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No effect of meat, meat cooking preferences, meat mutagens or heme iron on lung cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial

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Abstract

Recent epidemiological studies have suggested that red and processed meat may increase the risk of lung cancer. Possible underlying mechanisms include mutagens produced during high temperature cooking or preservation, or formed endogenously from heme iron in meat. We used data from 99,579 participants of both screened and non-screened arms of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO), aged 55–74 years, to investigate whether meat type, cooking method, doneness level, intake of specific meat mutagens 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline] (DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), and benzo(a)pyrene (B(a)P)] and heme iron are associated with lung cancer. Participants' diet was assessed prospectively using a 124-item food frequency questionnaire and an additional meat-cooking module. Dietary data were used in conjunction with a database to estimate intake of MeIQx, DiMeIQx, PhIP, B(a)P and heme iron. After up to 8 years of follow-up, 782 incident lung cancer cases were ascertained. Lung cancer risk was not associated with the consumption of either red (men: $HR_{Q5vs.Q1}$ = 1.11, 95% CI = 0.79–1.56, P_{trend} = 0.42; women: $HR_{Q5vs.Q1}$ = 1.30, 95% CI = 0.87–1.95, P_{trend} = 0.65) or processed meat (men: $HR_{Q5vs.Q1}$ = 1.12, 95% CI = 0.83–1.53, P_{trend} = 0.22; women: $HR_{Q5vs.Q1}$ = 0.98, 95% CI = 0.68–1.41, P_{trend} = 0.32) in multivariable models. High temperature cooking methods, level of meat doneness, meat mutagens and heme iron had no effect on lung cancer risk. In this population, we found no association between meat type, cooking method, doneness level, or intake of specific meat mutagens or heme iron and lung cancer risk.

Keywords

Meat; diet; lung cancer; meat mutagens; heme iron; PLCO

Introduction

Epidemiological evidence on the association between red and processed meat and lung cancer has been suggestive, yet limited, inconsistent, and mainly based on retrospective

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data1. Diets high in red and processed meat may increase the risk of lung cancer and there are several possible mechanisms that may explain this association. High temperature cooking of meat results in the formation of heterocyclic amines (HCAs)2, 3, and polycyclic aromatic hydrocarbons (PAHs), which are also present in cured and smoked foods4–6. Both HCAs and PAHs are potent lung carcinogens in animal studies7–9. *N*-nitroso compounds (NOCs), another group of known carcinogens10, are found in preserved meat and are endogenously produced when red meat is consumed11, 12. Once absorbed, NOCs can have a systemic effect as a tissue specific carcinogen, directly or after metabolic activation13. Heme iron can act as a pro-oxidant and can catalyze lipid peroxidation and cause DNA damage in tissue14, 15, while it was also shown to induce NOCs endogenous formation11, 12. Fat content of meat, specifically, may also play a role, although no plausible mechanisms that relate to lung carcinogenesis have been suggested.

The majority of the previous epidemiological studies have limited dietary data that do not allow for appropriate categorization of meats to investigate the possible underlying mechanisms for the role of meat in lung cancer pathogenesis. Moreover, grouping meats with potentially different content of carcinogens, if indeed important, may dilute the carcinogenic effect of meat and therefore lead to inconsistency in findings between studies. So far, only two case-control studies16–18 and one cohort study19 collected detailed information on meat cooking preferences17, 19 and were able to estimate intake of HCAs16, 18, 19, PAHs18, 19 or heme iron19. Positive associations observed between well-done17, 19 and fried meat17, as well as with intake of individual HCAs16, 18, 19, PAHs18 and heme iron19 and lung cancer in these studies, suggest that with detailed dietary data we may be able to identify possible mechanisms of how meat in diet may affect lung carcinogenesis.

In current analysis, we used data from a large cohort, the Prostate Lung Colorectal and Ovarian (PLCO) Cancer Screening Trial, to investigate whether meat type, cooking method, doneness level, intake of specific HCAs, such as 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline] (DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP); PAH, benzo(a)pyrene (B(a)P); and heme iron are associated with lung cancer. Besides prospectively collected detailed dietary data, the PLCO trial has comprehensive smoking information, which allowed appropriate adjustment for this powerful confounder, as well as detailed pathohistological data for investigation of the meat effect by lung cancer cell type.

Material and methods

Study population

The PLCO Cancer Screening Trial is a randomized two-armed trial of 154,910 participants aged 55–74 years designed to investigate the efficacy of screening methods for prostate, lung, colorectal and ovarian cancer. Participants were healthy volunteers recruited from ten centers throughout the United States between 1993 and 2001 and randomized to the intervention (screened) or control (non-screened) arm. Further details of the recruitment and the study design are reported else where20. Briefly, at enrollment, all participants completed a self-administered questionnaire, which included questions on demographic characteristics, personal and family medical history and lifestyle factors. Screening for lung cancer involved a posteroanterior chest x-ray at the initial visit, and once every year for the next 2 years for never smokers and for the next 3 years for ever smokers. On an annual basis, all participants were sent a study update form inquiring whether they had been diagnosed with cancer over the past year. Written informed consent was obtained from all participants. The trial was approved by the institutional review board of the National Cancer Institute and the participating ten centers.

Dietary assessment

A self-administered semi-quantitative food frequency questionnaire (FFQ) with 124 food items[21, 22] and a meat-cooking module[23], was used to assess participants' usual diet over the previous 12 months. The questionnaire was sent to participants in both arms of the trial at the end of 1998 or at time of recruitment, if recruitment occurred after this point. The DHQ was validated in a study population of a similar structure with 1,415 participants using two non-consecutive 24-hour dietary recalls[24]; the estimated correlations between red meat intake reported from a single DHQ and true intake (assuming 24-hour recalls measure true intake with classical error), were 0.62 in men and 0.70 in women. Meat intake was calculated in grams per day from the frequency and portion size information determined by the DHQ. Definition of red, white and processed meat is given in footnote of Table 1.

The meat-cooking module contained detailed questions on cooking methods (barbequed, pan-fried, broiled or sautéed/microwaved/baked) and doneness level (rare, done, well done or very well done) of certain meats (i.e. hamburgers, steak, bacon, chicken and sausage). Cooking method and doneness information was used along with the CHARRED-database (http://charred.cancer.gov/) to estimate intake of the HCAs: MeIQx, DiMeIQx and PhIP; and PAH: (B(a)P); as well as the overall mutagenic activity (revertant colonies per gram of meat intake)[25]. The CHARRED database has been developed from laboratory analyzed values of HCAs, B(a)P, and overall mutagenic activity from approximately 120 categories of meat samples prepared by different cooking methods with varying doneness levels[2, 3, 23, 26]. The relative validity of the meat cooking module to estimate the intake of HCAs was investigated in 165 healthy participants; the de-attenuated correlation coefficients were 0.60 and 0.36 for MeIQx and PhIP, respectively[27]. Heme iron was estimated using preliminary data from an NCI database, which includes measured values of heme iron in bacon, chicken (not in mixed dishes), cold cuts, hamburgers, hot dogs (regular), pork chops, roast beef, sausage (regular), and steak cooked by a variety of cooking methods to a range of doneness levels[23].

Cohort follow-up and case ascertainment

Incident lung cancer cases were identified by chest x-ray screening, self-report in the annual study update form, state cancer registries, death certificates, physician reports, or reports by next of kin. Follow-up was calculated starting one year after completion of the DHQ until censoring on December 31, 2006, or when the participant had a cancer diagnosis, withdrew from the study or died, whichever came first. The non-cases were censored at the date of the most recent annual study update questionnaire without a report of lung cancer. All lung cancer cases were pathologically confirmed. For this analysis, we included all cases of primary epithelial lung and bronchial carcinoma (ICD 34.0 - ICD 34.9)[28]. By histological sub-type, the cases were grouped as *small cell* (including intermediate cell and combined oat cell carcinoma), *adenocarcinoma* (bronchoalveolar, acinar, papillary, and adenocarcinoma with mucus formation), *squamous* (spindle cell carcinoma), *undifferentiated/large cell* and *other or not otherwise specified carcinoma (NOS)* (adenosquamous, neuroendocrine tumors, clear cell, non-small cell, mixed small, non-small cell carcinoma). Sarcoma and carcinoid tumors were excluded because of their potentially different etiology.

Assessment of smoking

The baseline questionnaire queried whether participants had smoked cigarettes regularly for six months or longer (ever smokers), at what age they started smoking, type of tobacco they used (cigarettes, pipes or cigars), whether they were currently smoking and how much they smoke or used to smoke (1–10, 11–20, 21–30, 31–40, 41–60, 61–80 or 81 or more cigarettes per day). Those who stopped smoking were asked about age of smoking cessation. Smoking duration was calculated from age of initiation and age of cessation. Packs of cigarettes was

derived from number of cigarettes smoked per day, where the maximum number of packs was assigned based on the range of cigarettes specified (i.e. 21–30 cigarettes per day grouped as 2 packs per day). Packs-years was calculated as a product of maximum number of packs smoked per day and smoking duration in years. Those who reported quitting within the past year were considered current smokers.

Statistical analysis

For our analysis we included participants from both screened and non-screened arms. We excluded those who failed to complete either the baseline questionnaire (n = 4,926) or the DHQ (n = 36,088) or were missing date of DHQ completion (n = 18). We further excluded participants who missed more than seven food items on the DHQ (n = 3,312) or those who reported implausible energy intake (i.e. the top and bottom 1% of the sex-specific energy intake distribution) (n = 2,378). From 111,515 participants with completed baseline questionnaire and a valid DHQ, we excluded those with extreme intakes (beyond twice the interquartile range above the 75th or below the 25th percentile of sex-specific Box-Cox transformed intake) of energy-adjusted total fat (n = 224), fruit (n = 207) and vegetable servings (n = 308). The Box-Cox family of transformations is similar to simple power transformations, and includes the logarithmic transformation as a limiting case [29, 30]. We further excluded prevalent cases of any cancer except non-melanoma skin cancer (n = 9,573), participants with no follow-up time (n = 18) or who provided no information on smoking (n = 882). Lung cancer cases with missing histology information were also excluded (n = 16), as well as participants who were diagnosed with lung cancer, who died or withdrew within the first year after baseline (n = 155). Our final analytical cohort consisted of 48,229 men and 51,350 women.

Sex-specific Cox proportional hazards regression models, with age as the underlying time metric, were used to estimate hazard ratios (HR) and 95% confidence intervals (CI). The baseline meat and meat mutagen variables were categorized into quintiles, based on sex-specific cut-points within the cohort, whereas meat intake by cooking method was categorized into tertiles due to the smaller intake range. The multivariable models were developed by individually entering potential confounders into a basic model with age, energy, and red and white meat. Variables remained in the model if they are established risk factors for lung cancer (race, education and smoking), or if they changed the risk estimate by 10% or more (body mass index (BMI) and fruit), or were considered potential risk factors for lung cancer (vegetables, alcohol and total fat intake) (for detailed definition of the covariates see footnote of Table 2). Five smoking variables were simultaneously entered in the multivariable models: smoking duration, packs of cigarettes smoked per day, years since smoking cessation, age of smoking initiation and pipe or cigar users. Different cut-off points were used to categorize packs of cigarettes per day and years since smoking cessation in men and women, due to gender difference in their distribution (for detailed definition of all smoking covariates see footnote of Table 2). All dietary variables, except alcohol, were energy-adjusted using the nutrient density method. The density method may be more robust to the possible presence of zero values than the residual method, although in many cases both methods perform similarly. After applying Box-Cox transformations to normalize their distributions, we assessed the correlation of energy-adjusted red and processed meat with smoking duration, packs of cigarettes per day, years since quit smoking, age of initiation, and intake of energy, alcohol and energy-adjusted fruit, vegetable and total fat, as well as the correlation between all investigated meat-related variables, using partial age- and sex-adjusted Pearson correlation coefficient. Stratified analysis by smoking status or interaction analyses were not conducted due to small numbers of cases in the never smokers (men = 20; women = 41). Prior to merging the data from the screened and non-screened arm, we checked for possible differences in the meat and lung cancer association between the two

arms. As no difference was found, the two arms were analyzed together. All analyses were performed using SAS version 9.1. The *P* values for statistical tests were two tailed and considered significant at a level of less than 0.05.

Results

Four hundred and fifty four male and 328 female lung cancer cases were identified after up to 8 years of follow-up (median (10^{th}; 90^{th} percentile) = 5.5 yrs (3.1; 6.7). Participants with higher red meat intake were more likely to be younger, Caucasians, less educated, have higher BMI and have more substantial smoking behaviors (higher frequency of current smokers, greater packs-years, have ceased smoking less than 20 years ago) (Table 1). Those eating more red meat also tended to eat more processed meat, had higher total fat and energy intake, but had lower fruit and vegetable intake, consumed less alcohol (only in men), and were less likely to take beta-carotene supplements. We did not find any of the investigated covariates to be strongly correlated with energy-adjusted red or processed meat intake. Besides energy-adjusted total fat (red meat: r = 0.42; processed meat: r = 0.28) and fruit (red meat: r = −0.36; processed meat: r = −0.25), other covariates showed a correlation of 0.14 or lower. The correlations of the smoking covariates ranged from 0.01 and 0.02 for years since quit smoking to 0.13 and 0.11 for duration, with red and processed meat, respectively. A correlation matrix of all investigated meat-related variables is provided as an online supplement (Supplement Table 1).

The risk for lung cancer by quintile of red and processed meat intake is presented in Table 2. We observed a mild increase in risk with higher intake of red and processed meat in men, however neither of the risk estimates were statistically significant, after multivariable adjustment. In women, there was a significant increased risk in those in the third quintile of red meat intake, which decreased and became non-significant in the highest quintile. No evidence of increased risk was found with higher processed meat intake in women.

None of the investigated meat cooking methods or the level of meat doneness had an effect on lung cancer risk in neither men nor women (Table 3). Furthermore, none of the investigated HCAs nor heme iron were associated with lung cancer risk (Table 4). However, there was a borderline inverse association for B(a)P intake and lung cancer risk in women, yet the risk estimate did not reach statistical significance.

We found no statistically significant increase in risk for any of the histological sub-types of lung carcinoma with high intake of red or processed meat in neither men nor women (data not shown). The frequency of lung carcinoma by histological sub-type is reported in footnote of Table 2.

In sex-combined analysis none of the investigated associations reached statistical significance, except for the association between red meat and lung cancer risk (HR_{Q5vsQ1} = 1.33; 95% CI = 1.01–1.73), yet with no significant dose-response effect (P_{trend} = 0.25).

In additional sensitivity analysis (data not shown), we found no effect of menopausal hormone use on the meat and lung cancer associations in women. Similarly, excluding participants who reported a history of emphysema did not change any of our findings.

Excluding total fat or BMI from the models only somewhat decreased the risk estimates for meat and meat-related exposures, however the pattern of associations remained the same.

Discussion

We found no statistically significant association between red or processed meat and lung cancer in the participants of the PLCO trial. Meat cooking methods and the level of doneness, as well as investigated mutagens and heme iron intake from meat, had no statistically significant effect on lung cancer risk.

In a recent analysis of 6,800 cases in another prospective U.S. study that used the same dietary questionnaire and meat mutagens database as this study, red and processed meat were positively associated with lung cancer in men and there was a suggestive positive association for women19. We, however, had much less cases (N = 782 cases). In addition, our participants were more educated and were more likely to be never smokers than the AARP participants19, although we carefully adjusted for both education and smoking. The evidence from other cohorts is also inconsistent31–35 and difficult to compare; cohorts used different definitions to identify meat exposures and different cut-points to categorize intake, employed dietary patterns rather than a single food approach to study the exposure effect31, studied relatively younger cohorts35 or investigated mortality rather than morbidity of lung cancer as an outcome32–34. Furthermore, some studies were conducted in countries with smoking and dietary habits different from the U.S.31, 33–35.

High temperature cooking methods and doneness of meat have been used as a surrogate measure for HCAs and PAHs exposures36. In our analysis, meat doneness level was not statistically significantly associated with lung cancer. In the NIH-AARP cohort, the risk increased with consumption of well/very-well done meat in men, whereas in women consuming rare meat was inversely associated with risk19. Higher risk with well-done meat intake was also observed in a case-control study of 1,216 non-smoking women who provided detailed dietary information on meat cooking preferences17. Furthermore, this study also found a borderline statistically significant increase in risk for lung cancer for fried and broiled red meat17, in contrast to our study where no statistically significant association was found with any of the cooking methods. We found a borderline significant decrease in risk in women with B(a)P intake >11.9 ng/1000 kcal compared to those with intake 0.9 ng/1000 kcal or less. Contrary to our finding, Lam et al18 reported 1.3 fold increase in risk in participants in the highest (≥85.4 ng/d) vs. lowest tertile of B(a)P intake (<0.2 ng/d) in a population-based case-control study from Italy. The range of B(a)P intake in our participants was, however, evidently narrower compared to one reported by Lam et al18, from their Italian population, where a great variety of processed meats are commonly consumed. They also found an increased risk with higher intake of MeIQx, PhIP and overall mutagenic activity. Two other studies observed elevated lung cancer risk with higher MeIQx intake; one in women16 and in one, risk was apparent only in men19. Given that the former study was done in non-smoking women only16, it may be that the effect of smoking is so large that small effects of diet are more likely to become apparent in non-smokers. Furthermore, a narrower intake range across meat mutagens compared to Sinha et al16 could have been a reason for null association in our cohort.

In our participants, heme iron intake was not statistically significantly associated with lung cancer risk. In a case-control study, Zhou et al37 observed a positive association between total iron and lung cancer, which was due to non-heme iron, whereas heme-iron was found to be protective. Contrary to these findings, two cohorts19, 38 observed increased risk with high heme iron intake, which became even more pronounced in participants with diet poor with fruit and vegetable intake19 or in former and current smokers taking vitamin C supplements38. However, with the exception of the AARP cohort19, none of the previous studies have estimated heme iron intake based on a specific database with measured values.

Investigation of the effect of meat by histological sub-type of lung cancer showed that similarly to our study, two studies found no difference in associations among different histological types39, 40. One study detected an increased risk for non-adenocarcinoma cell tumors31. in another study, risk was increased for all histological types, but was strongest for adenocarcinoma41, whereas two studies detected an increased risk for squamous cell carcinoma42, 43. Lam et al18 observed an elevated risk for both squamous and adenocarcinomas of the lung, whereas the NIH-AARP study detected an increased risk for squamous carcinoma in men and small cell carcinoma in women, although the risk between histological types was not statistically different19.

While we carefully controlled for multiple smoking characteristics, we lacked information on passive smoking, types of cigarettes smoked and depth of inhalation, which might have limited our ability to fully control for this powerful confounder. Thus, we acknowledge that even the modest non-significant effect of red and processed meat we are seeing may be due to residual confounding by smoking, given its strong effect. Nevertheless, we found no correlation between any of the smoking characteristics, and red or processed meat intake.

Only fruit and total fat intake were correlated with meat consumption in our analysis, however, neither of the two exposures was associated with lung cancer (data not shown). The fat content of meat has been postulated as one of the potential mechanisms relating meat to lung carcinogenesis, yet a pooled analysis of eight prospective studies observed no association of total fat, or any of the specific types of fat, with lung cancer risk44.

The prospective design of the PLCO trial, with diet being assessed prior cancer diagnosis, avoided the effect of recall or selection bias. To further limit the effect of reverse causality, we excluded all cases diagnosed within the first year of follow-up. Detailed information on meat cooking practicies, and the meat mutagen database allowed us to investigate different possible mechanisms for the effect of meat on lung cancer. However, we lacked information on B(a)P exposure from other dietary sources, which may be considerable45. FFQs are known to have substantial measurement error that can lead to bias in estimated diet-disease risks and, in multivariable models can lead to distorted confidence intervals. The measurement error related to the meat mutagen database is also likely to have caused further attenuation of the risk estimates. In addition, a narrow range of intake of high temperature cooked meats may have affected our ability to detect an association. On average, only approximately 20% of meats consumed in our cohort were meats cooked at high temperature. Participants enrolled in the PLCO trial were found to be better educated, more physically active, more likely to be married, less likely to smoke, and had lower all-cause and cause-specific standardized mortality than the general population46. They were also recruited among volunteers, hence it is reasonable to expect that they may have had healthier dietary behaviors. The FFQ was administered up to 4 years after baseline, at which time point no updates on participants' baseline characteristics were collected; some of those might have changed, therefore causing residual confounding in our analyses. The follow-up period in our cohort was relatively shorter compared to other cohorts19, 31, 32, 33, 34, 35, which certainly affected the statistical power of this analysis.

In conclusion, consumption of a diet high in red or processed meat was not associated with lung cancer in our population. We observed no effect of high temperature cooking methods, level of meat doneness, intake of meat mutagen or heme iron on risk of lung cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Baseline characteristics by quintile of red meat intake in participants of the PLCO Cancer Screening Trial*

	Men (N = 48,229)					Women (N = 51,350)				
Characteristics	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**
Red meat intake† (range and median, g/ 1000 kcal)	≤ 20.9 (14.5)	21.0 – 31.0 (26.2)	31.1 – 41.0 (35.8)	41.1 – 54.8 (47.1)	> 54.8 (67.1)	≤ 14.6 (9.4)	14.6– 22.8 (18.8)	22.9 – 31.0 (26.7)	31.1– 42.5 (36.1)	> 42.5 (52.8)
Age (y)	66.8~	66.3	65.9	65.3	64.5	66.0	65.8	65.3	64.9	64.5
BMI (kg/m^2)	26.3	27.1	27.5	28.0	28.7	25.6	26.5	27.0	27.6	28.3
1st degree relative with cancer (%)	51.8	51.9	52.6	53.3	53.3	59.9	60.2	60.5	60.6	59.7
Race (%)										
Non-Hispanic White	84.5	90.7	92.6	92.8	93.3	85.4	91.0	92.6	93.5	93.8
Non-Hispanic Black	4.3	2.8	2.2	2.3	1.9	6.8	4.0	3.2	2.7	2.2
Education, College graduate (%)	51.4	44.8	41.9	38.6	33.7	38.8	32.5	31.5	28.1	22.9
Smoking status (%)										
Never smokers	43.9	40.0	37.9	35.6	33.1	59.8	58.8	58.9	56.8	54.9
Former smokers, quit ≥ 1y ago	49.6	50.3	51.3	52.0	51.6	34.3	33.2	32.6	32.8	32.3
Current/former smoker quit <1 y ago	6.5	9.7	10.8	12.4	15.3	5.9	8.0	8.6	10.4	12.9
Age at smoking initiation (y)	18.2	17.9	17.8	17.8	17.6	19.6	19.7	19.4	19.5	19.4
Packs-years	33.5	36.4	38.0	39.2	43.3	25.8	28.0	28.5	30.1	32.3
Time since cessation (%)										
Quit ≥ 20 yrs ago	61.8	57.6	56.9	55.0	49.5	56.4	52.3	51.1	47.5	43.8
Quit <20 yrs and ≥ 10 yrs ago	23.8	25.6	25.5	26.2	28.8	26.1	26.1	25.7	29.1	29.1
Quit <10 yrs and ≥ 5 yrs ago	8.6	9.9	10.1	11.1	12.1	10.3	12.9	14.1	13.9	15.7
Quit <5 yrs and ≥ 1 yr ago	5.9	6.8	7.5	7.8	9.6	7.2	8.0	9.2	9.6	11.4
Married (%)	82.9	85.8	86.4	86.0	84.8	61.7	69.2	73.6	76.3	78.8

	Men (N = 48,229)					Women (N = 51,350)				
Characteristics	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**
Dietary intake										
Energy (kcal/d)	1905	1930	1981	2023	2137	1476	1453	1495	1512	1546
White meat‡ (g/1000 kcal)	28.5	25.0	25.3	25.9	26.7	35.4	32.7	32.4	32.9	33.6
Processed meat§ (g/1000 kcal)	5.6	8.6	10.8	13.2	18.0	3.8	5.7	7.4	9.3	12.6
Vegetables (servings/1000 kcal)	1.1	1.0	1.0	1.0	1.0	1.4	1.3	1.3	1.3	1.3
Fruits (servings/1000 kcal)	1.3	1.0	0.9	0.8	0.7	1.7	1.4	1.3	1.2	0.9
Total fat (g/1000 kcal)	30.2	34.2	36.0	37.8	41.0	30.0	33.0	34.9	36.5	39.4
Alcohol (servings/day)	1.3	1.1	1.0	0.9	0.8	0.3	0.4	0.4	0.4	0.3
β-carotene supplements (μg/day)	217	173	165	147	138	243	208	188	180	170

*P for trend < 0.01 for all variables except for age at smoking initiation (P = 0.02), alcohol intake (P = 0.39) and 1st degree relative with cancer (P = 0.99) in women, estimated by the Cohrain-Armitage tests for categorical variables and the *t* test for slope in generalized linear models for continuous variables.

†Included bacon, beef (including meat added to mixed dishes, such as pizza, chili, lasagna, stew), cold cuts, ham, hamburger, regular hotdogs, liver, pork, sausage, and steak.

‡All forms of poultry (chicken, cold cuts, ground, turkey), fish (fresh, frozen, canned), and low-fat hotdogs and sausages, which are usually made from turkey.

§All types of cold cuts, bacon, ham, hotdogs, and sausages from red and white meats.

~Mean (all such values).

Table 2

HRs and 95% CI for lung cancer across quintiles of red and processed meat intake in the PLCO Cancer Screening Trial participants (men = 48,229; women = 51,350)

	Quintiles of baseline meat intake					
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	P_{trend}
Men (Cases[†] = 454)						
Red meat[*] (g/1000 kcal)	≤ 20.9 (14.5)	> 20.9 and ≤ 31.0 (26.2)	> 31.0 and ≤ 41.0 (35.8)	> 41.0 and ≤ 54.8 (47.1)	> 54.8 (67.1)	
Age-and energy-adjusted HR[§]	1	1.27 (0.93, 1.72)	1.30 (0.96, 1.78)	1.49 (1.10, 2.02)	1.71 (1.26, 2.32)	<0.001
Multivariable HR[~]	1	1.02 (0.75, 1.41)	1.00 (0.72, 1.38)	1.06 (0.76, 1.47)	1.11 (0.79, 1.56)	0.42
Processed meat[*] (g/1000 kcal)	≤ 4.2 (2.6)	> 4.2 and ≤ 7.1 (5.6)	> 7.1 and ≤ 10.8 (8.7)	> 10.8 and ≤ 16.8 (13.3)	> 16.8 (22.6)	
Age-and energy-adjusted HR[§]	1	0.97 (0.71, 1.33)	1.28 (0.95, 1.72)	1.20 (0.89, 1.64)	1.52 (1.13, 2.03)	0.002
Multivariable HR[~]	1	0.85 (0.62, 1.18)	1.04 (0.76, 1.41)	0.97 (0.70, 1.33)	1.12 (0.83, 1.53)	0.22
Women (Cases[‡] = 328)						
Red meat (g/1000 kcal)	≤ 14.6 (9.4)	> 14.6 and ≤ 22.8 (18.8)	> 22.8 and ≤ 31.0 (26.7)	> 31.0 and ≤ 42.5 (36.1)	> 42.5 (52.8)	
Age-and energy-adjusted HR[§]	1	1.50 (1.04, 2.17)	1.85 (1.29, 2.65)	1.52 (1.04, 2.23)	1.81 (1.25, 2.62)	0.02
Multivariable HR[~]	1	1.33 (0.91, 1.94)	1.60 (1.10, 2.33)	1.24 (0.84,1.85)	1.30 (0.87, 1.95)	0.65
Processed meat[*] (g/1000 kcal)	≤ 2.4 (1.4)	> 2.4 and ≤ 4.4 (3.4)	> 4.4 and ≤ 6.9 (5.5)	> 6.9 and ≤ 11.6 (8.8)	> 11.6 (16.9)	
Age-and energy-adjusted HR[§]	1	1.42 (1.00, 2.00)	1.31 (0.92, 1.88)	1.05 (0.72, 1.53)	1.35 (0.95, 1.93)	0.51
Multivariable HR[~]	1	1.22 (0.86, 1.73)	1.09 (0.76, 1.57)	0.83 (0.56, 1.22)	0.98 (0.68, 1.41)	0.32

[*] Range (median).

[†] Adenocarcinomas (38%), squamous cell (21%), small cell (14%), large cell undifferentiated carcinomas (4%) other or not otherwise specified carcinoma (NOS) carcinomas (23%).

[‡] Adenocarcinomas (47%), squamous cell (14%), small cell (14%), large cell undifferentiated carcinomas (3%) and other or NOS carcinomas (22%).

[§] Cox proportional hazards regression model adjusted for age and energy intake as continuous variable, using age as the underlying time metric. Meats are computed as grams per 1000 kcal of total energy intake. Models with red and processed meat are adjusted for white and non-processed meat, respectively, summing to total meat.

[~] Cox proportional hazards regression model additionally adjusted for BMI, kg/m^2 (<18.5, ≥ 18.5 to <25, ≥ 25 to <30, ≥ 30, missing), race (Caucasian, Black or Hispanic/Asian/Pacific Islander/American Indian/Alaskan native/unknown), education (Less than high school or unknown, High school graduate, College graduate), years since quit smoking (*men*: quit ≥1 to <5 yrs ago, quit ≥ 5 to < 10 yrs ago, ≥10 to <20 yrs ago, ≥20 yrs ago vs. current and quit <1 yr ago and never smokers; *women*: quit ≥1 to <10 yrs ago, quit ≥ 10 yrs ago vs. current and quit <1 yr ago and non-smokers), packs of cigarettes smoked per day (*men*: ≤ 1 packs, > 1 to ≤ 2 packs, > 2 packs/day vs. never smokers; *women*: ≤ 1 packs, > 1 packs/day vs. never smokers), age started smoking (continuous variable, 0 for never-smokers), number of

years smoked (continuous variable, 0 for never-smokers), using pipe or cigar (current vs. former and never user) and intake of alcohol (servings/day) (categorical), energy-adjusted box-cox transformed vegetable and fruit servings, and total fat (continuous).

Table 3

HRs and 95% CI across tertiles of meat intake by cooking methods and level of doneness in the PLCO Cancer Screening Trial participants (men = 48,229; women = 51,350)

	Men (Cases = 454)				Women (Cases = 328)			
	T1	T2	T3	P_{trend}	T1	T2	T3	P_{trend}
Meat by meat-cooking methods								
Grilled/barbecued* (g/1000 kcal)	≤ 2.2 (0.8)	> 2.2 to ≤ 6.5 (4.0)	> 6.5 (11.2)		≤ 1.4 (0.5)	> 1.4 to ≤ 4.8 (2.8)	> 4.8 (8.7)	
Age-and energy-adjusted HR †	1	1.01 (0.81, 1.25)	0.89 (0.70, 1.13)	0.32	1	0.98 (0.76, 1.27)	0.96 (0.73, 1.28)	0.59
Multivariate HR‡	1	1.01 (0.81, 1.27)	0.88 (0.69, 1.14)	0.30	1	1.04 (0.80, 1.35)	0.89 (0.66, 1.19)	0.23
Pan-fried* (g/1000 kcal)	≤ 1.4 (0.6)	> 1.4 to ≤ 4.5 (2.7)	> 4.5 (8.0)		≤ 0.8 (0.3)	> 0.8 to ≤ 3.0 (1.7)	> 3.0 (5.7)	
Age-and energy-adjusted HR †	1	1.06 (0.84, 1.35)	1.45 (1.15, 1.82)	0.0004	1	1.11 (0.85, 1.47)	1.44 (1.10, 1.89)	0.01
Multivariate HR‡	1	0.89 (0.69, 1.14)	0.97 (0.76, 1.25)	0.82	1	0.91 (0.69, 1.22)	1.01 (0.76, 1.36)	0.87
Oven-broiled* (g/1000 kcal)	0	> 0 to ≤ 2.0 (0.9)	> 2.0 (4.3)		0	> 0 to ≤ 1.1 (0.5)	> 1.1 (2.9)	
Age-and energy-adjusted HR †	1	0.97 (0.77, 1.23)	1.06 (0.85, 1.32)	0.55	1	1.36 (1.04, 1.78)	1.12 (0.87, 1.46)	0.81
Multivariate HR‡	1	0.98 (0.77, 1.24)	0.98 (0.78, 1.23)	0.90	1	1.24 (0.94, 1.62)	0.96 (0.74, 1.25)	0.38
Sautéed/microwaved/baked* (g/1000 kcal)	≤ 0.7 (0)	> 0.7 to ≤ 2.9 (1.6)	> 2.9 (5.5)		≤ 1.1 (0.2)	> 1.1 to ≤ 3.8 (2.1)	> 3.8 (6.9)	
Age-and energy-adjusted HR †	1	0.99 (0.79, 1.25)	0.99 (0.78, 1.24)	0.91	1	0.89 (0.68, 1.17)	1.05 (0.80, 1.38)	0.51
Multivariate HR‡	1	0.99 (0.79, 1.25)	1.01 (0.80, 1.28)	0.89	1	0.96 (0.73, 1.26)	1.12 (0.85, 1.48)	0.34
Meat by level of doneness								
Rare/medium done* (g/1000 kcal)	≤ 1.5 (0.1)	> 1.5 to ≤ 5.6 (3.2)	> 5.6 (9.9)		≤ 0.6 (0)	> 0.6 to ≤ 2.9 (1.5)	> 2.9 (5.7)	
Age-and energy-adjusted HR †	1	1.12 (0.90, 1.41)	1.13 (0.90, 1.43)	0.31	1	1.17 (0.90, 1.53)	1.27 (0.97, 1.68)	0.12
Multivariate HR‡	1	1.05 (0.83, 1.32)	0.94 (0.74,1.21)	0.59	1	1.09 (0.83, 1.44)	1.08 (0.81, 1.44)	0.77
Well/very well done* (g/1000 kcal)	≤ 2.1 (0.9)	> 2.1 to ≤ 5.7 (3.6)	> 5.7 (9.5)		≤ 1.8 (0.7)	> 1.8 to ≤ 5.1 (3.2)	> 5.1 (8.7)	
Age-and energy-adjusted HR †	1	1.11 (0.88, 1.38)	1.11 (0.88, 1.40)	0.47	1	1.15 (0.89, 1.50)	1.05 (0.79, 1.39)	0.96
Multivariate HR‡	1	1.05 (0.84, 1.32)	0.90 (0.71, 1.15)	0.31	1	1.10 (0.84, 1.43)	0.89 (0.67, 1.18)	0.25

* Range (median).

[†]Cox proportional hazards regression model adjusted for age and energy intake as continuous variable, using age as the underlying time metric. Meats are computed as grams per 1000 kcal of total energy intake. The relevant meat groups by cooking methods and doneness are simultaneously entered in the models and adjusted for meat with no information on cooking and doneness level, respectively, to account for total meat. i.e. **Model 1**: grilled/BBQ, pan-fried, oven-broiled, sauteed/microwaved/baked adjusted for meat with no cooking information; **Model 2**: rare, well/very-well done meat adjusted for meat with no doneness level information).

[‡]Cox proportional hazards regression model additionally adjusted for BMI, kg/m^2 (<18.5, ≥ 18.5 to <25, ≥ 25 to <30, ≥ 30, missing), race (Caucasian, Black or Hispanic/Asian/Pacific Islander/American Indian/Alaskan native/unknown), education (Less than high school or unknown, High school graduate, College graduate), years since quit smoking (*men*: quit ≥1 to <5 yrs ago, quit ≥ 5 to < 10 yrs ago, ≥10 to <20 yrs ago, ≥20 yrs ago vs. current and quit <1 yr ago and never smokers; *women*: quit ≥1 to <10 yrs ago, quit ≥ 10 yrs ago vs. current and quit <1 yr ago and non-smokers), packs of cigarettes smoked per day (*men*: ≤ 1 packs, > 1 to ≤ 2 packs, > 2 packs/day vs. never smokers; *women*: ≤ 1 packs, > 1 packs/day vs. never smokers), age started smoking (continuous variable, 0 for never-smokers), number of years smoked (continuous variable, 0 for never-smokers), using pipe or cigar (current vs. former and never user) and intake of alcohol (servings/day) (categorical), energy-adjusted box-cox transformed vegetable and fruit servings, and total fat (continuous).

Table 4

HRs and 95% CI for lung cancer across quintiles of intake of meat mutagens and heme iron in the PLCO Cancer Screening Trial participants (men = 48,229; women = 51,350)

	Men (Cases = 454)				Women (Cases = 328)			
	Q1	Q3	Q5	P_{trend}	Q1	Q3	Q5	P_{trend}
Meat mutagens								
Overall mutagenic activity* (revertant colonies/1000 kcal)	≤ 465 (282)	> 836 to ≤ 1317 (1055)	> 2182 (3186)		≤ 347 (186)	> 688 to ≤ 1147 (893)	> 2025 (3048)	
Age-and energy-adjusted HR†	1	0.96 (0.72, 1.29)	1.21 (0.91, 1.62)	0.22	1	1.13 (0.80, 1.59)	1.23 (0.86, 1.76)	0.50
Multivariable HR‡	1	0.89 (0.66, 1.20)	0.98 (0.72, 1.33)	0.79	1	1.05 (0.73, 1.50)	1.02 (0.70, 1.47)	0.64
MeIQx* (ng/1000 kcal)	≤ 2.9 (1.7)	> 5.5 to ≤ 8.9 (7.0)	> 15.1 (22.0)		≤ 2.2 (1.2)	> 4.2 to ≤ 7.1 (5.5)	> 12.2 (17.9)	
Age-and energy-adjusted HR†	1	1.07 (0.79, 1.45)	1.48 (1.11, 1.98)	0.01		0.88 (0.61, 1.28)	1.29 (0.91, 1.83)	0.13
Multivariable HR‡	1	0.89 (0.65, 1.22)	1.01 (0.74, 1.37)	0.90		0.81 (0.55, 1.19)	0.95 (0.66, 1.38)	0.70
DiMeIQx* (ng/1000 kcal)	≤ 0.10 (0.02)	> 0.29 to ≤ 0.57 (0.42)	> 1.1 (1.70)		≤ 0.08 (0.02)	> 0.25 to ≤ 0.52 (0.37)	> 1.04 (1.65)	
Age-and energy-adjusted HR†	1	1.05 (0.78, 1.42)	1.24 (0.92, 1.66)	0.40		0.96 (0.69, 1.35)	1.07 (0.76, 1.51)	0.47
Multivariable HR‡	1	0.99 (0.73, 1.34)	1.07 (0.79, 1.44)	0.94		0.99 (0.71, 1.39)	1.02 (0.72, 1.45)	0.67
PhIP* (ng/1000 kcal)	≤ 8.8 (4.8)	> 17.9 to ≤ 30.8 (23.5)	> 55.8 (86.1)		≤ 5.8 (2.8)	> 13.8 to ≤ 25.8 (19.0)	> 50.3 (80.5)	
Age- and energy-adjusted HR†	1	0.92 (0.70, 1.22)	0.93 (0.70, 1.24)	0.94		0.92 (0.65, 1.31)	1.24 (0.88, 1.74)	0.22
Multivariable HR‡	1	0.94 (0.71, 1.24)	0.85 (0.63, 1.15)	0.46	1	0.86 (0.60, 1.23)	1.03 (0.72, 1.47)	0.93
B(a)P* (ng/1000 kcal)	≤ 1.3 (0.6)	> 3.9 to ≤ 8.3 (5.7)	> 17.5 (28.1)		≤ 0.9 (0.4)	> 2.6 to ≤ 5.6 (3.9)	> 11.9 (19.8)	
Age-and energy-adjusted HR†	1	0.88 (0.67, 1.17)	1.03 (0.78, 1.37)	0.35	1	0.83 (0.60, 1.15)	0.71 (0.50, 1.01)	0.07
Multivariable HR‡	1	0.95 (0.72, 1.26)	0.99 (0.74, 1.33)	0.60		0.99 (0.71, 1.36)	0.72 (0.50, 1.04)	0.07
Heme iron* (μg/1000 kcal)	≤ 106 (72)	> 162 to ≤ 224 (191)	> 317 (411)		≤ 72 (47)	> 114 to ≤ 160 (136)	> 227 (292)	
Age-and energy-adjusted HR†	1	1.07 (0.78, 1.46)	1.26 (0.93, 1.72)	0.24	1	1.06 (0.75, 1.49)	1.22 (0.86, 1.71)	0.11
Multivariable HR‡	1	0.88 (0.64, 1.22)	0.91 (0.65, 1.28)	0.37	1	0.90 (0.63, 1.28)	0.89 (0.62, 1.29)	0.78

*Range (median).

§Cox proportional hazards regression model adjusted for age and energy intake as continuous variable, using age as the underlying time metric.

‡Cox proportional hazards regression model additionally adjusted for BMI, kg/m^2 (<18.5, ≥ 18.5 to <25, ≥ 25 to <30, ≥ 30, missing), race (Caucasian, Black or Hispanic/Asian/Pacific Islander/American Indian/Alaskan native/unknown), education (Less than high school or unknown, High school graduate, College graduate), years since quit smoking (*men*: quit ≥1 to <5 yrs ago, quit ≥ 5 to < 10 yrs ago, ≥10 to <20 yrs ago, ≥20 yrs ago vs. current and quit <1 yr ago and never smokers; *women*: quit ≥1 to <10 yrs ago, quit ≥ 10 yrs ago vs. current and quit <1 yr ago and non-smokers), packs of cigarettes smoked per day (*men*: ≤ 1 packs, > 1 to ≤ 2 packs, > 2 packs/day vs. never smokers; *women*: ≤ 1 packs, > 1 packs/day vs. never smokers), age started smoking (continuous variable, 0 for never-smokers), number of years smoked (continuous variable, 0 for never-smokers), using pipe or cigar (current vs. former and never user) and intake of alcohol (servings/day) (categorical), energy-adjusted box-cox transformed vegetable and fruit servings, and total fat (continuous).