements combined (C). OR and 95% CI were adjusted for age, gender, family history of colorectal cancer, family history of cancer, education, ethnicity, use of NSAIDs, smoking, intake of vitamins C, A, E,  $\beta$ -carotene, and folate.

urinary mutagenicity and estimated intake of meat-derived mutagenicity.

## Results and Discussion

The distribution of risk factors has been described previously (1, 2, 7). Briefly, cases were slightly older, more likely to be male, and more likely to have a family history of colorectal cancer compared with controls (Table 1). Relative to controls, the cases also smoked more, used NSAIDs less often, and had a lower intake of folate. Their intake of total meat, red meat, well-done red meat, and meat-derived mutagenicity was higher than controls (Refs. 1, 2, 7; Table 1).

The mean urinary mutagenicity was higher in cases than in controls, regardless of whether mean urinary mutagenicity was expressed as rev/ml or rev/12-h overnight urine sample (Table 1). Urinary mutagenicity (rev/ml) was associated with a significant increase in risk for colorectal adenomas based on a comparison of the highest with the lowest quintile of urinary mutagenicity (OR = 2.4; 95% CI = 1.1–5.1; Fig. 1). A similar increase in risk was obtained when using total rev in the 12-h overnight urine (OR = 1.8; 95% CI = 0.8–3.8; comparing the highest with the lowest quintile).

We have shown previously, in a study of benzidine-exposed workers, that the levels of urinary mutagenicity can correlate strongly with the levels of other genotoxic endpoints, such as mutagenic urinary metabolites and urothelial cell DNA adducts (6). Our finding here that urinary mutagenicity predicts risk for colorectal adenoma is consistent with our previous findings of an association between urinary mutagenicity and genotoxic endpoints (6) and those of other (8).

Because the participants in this study were predominantly white-collar workers employed for or retired from the United States Navy and were current nonsmokers, occupational exposure or active smoking were unlikely to be an important source of mutagenic exposure for this population. Given these study population characteristics, and because consumption of meat cooked at high temperatures is known to increase the levels of urinary mutagenicity (9, 10), we infer that most of the mutagenic activity measured in this study population was likely attributable to dietary sources. However, other exposures, such as passive smoking, may have made a minor contribution to the measured urinary mutagenicity.

In support of this inference, we note our previous finding in this same case-control study that high intake of meat-derived mutagenicity is a risk factor for colorectal adenoma (2). In addition, the levels of urinary mutagenicity among 85.3% of the control subjects in this study were low and similar to the levels of urinary mutagenicity detected among subjects in a metabolic study who were fed red meat fried at low temperatures (9). This may explain why we found no association between adenoma risk and the four lowest quintiles of urinary mutagenicity; probably mutagenic exposure was only high enough in the fifth quintile to have an effect on adenoma risk.

A likely chemical class responsible for the observed urinary mutagenicity is HCAs, which are produced when meats are cooked at high temperatures (9) and are detected particularly well by the strain of *Salmonella* used in this study, YG1024 (11). In addition, HCAs produce colon tumors in rodents, as well as tumors at other sites in rodents, that are associated commonly with diet in human cancer studies, such as liver, breast, and lymphoma (12).

As shown previously for this case-control study (2), the estimated intake of meat-derived mutagenicity was associated with a significantly increased risk for colorectal adenoma, when

comparing the highest with the lowest quintile of meat-derived mutagenicity (OR = 2.2; 95% CI = 1.0–4.9; Fig. 1). A goal of the present study was to see whether a direct measure of mutagenic exposure (urinary mutagenicity) could be a useful adjunct to this questionnaire-based exposure estimate. Comparison of the previously determined meat-derived mutagenicity intake (2) with the present urinary mutagenicity data showed no correlation (Spearman,  $r = 0.09$ ). Because both of these measures capture different aspects of the mutagenicity activity, we did not expect to find a high correlation; however, such a low correlation was unexpected. We reasoned that the combination of the two measurements would categorize the exposure more accurately because they measure intake and excretion of mutagenic activity. Combining the measurements of urinary mutagenicity and estimated intake of meat-derived mutagenicity gave a stronger positive association with risk for colorectal adenomas than did either variable separately, when comparing the highest with the lowest quintile of combined mutagenicity (OR = 5.6; 95% CI = 2.3–13.9; Fig. 1).

Because controls were confirmed of not having an adenoma by sigmoidoscopy only, it is possible that some controls had undetected right-sided adenoma. However, restricting our analysis to left-sided cases resulted in similar risk estimates (data not shown), suggesting that it was unlikely that the misclassified controls influenced the finding substantially. A potential bias in this study was the determination of exposure by urinary mutagenicity after diagnosis of the outcome, colorectal adenoma. However, in this study, we used colorectal adenoma as an intermediate biomarker of colorectal cancer. It is less likely that urinary mutagenicity and dietary assessment were affected by case status in a case-control study of this early neoplastic lesion (adenoma), which, in addition, was removed before enrollment (urine was collected, on average, 2.6 months after enrollment), than by case status in a study of carcinoma. Thus, the elevated urinary mutagenicity among the cases was most likely an indicator of exposure and not attributable to the presence of an adenoma. On the basis of the concept of the case-control study design, we assessed exposure after case diagnosis to reflect past exposure during the relative time of adenoma development. It is possible that cases may have changed their diet to a healthier diet with reduced meat intake after their diagnosis. Such dietary changes would have likely resulted in a reduced urinary mutagenicity in cases and would have attenuated the observed risk estimate toward the null. Furthermore, urinary mutagenicity is a short-term measure that reflects exposure <24 h before collection (13). Consequently, with one 12-h urine collection, we would have expected that the exposure of some of the subjects was misclassified, which would have likely attenuated the underlining risk estimate toward the null. Despite these potential biases toward the null and the limited sample size of the study, we found a positive association between urinary mutagenicity and colorectal adenoma. This interesting result requires independent confirmation and further evaluation of urinary mutagenicity as a potential biomarker, including intra-individual variability and improvement of reproducibility of the biomarker. However, a constraint is the relatively large sample size needed and the cost associated with the assay.

An estimate of meat-derived mutagenicity and a measure of urinary mutagenicity capture and characterize different aspects of mutagenic exposure, and combining them into an integrated measure can compensate for the limitations and complement the strengths of each measure. Urinary mutagenicity is a short-term measure of exposure to mutagens. In contrast, estimated intake of meat-derived mutagenicity is a

long-term measure, reflecting exposure of the last 12 months before diagnosis. Nonetheless, the intake of meat-derived mutagenicity is an estimate based on the ability of participants to recall food intake, cooking methods, and doneness level of meat. Thus, as posed in the "Introduction," a biological measure of exposure, such as urinary mutagenicity, is a useful adjunct to questionnaire data and may have value as a direct biomarker of exposure and/or risk for colorectal adenoma.

The results presented here support the view that exposure to mutagens is associated with risk for colorectal adenoma and suggest that diet may contribute to the mutagenic exposure in this study population.

### Acknowledgments

We thank Drs. Larry Claxton, Daniel Shaughnessy, Russell Owen, and Julian Preston for their helpful comments.

### References

1. Sinha, R., Chow, W. H., Kulldorff, M., Denobile, J., Butler, J., Garcia-Closas, M., Weil, R., Hoover, R. N., and Rothman, N. Well-done, grilled red meat increases the risk of colorectal adenomas. *Cancer Res.*, *59*: 4320–4324, 1999.
2. Sinha, R., Kulldorff, M., Chow, W. H., Denobile, J., and Rothman, N. Dietary intake of heterocyclic amines, meat-derived mutagenic activity, and risk of colorectal adenomas. *Cancer Epidemiol. Biomarkers Prev.*, *10*: 559–562, 2001.
3. Ishibe, N., Sinha, R., Hein, D. W., Kulldorff, M., Strickland, P., Fretland, A. J., Chow, W. H., Kadlubar, F. F., Lang, N. P., and Rothman, N. Genetic polymorphisms in heterocyclic amine metabolism and risk of colorectal adenomas. *Pharmacogenetics*, *12*: 145–150, 2002.
4. Baker, R., Arlauskas, A., Bonin, A., and Angus, D. Detection of mutagenic activity in human urine following fried pork or bacon meals. *Cancer Lett.*, *16*: 81–89, 1982.
5. DeMarini, D. M., Hastings, S. B., Brooks, L. R., Eischen, B. T., Bell, D. A., Watson, M. A., Felton, J. S., Sander, R., and L. Kohlmeier. Pilot study of free and conjugated urinary mutagenicity during consumption of pan-fried meats: possible modulation by cruciferous vegetables, glutathione *S*-transferase, and acetyltransferase-2. *Mutat. Res.*, *381*: 83–96, 1997.
6. DeMarini, D. M., Brooks, L. R., Bhatnagar, V. K., Hayes, R. B., Eischen, B. T., Shelton, M. L., Zenser, T. V., Talaska, G., Kashyap, S. K., Dosemeci, M., Kashyap, R., Parikh, D. J., Lakshmi, V., Hsu, F., Davis, B. B., Jaeger, M., and Rothman, N. Urinary mutagenicity as a biomarker in benzidine-exposed workers: correlation with urinary metabolites and urothelial DNA adducts. *Carcinogenesis (Lond.)*, *18*: 981–988, 1997.
7. Peters, U., McGlynn, K. A., Chatterjee, N., Gunter, E., Garcia-Closas, M., Rothman, N., and Sinha, R. Vitamin D, calcium, and vitamin D receptor polymorphism in colorectal adenomas. *Cancer Epidemiol. Biomarkers Prev.*, *10*: 1267–1274, 2001.
8. Talaska, G., Schamer, M., Skipper, P., Tannenbaum, S., Caporaso, N., Unruh, L., Kadlubar, F. F., Bartsch, H., Malaveiklle, C., and Vineis, P. Detection of carcinogen-DNA adducts in exfoliated urothelial cells of cigarette smokers: association with smoking, hemoglobin adducts, and urinary mutagenicity. *Cancer Epidemiol. Biomarkers Prev.*, *1*: 61–66, 1991.
9. Peters, U., Sinha, R., Bell, D. A., Rothman, N., Grant, D. J., Watson, M. A., Kulldorff, M., Brooks, L. R., Warren, S. H., and DeMarini, D. M. Urinary mutagenesis and fried red meat intake: Influence of cooking temperature, phenotype, and genotype of metabolizing enzymes in a controlled feeding study. *Environ. Mol. Mutagen.* 2003 (in press).
10. Doolittle, D. J., Rahn, C. A., Burger, G. T., Lee, C. K., Reed, B., Riccio, E., Howard, G., Passananti, G. T., Vesell, E. S., and Hayes, A. W. Effect of cooking methods on the mutagenicity of food and on urinary mutagenicity of human consumers. *Food Chem. Toxicol.*, *27*: 657–666, 1989.
11. Watanabe, M., Ishidate, M., Jr., and Nohmi, T. Sensitive method for the detection of mutagenic nitroarenes and aromatic amines: new derivatives of *Salmonella typhimurium* tester strains possessing elevated *O*-acetyltransferase levels. *Mutat. Res.*, *234*: 337–348, 1990.
12. Sugimura, T. Overview of carcinogenic heterocyclic amines. *Mutat. Res.*, *376*: 211–219, 1997.
13. Sousa, J., Nath, J., Tucker, J. D., and Ong, T. M. Dietary factors affecting the urinary mutagenicity assay system. I. Detection of mutagenic activity in human urine following a fried beef meal. *Mutat. Res.*, *149*: 365–374, 1985.