Short Communication

Dietary Intake of Heterocyclic Amines and Benzo(a)Pyrene: Associations with Pancreatic Cancer

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Abstract

Objective: Heterocyclic amines (HCA) and polycyclic aromatic hydrocarbons, formed in temperature- and time-dependent manners during the cooking of meat, are mutagens and carcinogens. We sought to assess the association between dietary intake of HCA and benzo(a)pyrene [BaP] and exocrine pancreatic cancer in a population-based case-control study.

Methods: Subjects (193 cases and 674 controls) provided information on their usual meat intake and preparation method, e.g., stewed, fried, or grilled/barbecued, etc. Meat doneness preferences were measured using photographs that showed internal doneness and external brownness. We used a meat-derived HCA, B(a)P, and mutagen database with a questionnaire to estimate intake of PhIP, DiMeIQx, MelQx, B(a)P, and mutagenic activity (revertants/g of daily meat intake). Data were analyzed with unconditional logistic regression.

Results: In analyses adjusted for age, sex, smoking, education, race, and diabetes, the odds ratio and 95% confidence interval for the highest compared with the lowest quintile were as follows: PhIP, 1.8 (1.0-3.1); DiMeIQx, 2.0 (1.2-3.5); MelQx, 1.5 (0.9-2.7); B(a)P, 2.2 (1.2-4.0); and mutagenic activity, 2.4 (1.3-4.3).

Conclusions: HCAs and B(a)P from well-done barbecued and pan-fried meats may be associated with increased risk for pancreatic cancer. (Cancer Epidemiol Biomarkers Prev 2005;14(9):2261–5)

Introduction

Pancreatic cancer is rapidly fatal in the majority of cases; there are no screening tests for early detection and about 90% of cases present with late stage disease (1, 2). The prognosis is generally dismal given that there are few therapeutic options. Identifying risk factors that can be modified is a potential means to reduce mortality from this cancer.

Numerous potential carcinogens are present in meat, including heterocyclic amines (HCA), polycyclic aromatic hydrocarbons (PAH), and nitrosamines. The HCAs and PAHs are formed during the cooking of meats and the levels formed depend on cooking temperature and degree of doneness (3-6). Whereas baked and stewed meats do not contain these compounds, well-done barbecued and pan-fried meats typically contain high levels (7).

Several HCAs and at least one PAH have carcinogenic effects on the pancreas in experimental rodent models—although the most well-characterized models of experimental pancreatic carcinogenesis employ various nitrosamines or azaserine (8). The HCA, 2-amino-3-methylimidazo[4,5-f]quinoxaline (IQ), produces benign tumors in rats (9), whereas the N-hydroxy heterocyclic arylamine, 4-hydroxymethylaminoquinoxaline 1-oxide induces both benign (10) and malignant (11) pancreatic tumors in rats. Two other HCAs, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-I) and 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (DiMeQx), have shown tumor-promoting activity in hamsters (12). The PAH, dimethylbenzanthracene, when implanted in rats, induces pancreatic ductal adenocarcinomas that are histologically similar to those seen in humans (13, 14).

To investigate the role of HCAs and PAHs as possible human pancreatic carcinogens, we conducted a population-based case-control study. In a previous report from this study, we found that total meat consumption and red meat consumption were higher in cases than in controls, but these measures were not statistically significant predictors of risk (15). Positive associations were observed for well-done meat intake and fried meat intake and a strong and robust association was observed with grilled/barbecued red meat intake. Grilled/barbecued red meat consumption was associated with a nonlinear increased risk; the 90th relative to the 10th percentile of intake was associated with an odds ratio of 1.8 [95% confidence intervals (CI), 1.4-2.4]. To explore the underlying cause for this association, we have examined the estimated excess risk of pancreatic cancer associated with dietary intake of HCAs and the PAH, benzo(a)pyrene [B(a)P]. In addition, we have measured the association between pancreatic cancer and a mutagenic activity index based on daily meat intake—a measure that integrates all classes of mutagens.

Patients and Methods

Study Design. The Institutional Review Boards of the University of Minnesota, Minneapolis, the Mayo Clinic, and the U.S. Food and Drug Administration (National Center for Toxicological Research) approved this study protocol. A population-based case-control study of cancer of the exocrine
Diabetes mellitus
Race
Sex
n (SD) or controls
We estimated intake of HCAs and mutagenic activity using
cooked meat include a variety of HCAs and B(PhIP).

The mutagenic activity of sample extracts were measured
method of Gross and Gruter (18) using a solid-phase
extraction/high-pressure liquid chromatography method.

various methods to different degrees of doneness by the
cooking module was also completed. For meats prepared with
fruits, cruciferous vegetables, fish, white meat, red meat,

Semiquantitative food frequency questionnaire similar to the
Willett food frequency questionnaire (17). Reported frequent
columns of consumption were used to estimate usual intake of
fruits, cruciferous vegetables, fish, white meat, red meat,
processed meat, coffee, tea, and alcohol. A detailed meat-
cooking module was also completed. For meats prepared with
variable cooking techniques, we obtained information on the
typical level of doneness and cooking method as previously
detailed by Sinha et al. (5).

The food composition database used to assign HCA and
B(PhIP) content values to meat items on the study questionnaire
were derived from previous analyses of meat samples as
described (5). Briefly, HCA content (PhIP, DiMeIQx, and
MelIQx) and B(PhIP) were determined in meat samples cooked by
various methods to different degrees of doneness by the
method of Gross and Gruter (18) using a solid-phase
extraction/high-pressure liquid chromatography method.

The mutagenicity of sample extracts were measured
using the standard plate incorporation assay with Salmonella
typhimurium strain TA98. (6, 19). Agents with mutagenic
activity corresponding to a crude odds ratio of 1.9 (1.2-3.0) associated
with pancreatic cancer.

Cases were more likely than controls to report a history of diabetes (24% versus 8%)
corresponding to a crude odds ratio of 1.9 (1.2-3.0) associated with pancreatic cancer.

Table 1. Characteristics of pancreatic cancer cases and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases, mean value (SD) or n (%)</th>
<th>Controls, mean value (SD) or n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>65.4 (11.6) 66.0 (12.5)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male 118 (61.1%) 380 (56.4%)</td>
<td>Female 75 (38.9%) 294 (43.6%)</td>
</tr>
<tr>
<td>Race</td>
<td>Whites 181 (93.8%) 662 (98.2%)</td>
<td>African-American 8 (4.2%) 5 (0.7%)</td>
</tr>
<tr>
<td></td>
<td>Other 4 (2.1%) 7 (1.0%)</td>
<td></td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>No 147 (76.2%) 622 (92.3%)</td>
<td>Yes 46 (23.8%) 52 (7.7%)</td>
</tr>
<tr>
<td></td>
<td>Edward 32 (16.6%) 85 (12.6%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High school graduate 69 (35.8%) 175 (26.0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post high school education 91 (47.1%) 414 (61.4%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alcohol (servings/wk) 3.1 (6.3) 4.6 (8.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total energy (kcal/d) 2,053 (820) 2,076 (811)</td>
<td></td>
</tr>
<tr>
<td>Dietary fat intake</td>
<td>Animal (g/d) 38.4 (19.4) 37.7 (23.0)</td>
<td>Vegetable (g/d) 32.8 (17.6) 32.7 (18.8)</td>
</tr>
<tr>
<td></td>
<td>Fruit intake (servings/wk) 20.7 (19.3) 20.4 (13.6)</td>
<td>Vegetable intake (servings/wk) 18.5 (12.3) 22.1 (14.5)</td>
</tr>
<tr>
<td></td>
<td>Fruit and vegetable intake (servings/wk) 39.3 (27.0) 42.4 (24.0)</td>
<td></td>
</tr>
</tbody>
</table>

aPercentages may not add to 100 where information is missing.

Results

Cases and controls in this analysis were restricted to those who completed the meat module: 193 cases and 674 controls. The study population was 97% Caucasian (Table 1). The mean ages of the cases and controls were 65.4 and 66.0 years, respectively. Sixty-one percent of the cases and 56.4% of the controls were males. More cases than controls reported current or past cigarette smoking. Compared with never smokers these were associated with elevated odds ratios of 2.0 (95% CI, 1.2-3.3) and 1.5 (95% CI, 1.0-2.1), respectively. Cases were more likely than controls to report a history of diabetes (24% versus 8%) corresponding to a crude odds ratio of 1.9 (1.2-3.0) associated with pancreatic cancer.

Table 2. Carcinogen intake and mutagenic activity index in pancreatic cancer cases and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases, mean (SD)</th>
<th>Controls, mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhIP (ng/d)</td>
<td>94.6 (117.8) 63.3</td>
<td>69.1 (106.1) 39.8</td>
</tr>
<tr>
<td>MelIQx (ng/d)</td>
<td>54.8 (54.7) 38</td>
<td>42.1 (50.3) 26.4</td>
</tr>
<tr>
<td>DiMeIQx (ng/d)</td>
<td>4.3 (5.0) 2.5</td>
<td>3.1 (4.2) 1.8</td>
</tr>
<tr>
<td>B(PhIP) (ng/d)</td>
<td>26.3 (50.3) 3.6</td>
<td>16.3 (33.7) 1.8</td>
</tr>
<tr>
<td>Mutagenic activity</td>
<td>6,618.4 (6,349.6)</td>
<td>4,921.9 (5,756.3)</td>
</tr>
</tbody>
</table>

[revertant colonies/gram (g/d) 4,625.6 3,289.6]
In this population-based case-control study, pancreatic cancer risk, based on 867 subjects.

**NOTE:** Based on 867 subjects.

<table>
<thead>
<tr>
<th>Attenuation (revertant colonies / grams of meat / d).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutagenic activity* 1.00 B( ) DiMeIQX (ng/d) 1.00 0.43 0.84 MeIQX (ng/d) 1.00 0.50 0.92 PhIP (ng/d) 1.00 0.43 0.84 Mutagenic activity* 1.00</td>
</tr>
</tbody>
</table>

Calculated that meat-derived HCAs and B( ) and mutagenic activity were positively associated with increased dietary intake of HCAs, PhIP, DiMeIQX, MeIQX, and the PAH, B( ). Risk estimates also increased with a mutagenic activity index, a biologically relevant and integrated measure of mutagenicity. The associations were robust to multivariable adjustment. We conclude that meat-derived HCAs and B( )—from well-done grilled and fried meat intake—are possible risk factors for pancreatic cancer.

In our previous study (15), we found that mean levels of calories, fat, fruits and vegetables, fiber, and alcohol generally increased the point estimates in each quintile, but decreased the precision of the estimates, and as the findings were not substantially altered, these variables were not included in final models.

**Discussion**

In this population-based case-control study, pancreatic cancer was positively associated with increased dietary intake of HCAs, PhIP, DiMeIQX, MeIQX, and the PAH, B( ). Risk estimates also increased with a mutagenic activity index, a biologically relevant and integrated measure of mutagenicity. The associations were robust to multivariable adjustment. We conclude that meat-derived HCAs and B( )—from well-done grilled and fried meat intake—are possible risk factors for pancreatic cancer.

In our previous study (15), we found that mean levels of calories, fat, fruits and vegetables, fiber, and alcohol generally increased the point estimates in each quintile, but decreased the precision of the estimates, and as the findings were not substantially altered, these variables were not included in final models.

**Table 3.** Spearman correlation coefficients for HCAs, B(a)P, and mutagenic activity

<table>
<thead>
<tr>
<th>Attenuation (revertant colonies / grams of meat / d).</th>
<th>PhIP (ng/d)</th>
<th>MelQx (ng/d)</th>
<th>DiMeIQx (ng/d)</th>
<th>B(a)P (ng/d)</th>
<th>Mutagenic activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhIP (ng/d)</td>
<td>1.00 0.63</td>
<td>0.60 0.72</td>
<td>0.80 1.00</td>
<td>1.00 0.64</td>
<td></td>
</tr>
<tr>
<td>MelQx (ng/d)</td>
<td>1.00 0.89</td>
<td>0.50 0.92</td>
<td>1.00 0.43</td>
<td>0.84 0.64</td>
<td></td>
</tr>
<tr>
<td>DiMeIQx (ng/d)</td>
<td>1.00 0.43</td>
<td>0.84 1.00</td>
<td>1.00 0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B(a)P (ng/d)</td>
<td>1.00 0.64</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** Mutagenic activity (revertant colonies / grams of meat / d).

If pancreatic cancer risk is associated with the carcinogens formed during meat preparation and not meat intake per se, inconsistencies between different study populations are not surprising. Populations and individuals vary greatly in meat-cooking practices and doneness preferences. Although grilling and frying could produce high levels of carcinogens such as HCAs and PAHs, baking, stewing or broiling form only negligible levels (5). Failure to consider cooking techniques and doneness preferences, or estimates of carcinogenic and mutagenic dose (which are closely correlated in the diet). Positive associations have been reported for the following: daily meat consumption (29); total meat, liver, ham, and sausages (30); red meat and salted/smoked meat (31); beef and bacon (32); pork and beef (33, 34); pork and fish, but not beef (35); beef, chicken, and pork (36), and fat (37-39). Null, inverse, and inconsistent associations have also been reported (40-47).

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B(a)P and the HCAs considered here are reasonable candidates for human pancreatic carcinogens (49), and they are a reasonable candidate for human pancreatic carcinogens (49). And they are a reasonable candidate for human pancreatic carcinogens (49).

**Table 4.** Odds ratios associated with carcinogen intake and mutagenic activity and pancreatic cancer

<table>
<thead>
<tr>
<th>Quintile of daily dietary intake</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhIP (ng/d)</td>
<td>1.0 (ref)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Median</td>
<td>419</td>
<td>1,652</td>
<td>3,290</td>
<td>5,771</td>
<td>11,045</td>
</tr>
<tr>
<td>Range</td>
<td>0-1,079</td>
<td>1,080-2,309</td>
<td>2,310-4,329</td>
<td>4,330-7,334</td>
<td>7,335-53,026</td>
</tr>
<tr>
<td>Cases</td>
<td>24</td>
<td>31</td>
<td>38</td>
<td>36</td>
<td>63</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>1.0 (ref)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>95% CI, P</td>
<td>0.006</td>
<td>0.006</td>
<td>0.006</td>
<td>0.006</td>
<td>0.006</td>
</tr>
<tr>
<td>B(a)P (ng/d)</td>
<td>1.0 (ref)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Median</td>
<td>0.3</td>
<td>0.8</td>
<td>1.8</td>
<td>4.0</td>
<td>10.4</td>
</tr>
<tr>
<td>Range</td>
<td>0-0.5</td>
<td>0.5-1.1</td>
<td>1.1-3.1</td>
<td>3.1-5.1</td>
<td>5.1-10.1</td>
</tr>
<tr>
<td>Cases</td>
<td>22</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>1.0 (ref)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>95% CI, P</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>Mutagenic activity*</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Median</td>
<td>59</td>
<td>0.7-2.5</td>
<td>0.9-2.7</td>
<td>1.4-2.5</td>
<td>2.5-4.2</td>
</tr>
<tr>
<td>Range</td>
<td>0-6.4</td>
<td>6.4-27.2</td>
<td>27.2-54.9</td>
<td>54.9-105.9</td>
<td>105.9-210.9</td>
</tr>
<tr>
<td>Cases</td>
<td>29</td>
<td>26</td>
<td>36</td>
<td>44</td>
<td>57</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>1.0</td>
<td>0.9</td>
<td>0.7-2.1</td>
<td>1.4</td>
<td>1.8</td>
</tr>
<tr>
<td>95% CI, P</td>
<td>0.006</td>
<td>0.006</td>
<td>0.006</td>
<td>0.006</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Quintiles were determined using the control data.

*Adjusted for age, sex, smoking (pack-years and pack-years squared), education, race, and diabetes.

*Mutagenic activity (revertant colonies / grams of meat / d).
represent a substantial portion of meat-derived mutagens/carcinogens in their respective classes (7, 22). In rodent models, PhIP, the most mass-abundant of these HCAs, forms high levels of DNA adducts in the pancreas (50), and is preferentially taken up by pancreatic acini (51). Of note, however, there are other known carcinogenic HCA and PAH mutagens in cooked meat (48, 21) that may contribute to pancreatic carcinogenesis. In addition, there are components of meat, such as fat and iron, that may be relevant to carcinogenesis in the pancreas as well (3, 49).

A strength of this study is that it was designed to address the hypothesis that dietary HCAs and B(a)P intake are associated with risk of pancreatic cancer. We collected detailed information, from direct interviews, on cooking practices and doneness levels for specific types of commonly consumed meats. This is essential to most accurately estimate the carcino men intake and mutagenicity index associated with meat intake. Estimates of dietary carcino men intake, like other dietary nutrient intakes, are imperfect. The HCAs and PAHs in cooked meat are correlated with each other and with other dietary nutrient intakes. Estimates of dietary mutagens, like other dietary nutrient intakes, are imperfect. The HCAs and PAHs in cooked meat are correlated with each other and with other dietary nutrient intakes. The proportion of all eligible cases enrolled was low (~30%), thus creating the potential for selection bias.

In addition, pancreatic cases that do enroll are usually quite ill, and as a result, may report their food intake history differently than controls. However, it is hard to imagine why selection bias or reporting bias would result in over-reporting by cases of meat preparation methods—particularly grilling, frying and well-done meat preferences that would result in the higher estimates of dietary HCAs and B(a)P.

Our evidence lends support to the view that HCAs and B(a)P, formed during the cooking of meat, are human carcinogens. These hypotheses should be replicated, ideally in a prospective study.

Acknowledgments

The authors are indebted to the following individuals for facilitating this project: Lois Murphy, Jane Curtin, and IMS, Inc., Jose Jesseruen, John Potter, Richard Severson, Eugene Di Magno, Gail Jolitz, and all of the Tumor Registrars in the Twin Cities seven-county metropolitan area of Minnesota; Judy Funkyo, Sally Bushhouse, Charles Murray, Trista Johnson, Ann Deshler, and Robin Bliss. This research would not have been possible without the many people with pancreatic cancer who gave of their precious time.

References

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