Process meat intake, CYP2A6 activity and risk of colorectal adenoma

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Red and processed meat intake is associated with increased risks of both colorectal adenoma and cancer. Processed meats contain nitrate and nitrite, precursors of N-nitroso compounds (NOCs); furthermore, meats cooked at high temperatures contain heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs). Specific NOC, HCA and PAH are mutagens and animal carcinogens. We conducted a case–control study of 146 cases of colorectal adenoma, diagnosed at sigmoidoscopy or colonoscopy, and 228 polyp-free controls. We calculated odds ratios (ORs) [and 95% confidence intervals (CIs)] and found a 2-fold increased risk in the highest, compared with the lowest, quartile of processed meat intake (95% CI 1.0–4.0). We estimated nitrate and nitrite intake from meat using published data from the literature as well as from actual measurements of meats analyzed recently. We evaluated the interaction of processed meat and nitrate plus nitrite intake with CYP2A6 activity, an enzyme able to metabolize some NOC to their carcinogenic form. Results for both methods of estimating nitrate and nitrite intake were similar; compared with the lowest, the highest quartile based on measured values was associated with a 2-fold elevated risk (95% CI 1.0–3.9). Adjust-ment for the HCA 2-amino-3,8-dimethylimidazo[4,5-\(\text{f}\)]quinoxaline (MeIQx) attenuated the association (OR 1.6, 95% CI 0.8–3.2), but other HCA and PAH had minimal effect. Higher CYP2A6 activity was not associated with risk and there was no evidence of an interaction of CYP2A6 activity with nitrate and nitrite intake. Our results suggest that nitrite and nitrate intake from processed meat intake increases the risk of colorectal adenoma after accounting for HCA and PAH.

Introduction

Colorectal cancer and the precursor colorectal adenoma are associated with higher consumption of red and processed meat. A meta-analysis of prospective studies of colorectal cancer showed significant increased risks of 12–17% for 100 g/day of red meat and a significant increased risk of 49% for only 25 g/day of processed meat (1). Another meta-analysis of both case–control and cohort studies found a 36% elevated risk for colorectal cancer associated with a 30 g/day increase in processed meat intake (2). Meat cooked by high-temperature cooking techniques is a source of several mutagens, including heterocyclic amines (HCA) and polycyclic aromatic hydrocarbons (PAH). Meat processed using nitrate or nitrite is also the main source of preformed N-nitroso compounds (NOC) in the diet (3). Additionally, red meat (that is not processed) produces a dose-dependent increase in endogenous NOC formation in humans, as measured by apparent total NOC concentration in feces (4,5). Epidemiologic and human biomonitoring studies suggest that HCA, PAH and NOC may all play an important role in colorectal cancer carcinogenesis (6–11). A few studies have estimated intake of individual HCA (7,9) and benzo[a]pyrene (BaP) (8), a carcinogenic PAH, in relation to risk of colorectal adenoma or colorectal cancer; however, no studies have adjusted the risk estimates for nitrate and nitrite levels in processed meats.

We previously reported that colorectal adenoma risk was associated with higher consumption of well-done red meat (12) and with higher consumption of certain HCAs (7) and BaP (8). Mutagenic activity from cooked meat, a measure that integrates effects from all classes of mutagens, explained the elevated risk associated with well-done red meat (7), but only somewhat attenuated the elevated risk associated with BaP (8). Here, we evaluate the association between processed meat intake, nitrite and nitrate levels in processed meat and colorectal adenoma risk taking into account HCA, BaP and meat-derived mutagenic activity. Further, we evaluate the potential interaction of processed meat and nitrate plus nitrite intake with CYP2A6 activity, an enzyme able to metabolize some NOC to their carcinogenic form (13).

Materials and methods

Study population

The study population and study design have been described previously (7,12). Briefly, we conducted a clinic-based case–control study of colorectal adenomas at the National Naval Medical Center in Bethesda, Maryland. Eligible cases were diagnosed with colorectal adenoma at sigmoidoscopy or colonoscopy, and eligible controls were individuals found not to have colorectal adenomas at sigmoidoscopy. Participation rates were 84% for cases and 74% for controls. Of the 244 participating cases, 93 were excluded due to previous history of adenomas. A total of 231 controls participated.

Dietary assessment

Participants completed a self-administered food frequency questionnaire to obtain information on usual diet during the 12 months prior to sigmoidoscopy/colonoscopy. Furthermore, a detailed meat-cooking module that included 23 meat items was administered at the same time as the food frequency questionnaire to estimate levels of HCA, BaP and mutagenicity in meats as described previously (7,12). We excluded two cases and three controls with implausible reported total caloric intakes and three cases for whom information on prior adenomas was not available, leaving 146 cases and 228 controls in the final analysis.

Total red meat was defined as hamburgers, cheeseburgers, beefsteaks, beef stew, potpie, liver, pork roast, beef roast, liverwurst, hot dogs, lunch meats, bacon, sausage, ham steaks/pork chops, veal, lamb and venison. Six questions were asked about processed meats, and thus the processed meat variable included: bacon; breakfast sausage; hot dogs/other sausage; ham steaks/pork chops; ham, bologna, salami and other luncheon meats and liverwurst. The fresh red meat variable included the remaining non-processed red meats. Variably cooked processed meats (meats that are often cooked by methods using high temperatures such as frying and grilling) included bacon, breakfast sausage and ham steak/pork chops. Processed meats cooked in a standard way (usually not at high temperatures) included hot dogs/other sausage, luncheon meats and liverwurst. Variably cooked fresh red meats included hamburgers, cheeseburgers and beefsteaks; the remaining fresh red meats were considered to be cooked in a standard way.

We estimated levels of nitrate and nitrite in processed meats by two methods. First, we used published values from two studies (14,15) in which >200 samples of processed meats from commercial outlets in the five largest metropolitan centers in Canada were analyzed for nitrate and nitrite. We computed means for fried bacon, breakfast sausage (fried pork sausage) and hot dogs from levels reported for individual meat samples and we converted the values to mg ion per 100 g. For other sausages (Polish, summer) and luncheon meats, we used the means as calculated by Choi (16) from the studies by Panalaks et al. (14,15).

Second, we used values from a recently developed database (17) of nitrite and nitrate content of 10 types of processed meats that represent 90% of the

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1210
processed meat consumed in USA according to the 1994–96 Continuing Survey of Food Intakes by Individuals database, a 24 h dietary recall survey (18). For each of the 10 meat types, a composite was prepared using brand name meats that were available from grocery stores in the Blacksburg, Virginia area in 2004. When major brand name products were not available, a local/ regional or store brand product was substituted. The meat composites were ground in liquid nitrogen and aliquots were capped with residual nitrogen gas, which is beneficial for preventing oxidative changes. Aliquots were shipped to Covance Laboratories in Madison, Wisconsin, for analysis of nitrate, nitrite and specific volatile nitrosamines [N-nitrosodimethylamine, N-nitrosodicycylamine, N-nitrosodibutylamine, N-nitroso-piperidine, N-nitrosopyrrolidine and N-nitrosophenol]. Before analysis for nitrate and nitrite, the samples were extracted with boiling water. Lipids were removed with chloroform extraction and proteins precipitated by centrifugation. The extract was filtered and appropriate dilutions were injected onto a high-performance anion exchange system equipped with an electrochemical detector using pulsed amperometric detection (19). Nitrates and nitrites were quantified against standards of known concentration. For analysis of volatile nitrosamines, the sample was first vacuum distilled and collected in a liquid nitrogen trap. The trap was allowed to warm to room temperature and then extracted with methylene chloride. The methylene chloride extract was concentrated to 1 ml and analyzed on a gas chromatography system equipped with a thermal energy analyzer (20). There were no detectable volatile nitrosamines in any of the meat composite samples (limit of detection = 1 ppb).

Because some questions on the food frequency questionnaire combined more than one processed meat type with differing nitrate and nitrite levels (e.g. hot dogs and other sausage), we assigned a weighted average of the nitrate and nitrite levels across all meat types included in the question, where the weights were based upon the reported frequency of consumption in the Continuing Survey of Food Intakes by Individuals database. One question combined both processed meat (ham steaks) and fresh meat (pork chops); nitrate and nitrite values were estimated by weighting values for fresh meat (21) with those for ham steaks according to Continuing Survey of Food Intakes by Individuals consumption patterns. Concentrations were not estimated for liverwurst since consumption was low and it was not one of the meat types that we measured for nitrate and nitrite.

We multiplied the frequency of consumption of each food item by the portion size and by the nitrate and nitrite concentration of each processed meat to estimate nitrate and nitrite intake (mg/day). Some of the nitrate added to processed meats may be converted to nitrite that can react with amines and amides in the meat to form N0OCS. Therefore, we evaluated nitrite concentrations and the total concentration of nitrate and nitrite combined in processed meats.

CYP2A6 caffeine phenotyping

As described previously (22), a urine caffeine collection kit, consisting of an overnight urine sample with cooler and ice packs, was delivered to the participant’s homes. Phenotyping is the preferred method of assessing CYP2A6 variation in Caucasians due to a low frequency of the allelic variants identified thus far for this gene; phenotyping was conducted as described by Nowell et al. (9,23). Study participants were instructed to abstain from methylxanthine-containing foods such as coffee, tea, chocolate and cola, from midnight before phenotyping until 5 h after the caffeine dose. In the morning, each participant took a caffeine dose in the form of a 200 mg tablet of 1,7-dimethyl uric acid to 1,7-dimethylxanthine. The dose consisted of 1.0–4.0 mg caffeine (Figure 1). As previously reported (12), this partitioned into a 16% increased risk per 10 g intake of variably cooked red meat (95% CI = 1.04–1.29) and a 5% increased risk for red meat cooked by standard methods (95% CI = 0.94–1.17). We evaluated intake of variably cooked red meat separately for processed red meat and fresh red meats. We found a 3% increased risk per 10 g for variably cooked processed meat (95% CI = 0.98–1.73; P for trend = 0.063) and a 10% increased risk per 10 g for variably cooked fresh red meat (95% CI = 0.94–1.27; P for trend = 0.23). Risks were lower for processed meats and fresh meats cooked by standard methods. We further partitioned variably cooked processed and fresh red meat into meats cooked well/very well done and rare/medium. We found that risk increased by 3% per 10 g intake for well/very well-done fresh red meats (95% CI = 0.98–1.73), whereas risk was increased 21% for well/very well-done processed meats (95% CI = 0.90–1.63) cooked to the same doneness level. Rare/medium doneness level was associated with a small non-significant 5% increased risk per 10 g intake of fresh red meat (95% CI = 0.90–1.23); in contrast, rare/medium processed meats were associated with a 56% increased risk for 10 g intake (95% CI = 0.94–2.59).

Nitrate concentrations in processed meats estimated from the published literature, which reflected levels in the 1970s, were somewhat lower for bacon and breakfast sausage compared with nitrate concentrations measured recently (Table II). In contrast, for hot dogs and anti-inflammatory drugs, physical activity and intakes of alcohol, fruit juice and fiber. Only pack-years of smoking changed the ORs by >20% compared with ORs adjusted for the matching factors (age and gender). We present results for processed meat intake, nitrite, and nitrate plus nitrite adjusted for age, gender, pack-years of smoking and total caloric intake (basic model). All models that included processed meat were further adjusted for other meats, so that the sum of the meat variables in each model represented total meat consumed. Furthermore, we investigated the potential for HCA, BaP and meat-derived mutagenic activity to confound the associations for nitrate and nitrite plus nitrite combined. We evaluated the statistical interaction of nitrate plus nitrite intake with MeIQx, the HCA that showed the strongest association with risk.

We also calculated ORs associated with a 10 g/day increase in consumption of different types of meat that allows for the direct comparison of ORs based on the same amount of intake (25). We evaluated three nested models. The first included processed meat, fresh red meat and white meat. For the second, we split processed meats into those cooked by variable methods and those prepared in a standard way. Finally, we further split variably cooked processed meats and fresh red meats by two doneness groups: well done/very well done and rare/medium. CYP2A6 phenotype activity was divided into tertiles according to the distribution among controls. We evaluated the interaction of CYP2A6 activity with processed meat and nitrate plus nitrite intake; for this analysis, we collapsed the second and third quartiles (interquartile range (IQR)) of intake into one level. We also evaluated the interaction for the continuous form of the variables.

Results

We observed a marginally significant 2-fold increased risk (95% CI = 1.0–4.0) among those in the highest quartile of total processed meat intake (>24 g/day) compared with those in the lowest quartile in the basic model (Table I). Adjustment for intake of other red meat and white meat attenuated the ORs; however, the risk was still elevated, although not significant, in the highest quartile. We calculated ORs per 10 g intake and found an 8% increased risk for processed meat (95% CI = 0.96–1.23).

The highest quartiles of intake of hot dogs and other sausages, ham steaks/pork chops and liverwurst were associated with an ~2-fold elevated risk of colorectal adenoma in the basic model; the association was statistically significant for ham steaks/pork chops and marginally significant for hot dogs/other sausages (95% CI = 1.0–3.7) (Table I). Risks were attenuated but remained elevated after adjustment for intake of other types of meats, including other processed meats. Consumption of bacon, breakfast sausage and lunch meats was not associated with elevated risk after adjustment for other meat intake.

The OR per 10 g intake of red meat was 1.10 (95% CI = 1.03–1.18) (Figure 1). As previously reported (12), this partitioned into a 16% increased risk per 10 g intake of variably cooked red meat (95% CI = 1.04–1.29) and a 5% increased risk for red meat cooked by standard methods (95% CI = 0.94–1.17). We evaluated intake of variably cooked red meat separately for processed red meat and fresh red meats. We found a 31% increased risk per 10 g for variably cooked processed meat (95% CI = 0.98–1.73; P for trend = 0.063) and a 10% increased risk per 10 g for variably cooked fresh red meat (95% CI = 0.94–1.27; P for trend = 0.23). Risks were lower for processed meats and fresh meats cooked by standard methods. We further partitioned variably cooked processed and fresh red meat into meats cooked well/very well done and rare/medium. We found that risk increased by 31% per 10 g intake for well/very well-done fresh red meats (95% CI = 0.98–1.73), whereas risk was increased 21% for well/very well-done processed meats (95% CI = 0.90–1.63) cooked to the same doneness level. Rare/medium doneness level was associated with a small non-significant 5% increased risk per 10 g intake of fresh red meat (95% CI = 0.90–1.23); in contrast, rare/medium processed meats were associated with a 56% increased risk for 10 g intake (95% CI = 0.94–2.59).

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Discussion

Previously, we reported that the risk of colorectal adenoma was associated with higher consumption of well-done red meat (12) and with higher consumption of certain HCA, mutagenic activity (7) and BaP (8). In those analyses, processed meats were not evaluated separately from other red meats. Here, we found that high intake of processed meat (>24 g/day) was associated with a 2-fold increased risk of colorectal adenoma, although risk was attenuated after adjustment for intake of fresh red meats and white meats. Intake of processed meat that was usually cooked at high temperatures
(variably cooked) was more strongly associated with risk than variably cooked fresh meats, and consumption of both processed and fresh meat cooked by standard methods was associated with lower risks. Intake of nitrite and nitrate plus nitrite from processed meats showed similar associations with risk as that for processed meat.

Two meta-analyses (1,2) of colorectal cancer concluded that there was an increased risk for both red meat and processed meats, with a stronger association for processed meat. The risk estimates were not mutually adjusted and were not adjusted for intake of poultry and fish because the data were not available from many studies to do so. In contrast to our results, several studies that adjusted processed meat intake for intakes of other meat types have found no attenuation of results upon adjustment (27,28). After adjustment for known risk factors and total meat consumption, Nowell et al. (23) found a 2-fold increased risk of colorectal cancer associated with processed meat consumption above the median intake level.

Most studies of colorectal adenoma (12,29) and colorectal cancer (9,30,31) that investigated meat-cooking method and doneness preference have reported increased risks associated with consumption of meat cooked well done or at high temperatures. None of these studies reported results for meat-cooking methods and doneness preference separately for processed and fresh red meats. Four case–control studies of colorectal cancer (9,23,32,33), our case–control study of colorectal adenoma (7,8,12) and a cohort study that evaluated colon adenoma (6) estimated exposure to specific HCA from meat. With the exception of the study by Augustsson et al. (32), increasing MeIQx intake was positively associated with risk. In the cohort study (6), the positive association with MeIQx was attenuated after adjustment for processed meat intake, whereas the highest quintile of processed meat intake remained significantly elevated after adjustment for MeIQx. None of the other studies adjusted HCA risk estimates for processed meat intake.

In these analyses, MeIQx (but not other HCA, BaP or meat-derived mutagenicity) appeared to explain some but not all of the elevated risk associated with nitrite and nitrate intake from processed meat. We observed an increased risk associated with variably cooked processed meats (bacon, breakfast sausage, ham steaks/pork chops), that was not related to the doneness level of the meats. Risks were lower for processed meats cooked by standard methods (luncheon meats, hot dogs/other sausages, liverwurst). Although the levels of nitrate plus nitrite did not vary substantially in these two groups of processed meats, NOC can vary across types of processed meats, particularly volatile nitrosamines (14,15,34). Historically, volatile nitrosamines were detected most frequently in bacon (35) and can be formed during the cooking process (35–37); fried bacon cooked well done contained higher levels of volatile nitrosamines than bacon.

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**Table II. Nitrate and nitrite concentrations in processed meats, average values from historical publications and recent measurements of meat samples**

<table>
<thead>
<tr>
<th>Processed meat question</th>
<th>Nitrate concentration (mg/100 g)</th>
<th>Nitrite concentration (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Published&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Measured&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Published&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bacon</td>
<td>2.70</td>
<td>5.26</td>
</tr>
<tr>
<td>Breakfast sausage</td>
<td>1.50</td>
<td>2.04</td>
</tr>
<tr>
<td>Hot dogs and other sausages, such as Polish</td>
<td>10.56</td>
<td>2.06</td>
</tr>
<tr>
<td>Ham steaks/pork chops</td>
<td>5.57</td>
<td>0.99</td>
</tr>
<tr>
<td>Ham, bologna, salami and other lunch meats</td>
<td>7.70</td>
<td>0.77</td>
</tr>
</tbody>
</table>

<sup>a</sup>Average nitrate and nitrite concentration in different types of processed meats [averages computed by Choi (1985) from measurements by Panalaks et al. (14,15,16)].

<sup>b</sup>Based on nitrate and nitrite concentrations measured in meat samples for the meat module questionnaire [Sinha et al. (8,17)].
cooked rare or medium (38) and microwaved bacon had the lowest concentrations (38,39). However, other variables besides the nitrate plus nitrite content and cooking methods influence NOC levels, including the amount of added ascorbate, concentrations of amines in meat and the other ingredients used in processing (e.g. salt, smoking). Thus, variation in volatile nitrosamine content or other differences in the types of processed meats in the variably cooked and standard preparation groups may explain the differences in risk that we observed. Alternatively, chance may be an explanation and these analyses should be repeated in larger studies. Processed meats have been associated with an increased risk of both colorectal adenoma and cancer in numerous studies and are a major source of NOC and NOC precursors, which can go on to form NOC endogenously in animals (40) and humans (4,5). NOCs are known mutagens and carcinogens able to cause tumors of the gastrointestinal tract in animals (41). Few studies have estimated dietary intakes of nitrite and NOC in relation to risk of colorectal cancer. A cohort study in Finland (10) estimated dietary intakes of nitrite (94% from cured meats) and nitrosodimethylamine (from cured meats and smoked/salted fish) and found a significant 2-fold increased risk for colorectal cancer in the highest quartile of N-nitrosodimethylamine intake, but no elevated risk associated with nitrite intake. We estimated nitrate and nitrite levels in processed meats using historical published values and our recent measurements of commercially available processed meats. Although the average historical values for nitrate and nitrate concentrations in processed meats were often higher than our recently measured levels, the intakes of nitrite and nitrate plus nitrite based on both methods were highly correlated ($r > 0.90$) indicating that both approaches to estimating intake ranked individuals similarly. In our study population, higher CYP2A6 activity was associated with a 40% increased risk of colorectal adenoma, which did not reach statistical significance. To our knowledge, there have been no previous studies of CYP2A6 phenotype and processed meat intake with respect to colorectal adenoma risk; however, one case–control study evaluated these factors for colorectal cancer (9,23), finding a 2.6-fold increased risk in the highest tertile of CYP2A6 activity and a 2-fold increased risk in those consuming above the median intake of processed meat (23). They observed an increased risk of colorectal cancer associated with medium and high CYP2A6 activity among those with high processed meat consumption, whereas only high CYP2A6 activity increased risk among those with lower processed meat intake. Our results differed from that of Nowell et al. (23) in that higher CYP2A6 activity was associated with a smaller non-significant elevated risk and there was no evidence for an interaction with processed meat consumption. The tertile categories of CYP2A6 activity were similar between our studies; however, processed meat intake was lower in our study population, higher CYP2A6 activity was associated with a statistically significant increased risk of colorectal adenoma and there was no evidence for an interaction with processed meat intake. Processed meat and nitrate plus nitrite intake by CYP2A6 activity.
Strengths of the study include the high response rates, and the fact that reporting of dietary intakes is less likely to be influenced by their diagnosis of adenoma compared with studies of colorectal cancer. The study was specifically designed to investigate the role of mutagens formed in different types of meats by variable cooking methods. We estimated nitrate plus nitrite for processed meat items on the questionnaire from historical published values and from recent measurements of commonly consumed processed meats. The measured values reflect current intake levels of processed meat sources of nitrate and nitrite as well as changes in processing procedures, such as the addition of ascorbate to reduce NOC formation.

Our ability to evaluate processed meat and nitrate plus nitrite intake in relation to colorectal adenoma risk was limited by the fact that the questionnaire was not originally designed to evaluate this hypothesis in detail. Several of the processed meat questions combined individual processed meats with varying levels of added nitrate and nitrite; although we weighted the assigned values by national consumption data, this could have induced misclassification. We only assessed nitrate from processed meats although the main dietary sources are vegetables (21), because we were interested in added nitrate as a source of nitrate that can react exogenously or endogenously with the amines and amides in meats to form NOC. We found no measurable volatile nitrosamines in processed meat products purchased recently and we did not measure total NOC; therefore, we are unable to evaluate the effects of individual or total NOC exposure from processed meats. Further, we did not evaluate meat levels of heme, the major determinant of endogenous intestinal N-nitrosation (4,5). Other limitations of the study were that controls only had a flexible sigmoidoscopy (and not colonoscopy) and, therefore, may have undetected adenomas in the proximal colon. If risk factors for adenoma on both sides of the colon are similar, having some controls with undetected proximal adenomas would attenuate our results. As previously reported (12), when the meat intake analyses were restricted to distal colon adenomas, the results were essentially unchanged.

Our results are consistent with numerous epidemiologic studies of colorectal adenoma and colorectal cancer that observe an increased risk with processed meat intake. The association with processed meat and nitrate plus nitrite levels was somewhat attenuated after adjustment for McIqX. To our knowledge, our study is the first to attempt to separate the effects associated with consumption of nitrate and nitrite in processed red meats from the effects of HCA in cooked meats. Additional studies with detailed assessment of processed meat, other meats and cooking methods are needed to further evaluate the contribution of each component of meat intake to risk of colon adenoma and colorectal cancer.

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Conflict of Interest Statement: None declared.

References


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