Intakes of Red Meat, Processed Meat, and Meat Mutagens Increase Lung Cancer Risk

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Abstract

Red and processed meat intake may increase lung cancer risk. However, the epidemiologic evidence is inconsistent and few studies have evaluated the role of meat mutagens formed during high cooking temperatures. We investigated the association of red meat, processed meat, and meat mutagen intake with lung cancer risk in Environment And Genetics in Lung cancer Etiology, a population-based case-control study. Primary lung cancer cases (n = 2,101) were recruited from 13 hospitals within the Lombardy region of Italy examining ~80% of the cases from the area. Noncancer population controls (n = 2,120), matched to cases on gender, residence, and age, were randomly selected from the same catchment area. Diet was assessed in 1,903 cases and 2,073 controls and used in conjunction with a meat mutagen database to estimate intake of heterocyclic amines (HCA) and benzo(a)-pyrene (BaP). Multivariable odds ratios (OR) and 95% confidence intervals (95% CI) for sex-specific tertiles of intake were calculated using unconditional logistic regression. Red and processed meat were positively associated with lung cancer risk (highest-versus-lowest tertile: OR, 1.8; 95% CI, 1.5–2.2; P trend < 0.001 and OR, 1.7; 95% CI, 1.4–2.1; P trend < 0.001, respectively); the risks were strongest among never smokers (OR, 2.4; 95% CI, 1.4–4.0; P trend = 0.001 and OR, 2.5; 95% CI, 1.5–4.2; P trend = 0.001, respectively). HCAs and BaP were significantly associated with increased risk of lung cancer. When separated by histology, significant positive associations for both meat groups were restricted to adenocarcinoma and squamous cell carcinoma but not small cell carcinoma of the lung. In summary, red meat, processed meat, and meat mutagens were independently associated with increased risk of lung cancer. [Cancer Res 2009;69(3):932–9]

Introduction

Lung cancer is the leading cancer-related cause of mortality worldwide (1). Although cigarette smoking is the dominant and indisputable risk factor for lung cancer, other environmental determinants, including dietary factors, may contribute to lung cancer risk. Fresh red meat (e.g., steak, hamburger, and pork chops) as well as processed meat (e.g., baloney, salami, and hot dogs) may be sources of mutagens (2, 3). Meats cooked well done at high temperatures develop carcinogenic heterocyclic amines (HCA) and polycyclic aromatic hydrocarbons (PAH). Concomitantly, *N*-nitroso compounds (NOC) form exogenously (via nitrates and nitrites added to processed/cured meats for color and flavor and to inhibit the growth of *Clostridium botulinum*) and endogenously (related to heme iron, inherently found in fresh red meats; refs. 2, 4). NOCs can induce tumors in a variety of organs, including the lung, in experimental studies and are universally carcinogenic across species (4, 5). Diets in developed countries contain a high proportion of meats; characterizing the role of meat intake and meat mutagens in carcinogenesis may provide additional insights into the etiology of lung cancer and modifiable risk factors.

Epidemiologic studies have linked consumption of meat, particularly fresh red meat and processed meat, to cancer risk (3, 6, 7). In a review of the epidemiologic literature, the World Cancer Research Fund and the American Institute for Cancer Research reported that the epidemiologic evidence for red or processed meat intake and lung cancer risk is limited and inconsistent (8). Recently, Cross and colleagues (3) reported increased risk of lung cancer for the highest-versus-lowest quintiles of red meat [hazard ratio (HR), 1.20; 95% confidence interval (95% CI), 1.10–1.31] and processed meat (HR, 1.16; 95% CI, 1.06–1.26) intake using data from a large prospective cohort in the United States. The findings corroborated previous results from some case-control studies (9–13) but not others (14–17). Lung cancer risk associated with red meat may vary according to histologic subtypes (18) as well as by smoking status. Previous studies have not fully investigated cell type differences in relation to processed meats for lung cancer risk, although there is equivocal evidence with respect to fresh red meat intake (11, 19). Notably, to our knowledge, only one study had published on the relationship between dietary HCAs and lung cancer risk (20) but none on dietary PAHs.

To address some of these gaps in the literature and to extend the body of evidence, we conducted an investigation on the association between red and processed meat intake and risk of lung cancer using the Environment And Genetics in Lung cancer Etiology (EAGLE) study (21). The EAGLE study contains comprehensive information on smoking exposure as well as dietary information with data on cooking methods and meat doneness levels, allowing for the estimation of HCAs and PAHs. Furthermore, because processed meats are commonly consumed in Italy, this study

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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population provided a range of meat types and intake data wider than in previous studies.

Materials and Methods

Study population. The EAGLE study has been previously described (21). Briefly, EAGLE is a large population-based case-control study conducted in the Lombardy region of Italy.[9] The catchment area covers 216 municipalities, which include five cities (Milan, Monza, Brescia, Pavia, and Varese) and surrounding towns and villages. Between April 2002 and February 2005, primary lung cancer cases (n = 2,101) were recruited from 13 hospitals that oversee ~80% of the incident lung cancer cases in the area. Cases' response rate was 86.6%. The majority of cases (95%) were confirmed pathologically or cytologically, and detailed histologic classification was recorded. The remaining 5% were confirmed on clinical history and imaging.

Controls were randomly selected from the Regional Health Service database, which contains demographic information for virtually all Italians from the catchment area, and were matched to cases on gender, age (5-y classes), and residence area where cases originated (21). At the study completion, 2,120 controls were successfully recruited with a participation rate of 72.4%. The study was approved by the Institutional Review Board of the National Cancer Institute and the local hospitals and universities. Each subject signed an informed consent form before participation.

Exposure assessment. At baseline, comprehensive information on demographic characteristics and risk factors was collected using both a computer-assisted personal interview and a self-administered questionnaire. Particular attention was given to the collection of data on tobacco exposure, including active smoking (number of cigarettes per day averaged over a life time, age at initiation/quit, pack-years) and passive smoking (during childhood, at work, and at home during adulthood).

Dietary information was obtained at baseline from a short, self-administered 58-item food frequency questionnaire (FFQ) designed to target specific types of food commonly consumed by this population over the year before the study. The FFQ queried frequency of consumption using 11 possible response categories, from "never" to "2 or more times a day." A list of relevant food groups queried can be found in Supplementary Table S1. Additional information was obtained on cooking methods, doneness, and degree of browning (using a series of color photographs to represent increasing degree of doneness and browning) for fresh red meats and chicken (Supplementary Table S2).

As portion size was not asked in the FFQ, we used average portion sizes obtained from 24-h recalls collected from participants resided in Varese, Italy, within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts to estimate intakes of HCAs and benzo(a)pyrene (BaP; ref. 22). We specifically requested portion size of participants from Varese, Italy, because Varese is one of the participating cities in the EAGLE study. We created variables for fresh red meat and processed meat consumption by summing the individual food item contributing to each meat group (Supplementary Table S1).

We used the EPIC portion size with the EAGLE cooking methods and doneness information in conjunction with the CHARRED database[10] (23) to estimate daily intake of HCAs {2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-3,8-dimethylimidazo-[4,5-b]quinoxaline (MeIQx), and 2-amino-3,4,8-trimethylimidazo-[4,5-f]quinoxaline (DiMeIQx)} and one PAH (BaP). The CHARRED software also calculates mutagenic activity of meat by applying values generated from the Ames *Salmonella* test (24, 25).

Statistical analysis. Of the 4,221 cases and controls, 245 participants (198 cases and 47 controls) did not complete the FFQ and were excluded from this analysis, resulting in a study population of 1,903 cases and 2,073 controls. We categorized dietary intake of food groups in tertiles based on the distribution of both cases and controls for each gender. Our results using sex-specific tertiles based on the distribution of controls only did not substantially differ. Odds ratios (OR) and 95% CI, within sex-specific tertiles of intake, were obtained using logistic regression. All models were adjusted for matching variables (age, gender, and area of residence), body mass index (BMI), education, alcohol consumption, cigarette intensity (quartiles; 0 for never smokers), smoking duration (continuous; 0 for never smokers), and years since last cigarette smoked for former smokers (continuous; 0 for never and current smokers because they did not experience any year since quitting smoking). Additional analyses included adjusting for dietary intake of fruits and vegetables (continuous; summary measures of fruits and vegetables; Supplementary Table S1) and mutually adjusting for different meat groups (continuous). Inclusion of family history of lung cancer, previous lung diseases, and passive smoke exposure did not alter the results appreciatively and were not included in the final models.

We conducted subgroup analyses, separated by smoking status (never, former, and current), smoking intensity (quartiles based on the distribution of the controls), the major histologic subtypes (adenocarcinoma, squamous cell carcinoma, and small cell lung cancer), and gender. For the subgroup analyses by histology, the ORs and 95% CIs were computed using the same control group and thus are not independent and correlation between estimates has to be considered. Therefore, to test the heterogeneity between histology-specific ORs and 95% CIs, we first used unconditional multinomial logistic models, comparing histology-specific cases with all controls, to derive the β estimates and the covariance matrix of the βs. Homogeneity was then assessed using the Wald χ^2 test. Interactions were evaluated using the likelihood ratio test. Test for dose-response trends across different categories of meat exposure was estimated by fitting the ordinal exposure variables as ordered categories. A two-tailed P value of <0.05 was considered to be statistically significant. All statistical analyses were carried out using STATA version 9.1.

Results

Compared with cases in the lowest tertile of weekly frequency of meat intake, cases in the highest tertile were more likely to be current smokers and to have higher smoking intensity (Table 1). Cases were more likely to smoke and consumed higher frequency of alcohol than controls. Smoking intensity and lifetime consumption of alcohol did not significantly correlate with intake of red or processed meats [Spearman correlation (r) = 0.1 and 0.1, respectively].

Fresh red meat and processed meat. Individuals in the highest compared with the lowest tertile of fresh red meat and processed meat weekly frequency of intakes had increased risks of lung cancer (OR, 1.8; 95% CI, 1.5–2.2; P trend < 0.001 and OR, 1.7; 95% CI, 1.4–2.1; P trend < 0.001, respectively; Table 2). The statistically significant increase in lung cancer risks remained even after additional adjustment of processed meat intake (for red meat) and fresh red meat intake (for processed meat), and intake of total fruits and vegetables in the models. Statistically significant positive associations comparing highest-versus-lowest tertile of intake frequency were observed for all fresh red meats (ORs ranged from 1.3 to 1.9) and all processed meat items (ORs ranged from 1.2 to 1.7), although the risk associated with salami did not reach statistical significance (data not shown).

Statistically significant positive associations were observed across all strata of smoking status (for red meat: ORs ranged from 1.7 to 2.4; for processed meat: ORs ranged from 1.6 to 2.5; Table 3). The greatest increase in lung cancer risk was observed among never smokers for both fresh red meat (OR, 2.4; 95% CI, 1.4–4.0; P trend = 0.001) and processed meat (OR, 2.5; 95% CI, 1.5–4.2; P trend = 0.001) compared with never smokers in the lowest tertile of intake (P interaction = 0.09). Further adjustment for passive smoking and analyses stratified by quartiles of cigarette smoking intensity did not substantially alter the positive associations (data not shown).

[9] http://dceg.cancer.gov/eagle
[10] http://www.charred.cancer.gov

When the analyses were separated by histology, statistically significant positive associations were observed for adenocarcinoma (for red meat: OR, 1.8; 95% CI, 1.4–2.3; P trend < 0.001 and for processed meat: OR, 1.9; 95% CI, 1.5–2.4; P trend <0.001) and squamous cell carcinoma (for red meat: OR, 2.1; 95% CI, 1.5–2.9; P trend < 0.001 and for processed meat: OR, 1.9; 95% CI, 1.4–2.6; P trend < 0.001). No statistically significant associations were observed for small cell lung cancer (Table 3). There was no evidence of heterogeneity by histology for both fresh red meat (P = 0.07) and processed meat (P = 0.10).

Meat mutagens and cooking preference. Intakes of MeIQx, PhIP, and DiMeIQx from total meat (including chicken) were highly correlated with one another (MeIQx and PhIP: r = 0.8; MeIQx and DiMeIQx: r = 0.8; PhIP and DiMeIQx: r = 0.6). Of the three HCAs, only MeIQx and PhIP were strongly correlated with BaP (r = 0.7), whereas moderate correlation was observed for DiMeIQx and BaP (r = 0.5).

The risk of lung cancer was increased for those in the highest (compared with the lowest) tertile of PhIP (OR, 1.5; 95% CI, 1.2–1.8; P trend < 0.001), MeIQx (OR, 1.4; 95% CI, 1.3–1.7; P trend < 0.001), and BaP (OR, 1.3; 95% CI, 1.1–1.6), but there was no association for DiMeIQx (OR, 1.0; 95% CI, 0.8–1.2; Table 4). The increased risks of lung cancer associated with those in the highest tertile of HCAs and BaP were consistent across all smoking strata, although in never smokers the associations did not reach statistical significance (Table 4).

Discussion

Our data from a large population-based case-control study in northern Italy, comparing highest-versus-lowest tertile of meat intake, found a smoking adjusted 80% and 70% increased risk of lung cancer for red and processed meat, respectively. In the

Table 1. Selected characteristics by sex-specific tertile of combined red and processed meat intake in EAGLE

Tertile*	Cases (n = 1,903)			Controls (n = 2,073)		
	T1	T2	T3	T1	T2	T3
n †	548	601	736	772	718	582
Intake of combined meats,‡ frequency per week, median (IQR)	3.1 (2)	6.4 (1.9)	12.3 (5.7)	2.9 (1.9)	6.4 (1.9)	11.2 (4.7)
Female	2.5 (1.9)	5.4 (1.9)	11.2 (4.8)	2.3 (1.7)	5.7 (1.6)	10.2 (3.8)
Male	3.2 (1.9)	6.7 (1.9)	12.6 (5.7)	3.1 (2.0)	6.7 (1.9)	11.4 (4.8)
Fresh red meat§	0.8 (1.0)	2.0 (1.5)	3.2 (2.9)	0.7 (0.9)	1.7 (1.6)	3.0 (2.5)
Processed meat‖	1.9 (1.6)	4.4 (2.2)	8.9 (5.0)	1.8 (1.5)	4.6 (1.9)	8.6 (4.1)
Female (n = 885), n (%) †	95 (23.8)	132 (33.1)	166 (41.6)	198 (40.7)	161 (33.1)	126 (25.9)
Male (n = 3,091), n (%) †	453 (30.1)	469 (312)	570 (37.9)	574 (36.2)	557 (35.1)	456 (28.7)
Age, mean ± SD (y)	67.8 ± 7.6	66.3 ± 8.1	65.0 ± 8.9	66.5 ± 8.2	65.6 ± 8.2	63.9 ± 9.3
BMI, mean ± SD (kg/m^2)	26.0 ± 4.5	25.8 ± 4.0	25.6 ± 4.2	25.8 ± 4.0	26.0 ± 3.9	26.4 ± 4.0
Education, n (%)						
None (n = 187)	27 (26.7)	27 (26.7)	45 (44.5)	26 (30.2)	25 (29.1)	35 (40.7)
Elementary school (n = 1,262)	193 (27.5)	222 (31.6)	279 (39.7)	216 (38.6)	184 (32.9)	159 (28.4)
Middle school (n = 1,133)	160 (29.9)	171 (31.9)	199 (37.1)	201 (33.7)	222 (37.2)	174 (29.2)
High school (n = 983)	117 (28.5)	128 (31.1)	164 (39.9)	222 (38.8)	204 (35.7)	146 (25.5)
University (n = 355)	38 (36.9)	31 (30.1)	34 (33.0)	105 (41.3)	82 (32.3)	67 (26.4)
Missing (n = 52)	13 (27.1)	22 (45.8)	13 (27.1)	2 (50.0)	1 (25.0)	1 (25.0)
Smoking status (%)						
Never (n = 797)	32 (5.8)	43 (7.2)	55 (7.5)	257 (33.3)	236 (32.9)	172 (29.6)
Former (n = 1,723)	247 (45.1)	285 (47.4)	290 (39.4)	334 (43.3)	319 (44.4)	242 (41.6)
Current (n = 1,444)	268 (48.9)	269 (44.8)	389 (52.9)	179 (23.2)	161 (22.4)	167 (28.7)
Missing (n = 12)	1 (0.2)	4 (0.7)	2 (0.3)	2 (0.3)	2 (0.3)	1 (0.2)
Smoking intensity¶ (pack-year per day)						
Quartile 1 (0 < range < 0.5; n = 462)	35 (6.8)	38 (6.8)	37 (5.4)	142 (27.6)	122 (25.3)	88 (21.5)
Quartile 2 (range: 0.5–0.8; n = 793)	100 (19.4)	116 (20.8)	141 (20.7)	155 (30.1)	161 (33.4)	118 (28.8)
Quartile 3 (range: 0.8–1.0; n = 793)	198 (38.4)	194 (34.8)	234 (34.4)	127 (24.7)	116 (24.1)	124 (30.2)
Quartile 4 (range: 1.0–5.0; n = 825)	160 (31.0)	180 (32.3)	229 (33.7)	88 (17.1)	81 (16.8)	79 (19.3)
Missing (n = 99)	23 (4.5)	30 (5.4)	40 (5.9)	3 (0.6)	2 (0.41)	1 (0.2)
Lifetime alcohol consumption (median, g per day)**	22.2 (35.0)	20.3 (31.3)	21.9 (32.2)	15.7 (20.4)	17.6 (21.1)	18.7 (21.4)

Abbreviation: IQR, interquartile range.

*Sex-specific tertile, based on the distribution of the entire study population for each gender: T1 = first tertile; T2 = second tertile; T3 = third tertile.

† Total number (n) or % may not add up to total number of cases and controls (N) or 100% due to missing data or rounding, respectively.

‡ Combined fresh red and processed meat: total fresh red meat + total processed meat.

§Total fresh red meat: summary measure of beef steak, hamburger, pork chops, and veal chop/cutlet.

‖Total processed meat: summary measure of cooked ham (prosciutto cotto), smoked ham (prosciutto crudo), cured ham (speck), salami, baloney (mortadella), wurstel, salted sliced beef, coppa, pancetta, and other types of processed meats.

¶Ever smokers only; quartile of smoking intensity based on the distribution of the controls.

**Weighted average daily lifetime alcohol from alcoholic beverages in grams.

Table 2. ORs and 95% CIs for the risk of lung cancer by sex-specific tertiles of fresh red meat and processed meat in the EAGLE study

Tertile*	Fresh red meat †				Processed meat ‡			
	T1	T2	T3	*P* trend	T1	T2	T3	*P* trend
Median, frequency per week (IQR)								
Female	0.36 (0.36)	1.4 (0.7)	3.5 (1.7)		1.5 (1.2)	3.8 (1.5)	7.7 (4.0)	
Male	0.55 (0.48)	1.7 (0.7)	3.7 (1.8)		1.9 (1.3)	4.5 (1.5)	9.0 (4.4)	
All participants								
Cases/controls§	539/779	614/706	719/587		548/770	604/710	721/592	
Multivariate, OR‖ (95% CI)	1.0 (reference)	1.3 (1.1–1.6)	1.8 (1.5–2.2)	<0.001	1.0 (reference)	1.3 (1.1–1.5)	1.7 (1.4–2.1)	<0.001
Multivariable‖ (+ red or processed meat intake)	1.0 (reference)	1.3 (1.1–1.5)	1.7 (1.4–2.0)	<0.001	1.0 (reference)	1.2 (1.0–1.5)	1.5 (1.2–1.8)	<0.001
Multivariable‖ (+ total fruit and vegetable intake)	1.0 (reference)	1.3 (1.1–1.6)	1.9 (1.5–2.2)	<0.001	1.0 (reference)	1.3 (1.1–1.6)	1.8 (1.5–2.2)	<0.001

Abbreviation: IQR, interquartile range.

*Sex-specific tertile, based on the distribution of the entire study population for each gender: T1 = first tertile; T2 = second tertile; T3 = third tertile.

†Fresh red meat: summary measure of beef steak, hamburger, pork chops, and veal chop/cutlet.

‡Processed meat: summary measure of cooked ham (prosciutto cotto), smoked ham (prosciutto crudo), cured ham (speck), salami, baloney (mortadella), wurstel, salted sliced beef, coppa, pancetta, and other types of processed meats.

§Due to missing data, numbers may not add up to total cases (n = 1,903) or controls (n = 2,073).

‖Adjusted for age, gender, area of residence, education, BMI, alcohol, smoking intensity in pack-year per day (quartiles, distribution of controls; 0 for never smokers), duration of cigarettes smoking (continuous; 0 for never smokers), and years since last cigarettes (continuous; 0 for never and current smokers).

analysis by histology, elevated risk for lung cancer risk was observed for adenocarcinoma and squamous cell carcinoma cases. Our data also showed that the elevated risks of lung cancer among high consumers of fresh red meat were associated with intakes of the meat mutagens PhIP, MeIQx, and BaP derived from high cooking temperatures.

Our findings are particularly timely as they corroborate some of the recent results from a large prospective cohort study, which reported a 20% higher risk for lung cancer for those in the highest quintile of red meat intake and 16% higher for processed meat (3). Previous case-control studies also reported elevated risks for higher red meat intake in relation to lung cancer risk (9–13), whereas others have not (14–17). Similarly, results from previous epidemiologic studies (16, 26–30) on processed meat intake and lung cancer risk have been inconsistent. The differences may be due to the small sample size of these studies [<500 cases in all studies but Cross and colleagues (3)] and possibly population differences in the amount and range of consumption of red and processed meat intake.

Several mechanisms have been hypothesized that could explain how red meat and processed meat contribute to cancer risk. Fresh red meat and processed meats are sources of saturated fats, iron, and several mutagens, including NOCs, HCAs, and PAHs (3). Each of these chemicals and mutagens could theoretically contribute to the associated increased risk of lung cancer observed. Saturated fats may be related to energy balance; however, the epidemiologic evidence has shown no relationship between dietary saturated fat intake and lung cancer risk (31). Conversely, dietary heme iron is associated with increased risk of lung cancer prospectively (32) and may be related to oxidative stress and endogenous formation of NOCs (2).

NOCs are potent carcinogens and had been shown in experimental studies to induce tumors at multiple sites via alkylative DNA damage (2, 4, 33). Humans may be exposed to NOCs endogenously through heme iron found in red meat or exogenously from the use of nitrates or nitrites for preservation and color of processed meats (2). HCAs are formed via the pyrolyzation of amino acids found in meat juices and creatine when meats are cooked at high temperatures (23, 34). PAHs are produced when meats are grilled or barbecued (35). Some investigators have used well-done meat as a surrogate for exposure to HCAs or PAHs in relation to various cancers (2, 36, 37).

The mechanistic bases by which meat mutagens contribute to carcinogenesis of the lung have not been fully characterized. One possible mechanism by which the lung is exposed to mutagens from ingested meats cooked at high temperature is through metabolic activation of HCAs, such as PhIP, by phase I hepatic cytochrome *P*450 (*CYP450*)–mediated *N*-hydroxylation (38). Carcinogenic metabolites may then circulate to the lung and covalently bind to DNA to induce lung tumors. Alternatively or in addition to this mechanism, HCAs could be metabolized directly in the lung by CYP1A1, as shown for PhIP in knockout mice (39). It is generally accepted that DNA adducts can initiate carcinogenesis and mutagenesis (40) and PhIP-DNA adducts have been detected in the lung of rats (41) and monkeys (42). Similarly, dietary NOCs undergo metabolic activation by CYP450 enzymes to form alkylating agents, which can then give rise to alkyl adducts (43, 44). Moreover, inherited polymorphisms in *CYP450*s may alter the inducibility of the genes and/or their activity, thus further adding to the complexity of the mechanism relating meat to cancer risk. Additional studies addressing the role of gene-meat mutagen interaction are warranted to further understand lung cancer etiology.

Studies have reported meat consumption and lung cancer risk stratified by cooking preference (11, 12). One reported no difference in risk by strata of cooking temperature (11), whereas the other found that consumption of fried or well-done red meat was

associated with an increased risk of lung cancer (12). To our knowledge, only one case-control study had published on the relationship between dietary HCAs and lung cancer risks (20). Sinha and colleagues (20) observed a 50% increased risk of lung cancer for MeIQx, but no association for DiMeIQx and PhIP in a population of American women from Missouri. Our findings confirmed the positive finding for MeIQx but also extended the increased risks of lung cancer to PhIP, BaP, and total mutagenic activity. The discrepancy between our positive finding for PhIP and the null association in the study of Sinha and colleagues may be due to smaller sample size of the U.S. study and differences in levels of meat intake and cooking practices.

In contrast to previous studies, which included only sausages, bacons, hams, and hot dogs as processed meats, EAGLE used a more comprehensive list of processed meats, characteristic of an Italian diet. These meats are generally cured, consumed uncooked, and frequently (≥3 times per week). Processed meats in the United States are more limited and are often cooked and the intake range is narrower. For example, Cross and colleagues (3) reported a smaller risk in the United States than what we observed in the present study. This may be due to the prospective design of the Cross and colleagues study and/or greater variability in the range of consumption by the Italians. Although we found consistent positive associations between total or specific processed meat intake and lung cancer risk, the magnitude of these findings may be unique to this study population.

High temperature cooking may influence the formation of HCAs (4). In EAGLE, as per the Italian tradition, processed meats were consumed without additional cooking preparations. Thus, the excess risks of lung cancer associated with processed meats in this study cannot be explained by high temperature cooking methods and related mutagens. Analyses of the lung cancer risk associated with individual processed meat items showed the greatest increase in risks for smoked, cured, and cooked ham. Intake of salami, which was consumed less frequently, was the only item not associated with lung cancer risk. A possible explanation may be that salami is usually aged and cured for a shorter time, although preparation varies by geographic region (45).

The lack of positive association for small cell lung cancer cases in the current study may be due to the smaller number of cases in this group. Moreover, small cell lung cancer is the histologic subtype that is more strongly correlated with cigarette smoking. In EAGLE, for example, the population attributable risk due to smoking is 94% (95% CI, 83–98%) for small cell carcinoma versus 67% (95% CI, 51–74%) for adenocarcinoma. Thus, the role of meat consumption may be stronger or more easily observed in the histologic subtypes less associated with tobacco smoking.

Table 3. ORs and 95% CIs for the risk of lung cancer by sex-specific tertiles of fresh red meat and processed meat in the EAGLE study, stratified by selected characteristics

Tertile*	Fresh red meat †				Processed meat ‡			
	T1	T2	T3	*P* trend	T1	T2	T3	*P* trend
Smoking status								
Current smokers								
Cases/controls	256/198	304/149	358/160		269/180	277/172	376/155	
Multivariate, OR§ (95% CI)	1.0 (reference)	1.5 (1.1–2.0)	1.7 (1.3–2.3)	<0.001	1.0 (reference)	1.2 (0.9–1.7)	1.77 (1.3–2.4)	<0.001
Former smokers								
Cases/controls	247/342	266/315	305/238		242/334	282/312	290/249	
Multivariate, OR§ (95% CI)	1.0 (reference)	1.2 (1.0–1.6)	1.7 (1.3–2.2)	<0.001	1.0 (reference)	1.3 (1.0–1.7)	1.6 (1.3–2.1)	0.002
Never smokers								
Cases/controls	34/237	42/240	53/188		34/254	41/225	55/186	
Multivariate, OR§ (95% CI)	1.0 (reference)	1.1 (0.7–2.0)	2.4 (1.4–4.0)	0.001	1.0 (reference)	1.5 (0.9–2.6)	2.5 (1.5–4.2)	0.001
Histologic subtypes								
Adenocarcinoma (n = 781)								
Cases/controls	218/779	247/706	305/578		214/770	245/710	311/592	
Multivariate, OR§ (95% CI)	1.0 (reference)	1.3 (1.0–1.6)	1.8 (1.4–2.3)	<0.001	1.0 (reference)	1.3 (1.0–1.7)	1.9 (1.5–2.4)	<0.001
Squamous cell carcinoma (n = 487)								
Cases/controls	122/779	166/706	190/587		134/770	160/710	184/592	
Multivariate, OR§ (95% CI)	1.0 (reference)	1.7 (1.2–2.3)	2.1 (1.5–2.9)	<0.001	1.0 (reference)	1.4 (1.0)	1.9 (1.4–2.6)	<0.001
Small cell lung cancer (n = 195)								
Cases/controls	68/779	65/706	59/587		64/770	65/710	63/592	
Multivariate, OR§ (95% CI)	1.0 (reference)	1.1 (0.7–1.6)	1.1 (0.7–1.7)	0.61	1.0 (reference)	1.0 (0.7–1.6)	1.2 (0.8–1.8)	0.51

*Sex-specific tertile, based on the distribution of the entire study population for each gender: T1 = first tertile; T2 = second tertile; T3 = third tertile.
†Fresh red meat: summary measure of beef steak, hamburger, pork chops, and veal chop/cutlet.
‡Processed meat: summary measure of cooked ham (prosciutto cotto), smoked ham (prosciutto crudo), cured ham (speck), salami, baloney (mortadella), wurstel, salted sliced beef, coppa, pancetta, and other types of processed meats.
§Adjusted for age, gender, area of residence, education, BMI, alcohol, smoking intensity in pack-year per day (quartiles, distribution of controls; 0 for never smokers), duration of cigarettes smoking (continuous; 0 for never smokers), and years since last cigarettes (continuous; 0 for never and current smokers).

Table 4. ORs and 95% CIs for the risk of lung cancer and sex-specific tertile of meat mutagens in the EAGLE study

	Median, frequency/week (IQR)		All participants		Smoking status					
					Current		Former		Never	
	Female	Male	Cases/ controls	OR (95% CI)	Cases/ controls	OR (95% CI)	Cases/ controls	OR (95% CI)	Cases/ controls	OR (95% CI)
PhIP (ng/d)										
T1	0 (2.3)	0 (8.9)	587/740	1.0 (reference)	276/183	1.0 (reference)	262/327	1.0 (reference)	45/230	1.0 (reference)
T2	36.4 (34.7)	49.8 (36.6)	618/708	1.1 (0.9–1.4)	326/174	1.2 (0.9–1.6)	250/300	1.1 (0.8–1.4)	37/231	1.0 (0.6–1.6)
T3	176.7 (155.5)	163.4 (118.0)	698/625	1.5 (1.2–1.8)	331/151	1.5 (1.1–2.0)	316/268	1.4 (1.0–1.8)	49/205	1.8 (1.0–3.0)
P trend				<0.001		0.013		0.03		0.04
DiMeIQx (ng/d)										
T1	0 (0)	0 (0)	666/671	1.0 (reference)	309/153	1.0 (reference)	293/285	1.0 (reference)	57/232	1.0 (reference)
T2	0.60 (0.61)	0.70 (0.6)	547/767	0.68 (0.6–0.8)	271/195	0.64 (0.5–0.9)	237/344	0.70 (0.5–0.9)	36/226	0.8 (0.5–1.3)
T3	3.10 (2.5)	2.9 (2.7)	690/635	1.0 (0.8–1.2)	353/160	1.0 (1.7–1.4)	298/266	1.0 (0.8–1.3)	38/208	0.91 (0.5–1.5)
P trend				0.09		0.90		0.94		0.67
MeIQx (ng/d)										
T1	1.4 (2.4)	1.7 (2.9)	574/752	1.0 (reference)	270/182	1.0 (reference)	253/326	1.0 (reference)	47/243	1.0 (reference)
T2	9.1 (6.6)	11.9 (7.0)	609/716	1.1 (0.9–1.3)	296/172	1.0 (0.76–1.4)	271/316	1.1 (0.84–1.4)	37/226	0.93 (0.56–1.6)
T3	34.5 (29.8)	36.1 (28.9)	720/605	1.4 (1.2–1.7)	367/154	1.4 (1.0–1.9)	304/253	1.4 (1.0–1.8)	47/197	1.5 (0.9–2.5)
P trend				<0.001		0.04		0.03		0.13
BaP (ng/d)										
T1	0.2 (0.3)	0.2 (0.4)	651/675	1.0 (reference)	318/170	1.0 (reference)	283/291	1.0 (reference)	45/214	1.0 (reference)
T2	14.2 (21.8)	18.9 (20.0)	536/789	0.8 (0.64–0.9)	275/197	0.8 (0.6–1.0)	222/344	0.7 (0.5–0.9)	37/246	1.0 (0.6–1.6)
T3	85.4 (86.6)	89.4 (74.0)	716/609	1.3 (1.1–1.6)	340/141	1.3 (1.0–1.8)	323/260	1.3 (1.0–1.7)	49/206	1.6 (1.0–2.7)
P trend				0.003		0.07		0.09		0.07
Mutagenic activity (per 1,000 revertant colonies)										
T1	55.6 (304.4)	177.4 (460.2)	578/748	1.0 (reference)	278/183	1.0 (reference)	251/326	1.0 (reference)	45/237	1.0 (reference)
T2	1,426.5 (1129.2)	1,738.9 (941.2)	633/692	1.2 (1.0–1.4)	304/165	1.0 (0.8–1.4)	274/293	1.2 (0.9–1.6)	49/234	1.3 (0.8–2.1)
T3	5,956.4 (6150.1)	5,617.0 (4965.4)	692/633	1.4 (1.1–1.7)	351/160	1.4 (1.0–1.9)	303/276	1.3 (1.0–1.8)	37/195	1.3 (0.8–2.2)
P trend				0.001		0.03		0.05		0.3

NOTE: Sex-specific tertile, based on the distribution of the entire study population for each gender: T1 = first tertile; T2 = second tertile; T3 = third tertile. ORs: adjusted for age, gender, area of residence, education, BMI, alcohol, smoking intensity in pack-year per day (quartile, distribution of controls; 0 for never smokers), duration of cigarettes smoking (continuous; 0 for never smokers), and years since last cigarettes. Daily intake of meat mutagens estimated from beef steak, hamburger, pork chops, veal chop/cutlet, and chicken using mean EPIC portion size.

Cigarette smoking is also correlated with a less healthy lifestyle, including higher alcohol consumption and a poor diet (46), although there was no correlation between cigarette smoking, alcohol consumption, and meat intake in the EAGLE study. The extensive data available within this study enabled rigorous control for cigarette smoking, alcohol, and other factors in our analyses. Although residual confounding can never be completely ruled out, it is unlikely that it could account for the results observed.

Our data suggest that the increased risk of lung cancer associated with fresh red meat, processed meat, and meat mutagen intake is independent of cigarette smoking. Our observation of statistically significant elevated risks of lung cancer across all smoking strata, particularly among never smokers, even after adjustment for passive smoking further buttresses this conclusion. Tobacco-related chemicals (e.g., PAHs) have been shown to induce CYP1A1 expression (47) and can theoretically influence the metabolism of meat mutagens and NOCs (48) and thereby lung cancer risk. However, as cigarette smoking also induces a constellation of other xenobiotic enzymes, including phase 2 enzymes that detoxify carcinogenic metabolites, it is difficult to predict the extent in which smoking-induced CYP450 levels may affect metabolism of meat mutagens and lung cancer risk (47).

In our analyses, mutually adjustment for fresh red meat and processed meat intake in the models did not alter the respective associations conferred to each meat group. This suggests that these two meat types act independently on the association with lung cancer risk.

Study limitations include the possibility of recall bias due to the case-control study design, although the rapid recruitment protocol that allowed study enrollment and interview at the time of the diagnosis and not when the patients were in terminal conditions was designed to minimize such issue. Moreover, although the FFQ in the EAGLE study was targeted to obtain information on specific foods common in this study population, it was relatively limited in scope and portion size was not asked. Hence, we were unable to adjust for total energy intake in our models. Energy adjustment, although not perfect for addressing measurement errors in FFQs, is a reasonable method by some (49), whereas others have proposed adjustment for body weight and physical activity as more appropriate methods (50). In this present study, we adjusted for

BMI, used sex-specific tertiles of meat intake, and conducted analyses stratified by gender but did not have information on physical activity. Dietary data derived from FFQs are prone to measurement errors that may be random and systematic (49), and thus, measurement error likely remains.

The results for meat mutagens need to be assessed with caution as values of HCAs and PAHs were estimated indirectly using a database (34) and surrogate portion size, albeit obtained from an Italian population residing in one of the cities included in the EAGLE study. We also cannot exclude the possibility that exposure to HCAs and PAHs includes a degree of misclassification and measurement errors. For example, there was no information on frequency of flipping meat slices during cooking, which can reduce the amount of HCAs formed (23).

Our study has several strengths. It is a large population-based case-control study with high participation rates and detailed information on smoking history, imperative when studying lung cancer, as well as many other risk factors. Cases were rapidly ascertained and thus eliminated the need to use surrogate participants. The large sample size permitted investigation by histologic subtypes and smoking status with adequate power.

In conclusion, higher frequencies of intake of fresh red meats and processed meats were independently associated with increased lung cancer risk in this Italian population. The association was concentrated in cases with non–small cell lung cancer and seemed to be independent of cigarette smoking status. The increased risk associated with fresh red meat consumption may be partially explained by meat mutagens. Our results, together with the recent findings from a prospective cohort study, provoke a thoughtful evaluation that red and processed meat intake may be independent etiologic risk factors for lung cancer. However, further studies are warranted to characterize the mechanisms by which meats and meat mutagens contribute to lung carcinogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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