Prediagnostic Calcium Intake and Lung Cancer Survival: A Pooled Analysis of 12 Cohort Studies

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Abstract

PURPOSE: Lung cancer is the leading cause of cancer death worldwide. Its prognosis is usually poor and little is known about whether prediagnostic nutritional factors may affect its survival. Using data from the Calcium and Lung Cancer Pooling Project, including 12 prospective cohort studies in the US, Europe, and Asia, we examined the associations of prediagnostic calcium intake from foods and/or supplements with lung cancer survival.

PATIENTS AND METHODS: The present analysis included 23,882 incident, primary lung cancer patients. Information on participants' sociodemographics, diets and lifestyles, medical history, and anthropometrics was collected at the baseline survey of each cohort. Dietary calcium intake was estimated based on the cohort-specific food frequency questionnaires linked with country-specific food composition tables and standardized to caloric intake of 2000 kcal/d for women and 2500 kcal/d for men. Lung cancer incidence, clinical tumor characteristics and subsequent vital status were ascertained per individual cohort follow-up protocol. Stratified, multivariable-adjusted Cox regression was used to compute hazard ratios (HRs) and 95% confidence intervals (CIs) for the associations between prediagnostic calcium intakes and survival among lung cancer patients.

RESULTS: A total of 19,538 lung cancer patients died during cohort follow-ups with 5-year survival rates of 56%, 21%, and 5.7% for cancer diagnosed at localized, regional, and distant stages, respectively. Low prediagnostic dietary calcium intake (<500-600 mg/d, less than half of the recommended intakes), was associated with a significant, although small, increase in risk of death compared with recommended calcium intakes (800-1200 mg/d); HR (95% CI) was 1.07 (1.01, 1.13) after adjusting for age, stage, histologic type, grade, smoking status, pack-years, and other potential prognostic factors. The association between low calcium intake and higher lung

cancer case mortality was evident primarily among patients diagnosed at localized and regional stages, with HR (95% CI) of 1.15 (1.04, 1.27). Among early-stage patients, the association between calcium intake and lung cancer survival seemed differed by sex. Compared with recommended intakes, low dietary calcium intake was associated with increased mortality in men (HR [95% CI] = 1.25[1.08, 1.45]), but not in women (HR [95% CI] = 1.08 [0.92, 1.25]). However, in women, a very high calcium intake (>1500-1800 mg/d) appeared to be associated with increased mortality (HR [95% CI] = 1.33 [1.05-1.70]). No association was found for prediagnostic supplemental calcium intake with lung cancer survival in the multivariable-adjusted model.

CONCLUSION: This large pooled analysis is the first, to our knowledge, to show that low and possibly very high prediagnostic dietary calcium intakes were associated with poorer survival among early-stage lung cancer patients, suggesting that calcium may play an important role in lung cancer prognosis.

Lung cancer is the most common cancer and the leading cause of cancer death in the world, accounting for approximately 1.8 million new cases (13% of all cancer cases) and 1.6 million deaths (20% of all cancer deaths) annually.[1] Most lung cancer patients are diagnosed at advanced stages when the possibility of cure is low, resulting in an overall 5-year survival rate of ~18% in the United States (US) and even lower in other countries.[2–4] While prognosis of lung cancer largely depends on clinical and pathological factors, such as stage, histologic type, treatment options, and patients' demographics and comorbidity status,[2, 5, 6] emerging evidence suggests that prediagnostic nutrition and lifestyle factors may also influence lung cancer survival.[7–9]

Both experimental and epidemiological studies have suggested potential roles of calcium in cancer development and progression.[10] Besides its well-known effects on bone health, calcium intake and calcium homeostasis can directly or indirectly affect cell proliferation, differentiation, and apoptosis, parathyroid hormone (PTH) and PTH-related peptide, vitamin D metabolism and signaling, angiogenesis, and immune response.[11, 12] Prospective cohort studies and meta-analyses of cohort studies have linked sufficient calcium intake with a decreased overall cancer risk and risks of specific cancers, including colorectal, breast, and prostate cancers.[13–19] Evidence, although limited, has also linked calcium intake with risk of lung cancer.[20, 21,22] To our knowledge, only a very few cohort studies have examined the association of prediagnostic calcium intake with cancer survival, and none have examined the association with lung cancer survival.[23, 24]

The aim of the present analyses is to investigate prediagnostic calcium intake from foods and supplements in relation to lung cancer survival. This is part of a large pooling project that collected individual-level data from nearly 1.9 million participants from 12 cohort studies in the US, Europe, and Asia (The Calcium and Lung Cancer Pooling Project). The present paper

focuses on the association of prediagnostic calcium intake with lung cancer survival among 23,882 incident cases who were diagnosed with primary lung cancer during cohort follow-ups. We examined the association among all cases combined and separately by major lung cancer prognostic factors, including age, stage, and histology.

Methods

Study Population

Twelve large, prospective cohort studies participated in The Calcium and Lung Cancer Pooling Project, including eight US cohorts: the National Institutes of Health-AARP study (NIH-AARP),[25] the Health Professionals' Follow-Up Study (HPFS),[26] the Nurses' Health Study I (NHS),[27] the Iowa Women's Health Study (IWHS),[28] the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO),[29] the Southern Community Cohort Study (SCCS),[30] the Vitamins and Lifestyle Cohort Study (VITAL),[31] and the Women's Health Initiative Observational Study (WHI);[32] one European cohort: the European Prospective Investigation into Cancer and Nutrition Cohort (EPIC);[33] and three Asian cohorts: the Japan Public Health Center-based Prospective Study cohort I and II (JPHC),[34] the Shanghai Men's Health Study (SMHS),[35] and the Shanghai Women's Health Study (SWHS).[36] Each study was approved by the Institutional Review Board at local institutions; and the pooling project was approved by the Vanderbilt University Institutional Review Board.

Assessment of Dietary and Supplemental Calcium Intake

Usual dietary intakes were assessed at baseline in each cohort using a self- or interviewer-administered food-frequency questionnaire (FFQ). The FFQs usually inquired about the average consumption of common food items over the past 12 months and were validated against 24-hour

dietary recalls, 7-day food records, or dietary biomarkers. Daily food intakes were estimated based on the frequency and amount of consumption and were linked to country-specific food composition tables to calculate intakes of energy (kcal/d), calcium (mg/d), and other nutrients. Details on the FFQs, calibration studies, and estimation of nutrient intake can be found in previous publications.[37–48] In the present study, dietary intakes were adjusted for total energy intake using the nutrient density method[49] and standardized to intakes per 2,000 kcal for women and per 2,500 kcal for men.

Intake of supplemental calcium was assessed in eight US cohorts. Participants were asked whether in the past year they generally took supplements (multivitamins and/or single calcium supplements); and if yes, how often (from less than once per week to every day) and how much they usually took (from less than 200 mg/d to more than 1000 mg/d for calcium). Most cohorts estimated supplemental calcium intakes from both calcium supplements and multivitamins, except that the SCCS asked only about the use of calcium supplements.

Assessment of Lung Cancer Incidence and Survival

Incident cancer cases and the vital status of cancer patients were identified in each cohort through linkages with regional or national cancer registries and death registries, follow-up interviews with cohort participants or their next of kin, review of medical records and/or death certificates, or these methods combined. Cancers of the bronchus and lung were ascertained by the *International Classification of Diseases* (ICD) codes: 162 (ICD-9) or C34 (ICD-10). Clinical tumor features were obtained when available, including stage, histologic type and grade. We harmonized the tumor information across studies. For stage, lung cancer cases were classified into localized, regional, distant, and unknown stages. For histologic type, lung cancer cases were classified into adenocarcinoma, squamous cell carcinoma, other non-small cell lung cancer,

small cell lung cancer, and all other types. For grade, lung cancer cases were classified into well-, moderately-, and poorly-differentiated, undifferentiated, and unknown grades. Lung cancer survival time was counted from the date of lung cancer diagnosis to the date of death or the end of follow-up, whichever came first. Information on year of lung cancer diagnosis and whether lung cancer was the underlying cause of death was acquired from all participating cohorts.

Assessment of Non-dietary Covariates

Each study collected baseline information on sociodemographics, lifestyles, medical history, and anthropometrics. We harmonized these data and generated uniform variables to be used in the statistical analyses, including age at baseline and at diagnosis (years, integer), sex (male or female), race/ethnicity (non-Hispanic white, Black, Asian, or other), educational attainment (≤ high school, vocational school or some college, college or graduate school), smoking status (never, former, or current use of cigarettes, cigars, or pipe), pack-years of cigarette smoking (continuous), alcohol drinking status (none, moderate, or heavy [>14 g/d for women and >28 g/d for men]), physical activity level (low, middle, or high [cutoffs: zero leisure-time physical activity and median of non-zero leisure-time physical activity assessed by metabolic equivalents in the EPIC, HPFS, VITAL, WHI, SCCS, SMHS, and SWHS or by hours in the NIH-AARP, IWHS, NHS, and PLCO; or tertile of total physical activity metabolic equivalents in the JPHC]), history of diabetes (yes or no), obesity status (body mass index [BMI] <18.5, 18.5-24.99, 25.0-29.99, or ≥30 kg/m^2), and in women, postmenopausal status (yes or no) and use of hormone therapy (never or ever).

The proportion of missing values was generally less than 10% in each cohort that measured the variable. If the proportion of missing values was <3%, we assigned the median non-missing

value for continuous variables (e.g. BMI) and the most frequent category for categorical variables (e.g. education). If the proportion of missing variables was ≥3%, we used a multivariate imputation to estimate missing value based on other covariates, calcium intake, energy intake, and lung cancer and death outcomes (fully conditional specification methods in the SAS PROC MI procedure). Missing data imputation was processed for each cohort separately. Specifically, in the JPHC, Cohort I did not have data on physical activity metabolic equivalents and Cohort II did not collect information on education level; we imputed these two variables using the above described method in JPHC Cohort I and II data combined.

Analytic population

Participants were excluded if they had 1) a history of any cancer except non-melanoma skin cancer prior to diagnosis of lung cancer, 2) missing diagnosis or survival time information, 3) missing calcium intakes or smoking status information, or 4) implausible total energy intake (beyond three standard deviations of the cohort- and sex-specific log-transformed mean energy intake or beyond the pre-determined range in six cohorts: HPFS, NHS, IWHS, SCCS, VITAL, and WHI). A total of 24,440 first, primary lung cancer cases diagnosed after the baseline survey among 1,679,842 eligible participants of the Calcium and Lung Cancer Pooling Project were considered eligible for the current study. We further excluded 11 cases with cancer in situ and 547 cases that had missing data on both stage and histology, leaving a total of 23,882 incident lung cancer cases in the present analyses.

Statistical Analysis

Usual dietary calcium intakes were calculated and compared among lung cancer patients with different baseline characteristics and tumor features using the general linear model (adjusted for age at baseline, sex, and total energy intake). Corresponding 5-year survival rates were estimated

by the life-table method and *P* for differences was evaluated via the log-rank test with Bonferroni correction.

The Cox proportional hazard model was used to estimate hazard ratios (HRs) and 95% confidence intervals (95% CIs) of death among lung cancer patients with different prediagnostic calcium intakes. The Cox model was stratified by cohort, year of lung cancer diagnosis (5-year intervals from earlier than 1990 to later than 2010), and time interval between dietary assessment and lung cancer diagnosis (<4, 4-7, 7-10, and >10 years, according to the quartile distribution). Potential confounding factors that were associated with calcium intake and/or lung cancer survival were adjusted for, including age at diagnosis, total energy intake, sex, race/ethnicity, education, smoking status, pack-years of cigarette smoking, alcohol consumption, physical activity level, history of diabetes, obesity status, use of hormone therapy in women, and the stage, histologic type, and grade of lung cancer. Considering the interplay of calcium, magnesium, vitamin D, and phosphorus, we further adjusted for, when data were available, dietary intakes of magnesium (in all cohorts), vitamin D (in 9 cohorts), and phosphorus (in all cohorts), individually or together, with or without interaction terms with calcium. However, the associations of dietary calcium with lung cancer survival were basically unchanged, so these nutrients were not included in the final model.

Calcium intakes were analyzed as categorical variables and as continuous variables. We used the *Dietary Reference Intakes* recommended by the US Institute of Medicine as project-wide cut points for dietary and total calcium intakes.[11] Briefly, for men age 19-70 years and women age 19-50 years, the estimated average requirement (EAR) of calcium is 800 mg/d and the recommended dietary allowance (RDA) is 1000 mg/d; and for men above age 70 years and women above age 50 years, the EAR is 1000 mg/d and the RDA is 1200 mg/d. Participants were

classified into five groups based on their calcium intakes: less than 0.5 RDA, 0.5 RDA to EAR, EAR to RDA, RDA to 1.5 RDA, or higher than 1.5 RDA. The cut points for supplemental calcium intake were 0, 200, 500, and 1000 mg/d. A joint analysis was conducted by dietary and supplemental calcium intakes to examine specifically the association for supplemental calcium among those who had low dietary calcium intake and the association for dietary calcium among those who had no or little supplemental calcium intake. Calcium intakes were also modeled continuously in restricted cubic spline analyses to examine dose-response associations. Men and women in the sex-specific top and bottom 1% of calcium intakes were excluded from the spline analyses. Three knots were chosen based on model fitness, at the 5^{th}, 50^{th}, and 95^{th} percentiles (correspondingly 425, 910, and 1625 mg/d). The referent intake was 900 mg/d and all potential confounders listed above were included in the spline regression.

Stratified analyses were performed by potential effect modifiers, including age at diagnosis, sex, race/ethnicity, education, smoking, other lifestyle factors, stage, histologic type, grade, and time interval between dietary assessment and cancer diagnosis. *P* for interaction was evaluated via likelihood ratio test comparing models with and without the interaction term (calcium intake category × stratification variable). A series of sensitivity analyses were conducted by excluding those diagnosed with lung cancer within two years after the baseline, by excluding those who died or were lost to follow-up within three months after lung cancer diagnoses, or by examining lung cancer-specific mortality. Meta-analysis was applied as an alternative approach to pooled analysis. Cohort-specific HRs and 95% CI were calculated and then combined using a fixed-effect model because no significant between-study heterogeneity was detected. Finally, we explored the associations of lung cancer survival with three major calcium food sources: dairy products, green leafy vegetables, and soy foods. Two-sided *P* values of <0.05 were considered

statistically significant. All analyses were conducted using SAS software, version 9.4 (SAS Institute, Inc.).

Results

Among ~1.7 million men and women from 12 cohort studies, 23,882 incident primary lung cancer cases were identified during a median follow-up of 7 years (interquartile range: 4-10 years). Among lung cancer patients, 19,538 died (16,279 due to lung cancer) with a median survival time of 11 months (interquartile range: 4-34 months). The overall 5-year survival rate was 21.3%. Higher survival rates were associated with younger age at diagnosis, female gender, never smoking, fewer pack-years if ever smoked, no history of diabetes, and a higher level of physical activity **(Table 1)**. Particularly low 5-year survival rates were found for small cell lung cancer (9.7%) vs. adenocarcinoma (27.7%), distant stage (5.7%) vs. localized or regional stage (56.4% or 21.1%, respectively), and undifferentiated (10.4%) vs. well- or moderately-differentiated tumor cells (57.9% or 37.3%, respectively). Usual dietary calcium intakes were higher among study patients in the US and European cohorts than in Asian cohorts **(Supplemental Table 1)**, and were positively associated with past smoking, physical activity, moderate alcohol consumption, BMI, history of diabetes, and use of hormone therapy in women. Prediagnostic dietary calcium intakes were similar among patients with different tumor characteristics. The mean dietary calcium intake was 927 and 945 mg/d in non-small cell and small cell cases, and 912 and 904 mg/d in early-stage and advanced-stage cases, respectively.

A majority of lung cancer patients (78.1%) reported dietary calcium intakes at baseline from half to 1.5-fold of the RDA (1000 or 1200 mg/d, based on age and sex); however, 15.5% of patients consumed less than 0.5 RDA and 6.4% consumed more than 1.5 RDA. A low dietary

calcium intake (<0.5 RDA) was associated with a small but significantly increased risk of death compared with the recommended calcium intake (800-1200 mg/d) **(Table 2)**; the corresponding HRs (95% CIs) were 1.14 (1.08, 1.20) in the model adjusted for age, sex, and total energy; and 1.07 (1.01, 1.13) in the model further adjusted for multiple risk factors, including stage, histologic type, grade, and all other characteristics listed in Table 1. Supplemental calcium intake was not associated with lung cancer survival, although in the age/sex/energy-adjusted model, 200-1000 mg/d calcium supplementation appeared to be associated with a reduced risk of death (Table 2).

Stratified analysis showed that the association of a low prediagnostic calcium intake with poor lung cancer survival was more evident in men than in women (*P* for interaction = 0.01), and in early-stage cases than in distant-stage cases (*P* for interaction = 0.006) **(Figure 1)**. In particular, a low calcium intake (<0.5 RDA) was associated with a 15% increased mortality in male patients (95% CI: 6-25%), and a 15% increase in localized or regional stage patients (95% CI: 4-27%) compared with the recommended level of calcium intake. The association appeared slightly stronger in White and Asian patients than in Black patients and in never smokers than in ever smokers; however, neither *P* for interaction was significant. We did not observe significant interactions by other potential effect modifiers (Figure 1).

We thereafter focused our analyses among early-stage lung cancer cases (n=8,103). The risks of death by different levels of prediagnostic calcium intake are shown in **Table 3**. A low dietary calcium intake (<0.5 RDA vs. RDA) was significantly associated with increased lung cancer mortality in early-stage patients, especially for men (HR [95% CI] = 1.25 [1.08, 1.45]) and never smokers (HR [95% CI] = 1.45 [1.01, 2.08]). Notably, we also observed that a very high calcium intake (>1.5 RDA vs. RDA) was associated with increased mortality in early-stage female

patients with HR (95% CI) of 1.33 (1.05, 1.70), although there were only 89 deaths and 134 female patients with a calcium intake this high. We did not observe a similarly increased risk in early-stage male patients. Among early-stage patients who had a low dietary calcium intake, supplemental calcium intake showed a possible trend of inverse association with death risk; compared with no or little supplemental calcium (<200 mg/d), HRs (95% CIs) were 0.90 (0.71, 1.13) and 0.68 (0.44, 1.07) for supplemental calcium of 200-1000 and >1000 mg/d, respectively. Meanwhile, among patients who had no or little supplemental calcium intake, the HR (95% CI) for a low dietary calcium intake (<0.5 RDA vs. RDA) was 1.17 (1.02, 1.35).

In cubic spline modeling, the lowest mortality among early-stage lung cancer patients was observed for dietary calcium intakes of 800-1200 mg/d (**Figure 2a,** P = 0.03). Consistent with the above findings, low dietary calcium intake was associated with increased mortality, especially among early-stage male patients (Figure 2b). Meanwhile, very high calcium intake might also be associated with increased mortality among early-stage female patients (Figure 2c), although the confidence interval was very wide.

Results were robust in sensitivity analyses and in meta-analysis. The HRs (95% CI) in early-stage cases for low dietary calcium intake were 1.14 (1.02, 1.28) after excluding those diagnosed with lung cancer within two years after baseline (n=6,362), 1.17 (1.05, 1.30), after excluding those who died within three months after lung cancer diagnosis (n=7,246), 1.15 (1.02, 1.28) for lung cancer-specific deaths, and 1.14 (1.02, 1.28) in a fixed-effect meta-analysis (P for heterogeneity = 0.39) **(Supplemental Figure 1)**. Among major calcium-contributing foods, a higher intake of green leafy vegetables was associated with a better lung cancer survival; the HRs (95% CIs) were 0.88 (0.81, 0.95) for intakes >40 vs. <10 g/d (approximately >0.5 serving/d

vs. <1 serving/week) and 0.97 (0.95, 0.99) for every 50 g/d increase. No significant associations were found for dairy products and soy foods.

Discussion

In this large pooled analysis of 12 cohort studies, we observed that a low prediagnostic dietary calcium intake (<500-600 mg/d) was associated with a slightly increased risk of death among lung cancer patients, after taking other prognostic factors into account. The lowest case mortality was observed for dietary calcium intakes of 800-1200 mg/d; any further increase in calcium intake did not offer additional benefit. The association between low prediagnostic calcium intake and lung cancer survival was primarily confined to patients diagnosed at early stages. No significant association was found for prediagnostic supplemental calcium intake with lung cancer survival.

For the first time, our study provides epidemiological evidence that a long-term insufficient calcium intake may influence lung cancer prognosis, especially for early-stage patients. Metastatic spread is the major reason for cancer-related deaths.[50] For lung cancer, one of the most frequent sites of metastasis is the bone, occurring in nearly 40% of patients.[51–53] It is possible that impaired bone metabolism and calcium homeostasis due to a longstanding calcium insufficiency may facilitate bone metastasis and promote tumor growth in lung cancer. Bone is a metabolically active tissue that undergoes a constant remodeling process via breaking down old and building up new skeletal tissues (bone resorption and formation). Calcium is an essential nutrient for this process. A prolonged calcium deficiency leads to increased bone resorption and compromised bone health.[11] Meanwhile, when the circulating calcium level drops because of a low calcium supply, multiple signaling pathways, hormones, and cytokines are affected.

Directly, calcium is a second messenger and calcium signaling regulates cell differentiation, proliferation, and apoptosis.[10, 54] Indirectly, to maintain a proper extracellular calcium level under calcium insufficiency, the body up-regulates secretions of PTH, 1,25-dihydroxyvitamin D, as well as several bone- or T cell-derived cytokines and growth factors, e.g. receptor activator of nuclear factor kappa-B ligand (RANKL), macrophage colony-stimulating factor (M-CSF), vascular endothelial growth factor (VEGF), and interleukin-6 (IL-6).[55] Activation of these pathways has been shown to enhance tumor growth, block apoptosis, promote angiogenesis, and accelerate metastasis.[52, 55] During tumor progression, bone remodeling and calcium homeostasis are further disturbed. Tumor cells secrete factors that increase RANKL expression and bone resorption, including PTH-related peptide, M-CSF, IL-6, and tumor necrosis factor; in turn, growth factors released from the bone stimulate tumor growth and metastasis. This vicious cycle of bone destruction and tumor progression has been well documented as an unfavorable prognostic factor of lung cancer.[53, 56, 57] Therefore, bone-targeted therapies have been used to reduce bone metastases and prolong lung cancer survival;[58, 59] and assessments of bone condition and bone metastasis have been recommended throughout the lung cancer treatment.[60, 61] Although our study did not have clinical information to identify and categorize patients according to bone or other metastasis status, our findings suggest that patients with habitually low calcium intakes may be at particular risk; and assessments of bone health and calcium homeostasis might be of benefit to these patients, especially for those who were diagnosed with an early-stage lung cancer for a proper intervention.

Previous studies have shown that poor calcium nutrition may contribute to development of several cancers, including lung cancer.[10] Low dietary calcium intake (e.g. <500 mg/d) has been linked to increased risks of colorectal cancer[13–15] and premenopausal breast cancer,[16, 17] and

possibly lung cancer, as shown in the SWHS, the NIH-AARP, and the EPIC-Heidelberg study.[20–22] However, very high calcium intake (e.g. >1800 mg/d) has also been associated with an increased risk of prostate cancer.[18, 19] A U-shaped relationship was found in recent meta-analyses of calcium intake with mortality from all causes, cardiovascular disease, and cancer.[62, 63] It is noteworthy that our results also suggest a possible U-shaped association between dietary calcium and lung cancer survival with an optimal intake around 1000 mg/d; while intakes lower than 500 mg/d (especially among early-stage male patients) or higher than 1800 mg/d (especially among early-stage female patients) were both associated with increased mortality. However, the number of patients with excessive dietary calcium intake was small. In addition, the increased risk among very high calcium consumers might reflect indirectly a history of poor calcium nutrition and compromised bone health, which may lead to a subsequent increase in calcium consumption, particularly for women, since they are more likely to be affected by and diagnosed with osteoporosis. Although the sex-differential associations between prediagnostic calcium intake and lung cancer survival observed in this study could be due to chance, they are intriguing and may be worth further investigation. Possible explanations include gender differences in calcium intake level, lung cancer histology, estrogen exposure, and lifestyle habits. Compared with female patients, male patients had on average a lower 5-y survival rate. While they were less likely to develop adenocarcinoma, they had higher proportions of smokers, heavy smokers (>50 pack-years), heavy drinkers, and diabetic patients. Meanwhile, estrogen plays an important role in regulating calcium metabolism, bone remodeling, and tumor progression;[11, 64] therefore the different estrogen exposure may interplay with calcium and modify its effects on cancer prognosis.

In the present study, we did not find a significant association between supplemental calcium intake and lung cancer survival among 18,137 incident cases from eight US cohorts that collected this information. The null association may be due to the fact that many fewer individuals would be calcium deficient when taking calcium supplements. If our observation was true that only a long-term low calcium intake was associated with cancer prognosis, a null association for supplemental calcium would be expected. We did observe a possible trend of beneficial association for supplemental calcium among early-stage patients who had low dietary calcium intakes. The null finding could also be due to a suboptimal measurement of usual supplement intake. Nevertheless, this finding is in line with results from randomized, controlled trials which found no significant effects of calcium supplements with or without vitamin D on cancer incidence or mortality.[65–67] Taken together, our results support the hypothesis that an optimal calcium intake from foods may play a beneficial, although small, role in cancer survival; however, evidence is weak or lacking for a recommendation to use calcium supplements to increase calcium intake, especially among individuals who already consume a sufficient amount of calcium, e.g. 800-1200 mg/d.[11]

We acknowledge several limitations of the present study. First, dietary intakes were measured via FFQs and food composition tables, both of which have non-negligible measurement errors. However, this is the most common and feasible choice for assessing usual dietary intakes in large observational studies. FFQs used in participating cohorts of this pooling project have each been validated by one or more diet assessment methods and have been shown to exhibit reasonably good validities.[37–45, 47, 48] Still, measurement errors can cause non-differential misclassifications of calcium intakes that are likely to bias our risk ratio estimates towards the null. Second, data on post-diagnostic calcium intake and lung cancer treatment were

not available, and data on tumor stage and grade were missing in a sizable fraction of patients. Most of the patients in our study were diagnosed before 2010 when targeted treatments were less common and lung cancer treatments were largely dependent on stage, histologic type, and patient sociodemographics. We have adjusted for all these factors and stratified by cohort (region) and year of diagnosis in the analyses. We also conducted a number of subgroup analyses and did not find significant effect modifications by these factors except stage. Moreover, we found little evidence that patient tumor characteristics, including stage and histology, were associated with prediagnostic calcium intake, suggesting that these clinical factors were unlikely to substantially confound the association between usual calcium intake and lung cancer survival. Third, despite the large number of lung cancer patients followed, statistical power remained limited in certain analyses, such as the interaction analysis by race/ethnicity (due to a small number of black participants) and the analysis among never smokers. Finally, we could not separate the effects of calcium from related nutrients, including vitamin D, magnesium, phosphorus, and other nutrients in calcium-rich foods (dairy products, soy foods, and green leafy vegetables), that may contribute to the observed associations. Particularly, the association of prediagnostic green leafy vegetable intake with better lung cancer survival is worth further investigation. We also could not rule out the possibility of residual confounding from unknown confounders and imperfectly measured covariates, which may be particularly challenging in pooling projects that use harmonized data from multiple studies.

Strengths of our study include its prospective design, large sample size, and pooled data analysis. By including only prospective cohort studies and first primary lung cancer cases, we minimized the reverse causality and biases of recall and selection. By obtaining individual-level data from 12 cohort studies in three continents, we established one of the largest cohort consortia

for investigation of nutrition on lung cancer risk and prognosis, with common variables on prediagnostic diet and lifestyle habits, cancer diagnosis, tumor characteristics, and survival status. This enabled us to evaluate the associations among populations with a broad range of exposures and clinical characteristics (such as extremely low and high calcium intake and early stage of lung cancer), which would be difficult for any single cohort study to investigate. Access to individual-level data also allowed us to examine calcium intake via multiple approaches, i.e. using project-wide cut points as continuous variables and in a series of sensitivity analyses. Results for low dietary calcium intake and poorer survival were largely consistent when different statistical approaches were used.

In summary, in this pooled analysis of 12 cohort studies, we found that low prediagnostic dietary calcium intake (<500-600 mg/d) was associated with a small but significantly increased risk of death among localized and regional stage lung cancer patients. Very high calcium intake (>1800 mg/d), a possible indication of previous history of calcium deficiency, was also associated with poorer survival for early-stage female patients. More studies are needed to explore biological mechanisms linking calcium nutrition, calcium homeostasis, bone remodeling, and bone metastasis with lung cancer progression, as well as to investigate modifiable nutrition and lifestyle factors to reduce risk and improve survival for lung cancer, the most deadly cancer of all.

Article Information

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Figure legend

Figure 1. Risk of death by prediagnostic dietary calcium intake (low vs. recommended intake) in subgroups of the Calcium and Lung Cancer Pooling Project. Low intake was defined as calcium intake less than half of the recommended dietary allowance (RDA), which is less than 500 mg/d for men ≤70 y and women ≤50 y, or less than 600 mg/d for men >70 y and women >50 y. Recommended intake was defined as calcium intake between the estimated average requirement (EAR) and RDA, which is 800-1000 mg/d for men ≤70 y and women ≤50 y, or 1000-1200 mg/d for men >70 y and women >50 y. The same stratified, multivariable-adjusted Cox model was used as shown in the footnote of Table 2.

Figure 2. Risk of death by prediagnostic dietary calcium intake in the Calcium and Lung Cancer Pooling Project (solid line: hazard ratio, dashed line: 95% confidence interval) among: a. early-stage lung cancer patients (P = 0.03); b. early-stage female patients (P = 0.39); and c. early-stage male patients (P = 0.02). The same stratified, multivariable-adjusted Cox model was used as shown in the footnote of Table 2.

Figure 1

Subgroups	Cases (deaths), n	Prediagnostic dietary calcium intake, mg/d	5-year survival rate, %	Multivariable-adjusted hazard ratios (95% CIs) for low vs. recommended dietary calcium intakes	P for interaction
Age at diagnosis					0.53
<65 years	6,439 (5,087)	883 ± 413	23.8		
65-75 years	13,263 (11,039)	930 ± 412	21.0		
>75 years	4,180 (3,412)	947 ± 412	18.0		
Sex					**0.01**
Female	11,574 (9,021)	897 ± 425	25.3		
Male	12,308 (10,517)	944 ± 424	17.5		
Race/Ethnicity					0.28
White	19,448 (16,137)	979 ± 389	21.1		
Black	1,037 (780)	789 ± 392	22.5		
Asian	3,140 (2,408)	593 ± 396	21.8		
Tobacco smoking status					0.51
Never	2,948 (2,063)	867 ± 415	31.6		
Former	9,589 (7,972)	975 ± 420	21.4		
Current	11,345 (9,503)	889 ± 415	18.6		
Tumor stage					**0.006**
Localized & regional stage	8,013 (5,536)	912 ± 411	36.0		
Distant stage	6,752 (6,327)	904 ± 412	5.7		
Histologic type					0.42
Adenocarcinoma	9,621 (7,324)	922 ± 411	27.7		
Squamous cell carcinoma	4,318 (3,468)	913 ± 411	24.7		
Other non-small cell carcinoma	3,414 (2,769)	958 ± 409	21.6		
Small cell carcinoma	3,210 (2,955)	945 ± 409	9.7		
Histologic grade					0.11
Well & moderately differentiated	4,004 (2,545)	898 ± 410	43.3		
Poorly & undifferentiated	6,398 (5,456)	900 ± 411	18.7		
Time interval from baseline survey to cancer diagnosis					0.64
<7 years	11,432 (9,912)	928 ± 418	19.9		
≥7 years	12,450 (9,626)	914 ± 417	22.6		

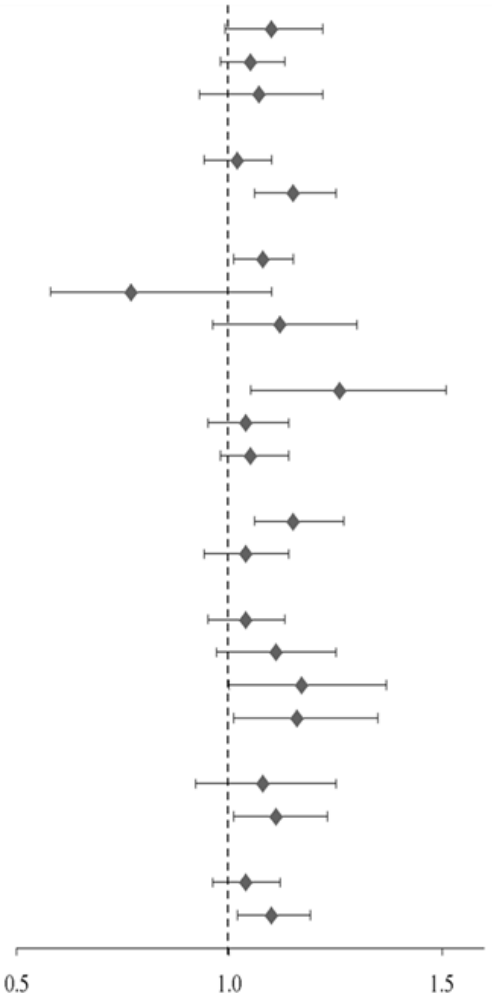

Figure 2

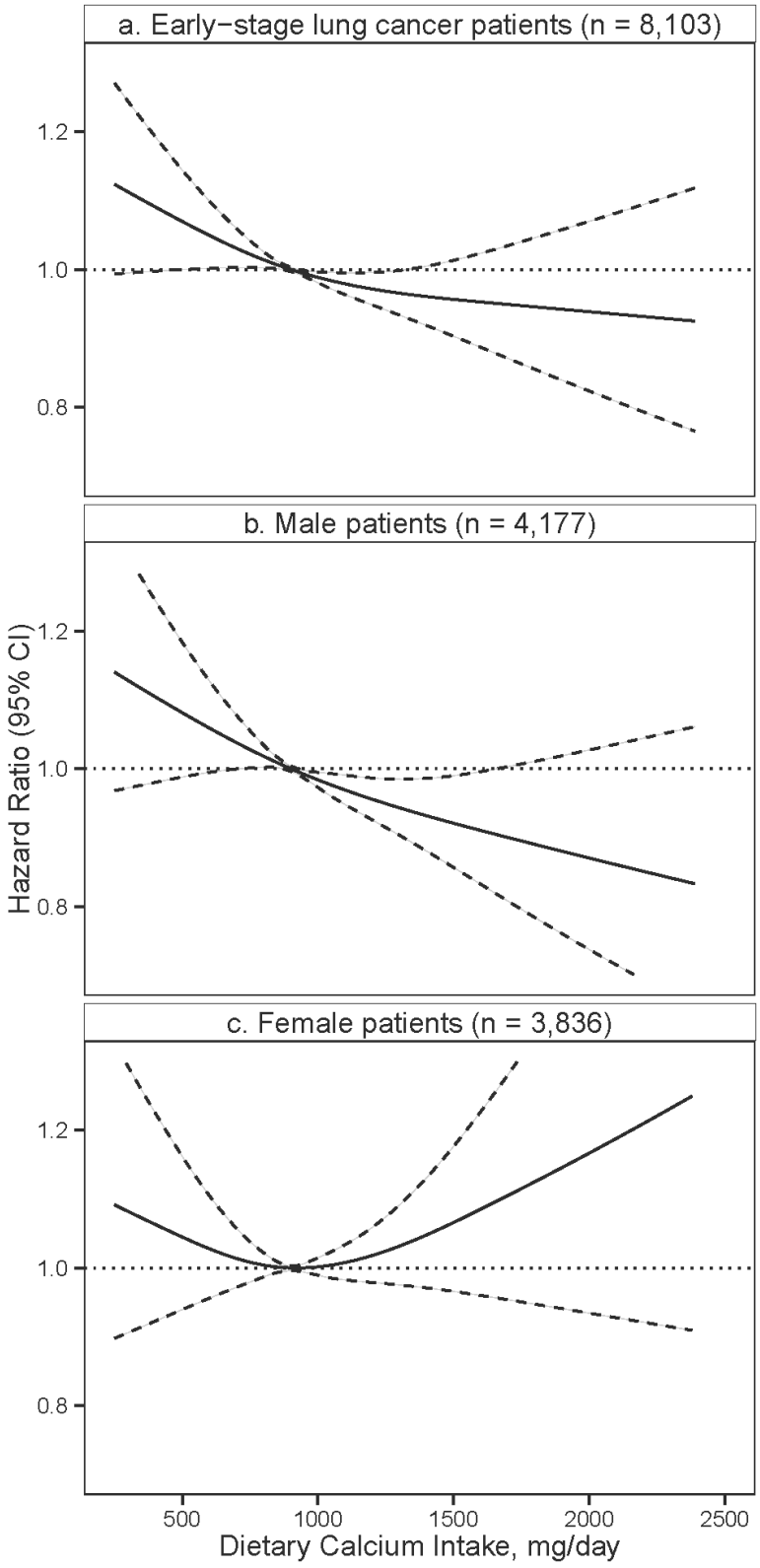
a. Early−stage lung cancer patients (n = 8,103)
b. Male patients (n = 4,177)
c. Female patients (n = 3,836)
Hazard Ratio (95% CI)
1.2
1.0
0.8
500
1000
1500
2000
2500
Dietary Calcium Intake, mg/day

Table 1. Characteristics, dietary calcium intake, and 5-year survival rate of lung cancer cases (*n*=23,882)

Characteristics	Cases, *n*	Deaths, *n*	Dietary calcium intake, mg/d[1]	*P* for calcium intake[2]	5-year survival rate (95% CI), %	*P* for survival rate[2]
Age at diagnosis						
< 65 years	6,439	5,087	883 ± 413	ref	23.8 (22.8, 24.9)	ref
65-75 years	13,263	11,039	930 ± 412	<0.0001	21.0 (20.3, 21.7)	<0.0001
> 75 years	4,180	3,412	947 ± 412	<0.0001	18.0 (16.8, 19.2)	<0.0001
Sex						
Female	11,574	9,021	897 ± 425	ref	25.3 (24.5, 26.2)	ref
Male	12,308	10,517	944 ± 424	<0.0001	17.5 (16.8, 18.2)	<0.0001
Race						
White	19,448	16,137	979 ± 389	ref	21.1 (20.6, 21.7)	ref
Black	1,037	780	789 ± 392	<0.0001	22.5 (19.9, 25.2)	0.99
Asian	3,140	2,408	593 ± 396	<0.0001	21.8 (20.3, 23.3)	0.76
Other	257	213	924 ± 388	0.14	20.0 (15.3, 25.2)	0.99
Education						
High school	9,947	8,181	889 ± 409	ref	18.8 (18.0, 19.6)	ref
Vocational school and some college	8,140	6,668	963 ± 410	<0.0001	22.2 (21.2, 23.1)	<0.0001
College and graduate school	5,795	4,689	912 ± 414	0.002	23.6 (22.5, 24.6)	<0.0001
Tobacco smoking						
Never	2,948	2,063	867 ± 415	ref	31.6 (29.8, 33.3)	ref
Former	9,589	7,972	975 ± 420	<0.0001	21.4 (20.6, 22.2)	<0.0001
Current	11,345	9,503	889 ± 415	0.04	18.6 (17.8, 19.3)	<0.0001

Smoking pack-years in cigarette smokers						
< 30 pack-years	6,187	4,876	946 ± 412	ref	22.8 (21.7, 23.9)	ref
30-50 pack-years	7,442	6,275	917 ± 410	<0.0001	19.4 (18.5, 20.3)	<0.0001
> 50 pack-years	7,134	6,173	923 ± 418	0.005	18.0 (17.1, 18.9)	<0.0001
History of diabetes						
No	22,028	17,918	913 ± 409	ref	21.8 (21.2, 22.3)	ref
Yes	1,854	1,620	1007 ± 411	<0.0001	15.5 (13.9, 17.2)	<0.0001
Physical activity						
Low	5,674	4,565	836 ± 407	ref	20.0 (18.9, 21.0)	ref
Middle	9,157	7,631	924 ± 407	<0.0001	20.7 (19.9, 21.6)	0.22
High	9,051	7,342	969 ± 406	<0.0001	22.6 (21.7, 23.5)	<0.0001
Body mass index						
<18.5 kg/m^2	525	423	796 ± 408	0.0002	21.7 (20.9, 22.5)	0.35
18.5-25.0 kg/m^2	10,758	8,768	889 ± 410	ref	20.3 (16.9, 24.0)	ref
25.0-30.0 kg/m^2	8,888	7,294	948 ± 411	<0.0001	21.0 (20.1, 21.9)	0.86
> 30.0 kg/m^2	3,711	3,053	963 ± 408	<0.0001	20.8 (19.5, 22.2)	0.68
Alcohol consumption						
None	7,265	5,849	909 ± 404	ref	21.4 (20.5, 22.4)	ref
Moderate	11,414	9,341	989 ± 404	<0.0001	21.9 (21.2, 22.7)	0.98
Heavy	5,203	4,348	786 ± 411	<0.0001	19.5 (18.5, 20.6)	0.02
Hormone therapy among women						
No	6,686	5,263	867 ± 388	ref	24.1 (23.1, 25.2)	ref
Yes	4,888	3,758	931 ± 388	<0.0001	27.0 (25.7, 28.3)	<0.0001

Histological type						
Adenocarcinoma	9,621	7,324	922 ± 411	ref	27.7 (26.8, 28.7)	ref
Squamous cell carcinoma	4,318	3,468	913 ± 411	0.99	24.7 (23.4, 26.0)	<0.0001
Other non-small cell carcinoma	3,414	2,769	958 ± 409	0.001	21.6 (20.2, 23.0)	<0.0001
Small cell carcinoma	3,210	2,955	945 ± 409	0.05	9.7 (8.7, 10.8)	<0.0001
All other	3,319	3,022	861 ± 410	<0.0001	9.6 (8.7, 10.7)	<0.0001
Tumor stage						
Localized	3,489	1,823	921 ± 410	ref	56.4 (54.6, 58.1)	ref
Regional	4,524	3,713	905 ± 410	0.54	21.1 (20.0, 22.3)	<0.0001
Distant	6,752	6,327	904 ± 412	0.31	5.7 (5.2, 6.3)	<0.0001
Unknown	9,117	7,675	940 ± 416	0.12	20.4 (19.5, 21.2)	<0.0001
Tumor grade						
Well differentiated	1,213	592	843 ± 409	ref	57.9 (54.9, 60.8)	ref
Moderately differentiated	2,791	1,953	921 ± 409	<0.0001	37.3 (35.5, 39.1)	0.66
Poorly differentiated	4,560	3,742	925 ± 411	<0.0001	22.2 (21.0, 23.4)	<0.0001
Undifferentiated	1,838	1,714	831 ± 409	0.99	10.4 (9.0, 11.8)	<0.0001
Unknown	13,480	11,537	938 ± 410	<0.0001	16.2 (15.5, 16.8)	<0.0001

[1] Mean ± SD, adjusted for age, sex, and total energy intake, per 2,000 kcal for women and per 2,500 kcal for men.

[2] *P*-values were corrected for multiple comparisons using the Bonferroni method.

Table 2. Pooled analyses of calcium intake and lung cancer survival (*n*=23,882)

Calcium intakes	Deaths / Cases, *n*	Hazard ratio (95% CI)	
		Age-, sex-, and energy-adjusted[1]	Multivariable-adjusted[2]
Dietary calcium intake, mg/d[3]			
<500 or <600	3,047 / 3,705	1.14 (1.08, 1.20)	1.07 (1.01, 1.13)
500-800 or 600-1000	7,463 / 9,190	1.05 (1.01, 1.09)	1.04 (1.00, 1.09)
800-1000 or 1000-1200	3,531 / 4,362	1.00 (ref)	1.00 (ref)
1000-1500 or 1200-1800	4,205 / 5,092	1.02 (0.97, 1.06)	1.05 (1.01, 1.10)
>1500 or >1800	1,292 / 1,533	1.02 (0.96, 1.09)	1.04 (0.97, 1.11)
Dietary and supplemental calcium intake, mg/d[3,4]			
<500 or <600	1,119 / 1,352	1.07 (1.00, 1.15)	1.02 (0.95, 1.10)
500-800 or 600-1000	4,333 / 5,212	1.02 (0.97, 1.07)	0.99 (0.94, 1.04)
800-1000 or 1000-1200	2,695 / 3,216	1.00 (ref)	1.00 (ref)
1000-1500 or 1200-1800	4,364 / 5,290	0.97 (0.92, 1.02)	1.01 (0.96, 1.06)
>1500 or >1800	2,507 / 3,067	0.95 (0.90, 1.01)	0.99 (0.94, 1.05)
Supplemental calcium intake, mg/d[4]			
None	7,850 / 9,387	1.00 (ref)	1.00 (ref)
0-200	3,524 / 4,158	0.99 (0.95, 1.03)	1.01 (0.97, 1.06)
200-500	1,557 / 1,969	0.92 (0.87, 0.97)	1.00 (0.94, 1.05)
500-1000	1,391 / 1,743	0.94 (0.89, 1.00)	1.00 (0.94, 1.06)

>1000	696 / 880	0.94 (0.87, 1.02)	1.01 (0.93, 1.10)

[1] Cox model was stratified by cohort, year of lung cancer diagnosis (5-year intervals from <1990 to >2010), and time interval between dietary assessment and lung cancer diagnosis (<4, 4-7, 7-10, and >10 years) and adjusted for age at diagnosis, sex, and total energy intake.

[2] Additionally adjusted for race, education, smoking status, pack-years of cigarette smoking, alcohol consumption, history of diabetes, physical activity level, obesity status, hormone therapy in women, and the histological type, stage, and grade of lung cancer.

[3] For men ≤70 y and women ≤50 y, the recommended dietary allowance (RDA) of calcium is 1000 mg/d and the estimated average requirement (EAR) is 800 mg/d. For men >70 y and women >50 y, RDA is 1200 mg/d and EAR is 1000 mg/d. Calcium intakes were categorized into 5 groups: less than 0.5 RDA, 0.5 RDA to EAR, EAR to RDA, RDA to 1.5 RDA, and higher than 1.5 RDA.

[4] Supplemental calcium intake data were only available in 8 US cohorts, *n*=18,137.

Table 3. Dietary calcium intake and lung cancer survival in early stage cases (*n*=8,103)

Dietary calcium intake, mg/d[2]	Deaths / Cases, n	Hazard ratio (95% CI)[1]	
		Age-, sex-, and energy-adjusted	Multivariable-adjusted
All early stage lung cancer cases			
<500 or <600	928 / 1,342	1.20 (1.09, 1.32)	1.15 (1.04, 1.27)
500-800 or 600-1000	2,083 / 3,065	1.08 (1.10, 1.16)	1.06 (0.98, 1.14)
800-1000 or 1000-1200	990 / 1,442	1.00 (ref)	1.00 (ref)
1000-1500 or 1200-1800	1,164 / 1,660	0.99 (0.91, 1.08)	1.04 (0.96, 1.14)
>1500 or >1800	371 / 504	0.99 (0.88, 1.12)	1.07 (0.95, 1.21)
Female			
<500 or <600	542 / 837	1.19 (1.02, 1.37)	1.08 (0.92, 1.25)
500-800 or 600-1000	1,076 / 1,727	1.10 (0.97, 1.26)	1.03 (0.90, 1.17)
800-1000 or 1000-1200	311 / 547	1.00 (ref)	1.00 (ref)
1000-1500 or 1200-1800	368 / 591	1.09 (0.94, 1.27)	1.12 (0.96, 1.31)
>1500 or >1800	89 / 134	1.24 (0.98, 1.58)	1.33 (1.05, 1.70)
Male			
<500 or <600	386 / 505	1.20 (1.04, 1.39)	1.25 (1.08, 1.45)
500-800 or 600-1000	1,007 / 1,338	1.05 (0.95, 1.16)	1.07 (0.97, 1.18)
800-1000 or 1000-1200	679 / 895	1.00 (ref)	1.00 (ref)
1000-1500 or 1200-1800	796 / 1,069	0.94 (0.85, 1.05)	0.99 (0.90, 1.10)
>1500 or >1800	282 / 370	0.93 (0.80, 1.06)	0.99 (0.86, 1.14)
Whites			
<500 or <600	507 / 691	1.19 (1.06, 1.33)	1.16 (1.03, 1.31)
500-800 or 600-1000	1,673 / 2,377	1.07 (0.99, 1.17)	1.06 (0.97, 1.15)
800-1000 or 1000-1200	873 / 1,254	1.00 (ref)	1.00 (ref)
1000-1500 or 1200-1800	1,103 / 1,539	1.02 (0.93, 1.11)	1.05 (0.96, 1.15)
>1500 or >1800	358 / 480	1.02 (0.90, 1.15)	1.09 (0.96, 1.23)
Asians			
<500 or <600	363 / 548	1.18 (1.00, 1.39)	1.20 (1.01, 1.43)
500-1000 or 600-1200	326 / 562	1.00 (ref)	1.00 (ref)
>1000 or >1200	33 / 76	0.72 (0.50, 1.05)	0.76 (0.52, 1.12)

Blacks			
<500 or <600	53 / 93	0.83 (0.60, 1.17)	0.79 (0.55, 1.14)
500-1000 or 600-1200	171 / 269	1.00 (ref)	1.00 (ref)
>1000 or >1200	25 / 47	0.58 (0.36, 0.91)	0.77 (0.46, 1.27)
Never smokers			
<500 or <600	143 / 260	1.32 (0.94, 1.85)	1.45 (1.01, 2.08)
500-800 or 600-1000	204 / 410	1.06 (0.73, 1.41)	1.12 (0.82, 1.53)
800-1000 or 1000-1200	71 / 146	1.00 (ref)	1.00 (ref)
1000-1500 or 1200-1800	100 / 181	1.01 (0.73, 1.40)	1.06 (0.76, 1.49)
>1500 or >1800	20 / 41	0.84 (0.50, 1.42)	0.86 (0.50, 1.47)
Former / Current smokers			
<500 or <600	785 / 1,082	1.12 (1.01, 1.25)	1.12 (1.01, 1.25)
500-800 or 600-1000	1,879 / 2,655	1.07 (0.99, 1.16)	1.05 (0.97, 1.14)
800-1000 or 1000-1200	919 / 1,296	1.00 (ref)	1.00 (ref)
1000-1500 or 1200-1800	1,064 / 1,479	1.00 (0.92, 1.10)	1.04 (0.95, 1.14)
>1500 or >1800	351 / 463	1.02 (0.90, 1.16)	1.09 (0.96, 1.23)
Localized stage cases			
<500 or <600	293 / 579	1.28 (1.07, 1.52)	1.16 (0.97, 1.40)
500-800 or 600-1000	696 / 1,346	1.15 (1.00, 1.33)	1.12 (0.97, 1.29)
800-1000 or 1000-1200	304 / 604	1.00 (ref)	1.00 (ref)
1000-1500 or 1200-1800	405 / 739	1.06 (0.91, 1.23)	1.10 (0.95, 1.28)
>1500 or >1800	125 / 221	0.94 (0.76, 1.16)	0.97 (0.78, 1.20)
Regional stage cases			
<500 or <600	635 / 763	1.11 (0.98, 1.25)	1.10 (0.97, 1.25)
500-800 or 600-1000	1,387 / 1,719	1.01 (0.92, 1.11)	1.02 (0.93, 1.12)
800-1000 or 1000-1200	686 / 838	1.00 (ref)	1.00 (ref)
1000-1500 or 1200-1800	759 / 921	0.99 (0.89, 1.10)	1.01 (0.90, 1.12)
>1500 or >1800	246 / 283	1.09 (0.94, 1.27)	1.11 (0.96, 1.29)

[1] The same covariates as shown in the footnote of Table 2. *P* for interaction was 0.02 for calcium intake levels with sex, 0.68 with race/ethnicity, 0.70 with smoking (never/ever), and 0.47 with stage (localized/regional).