Vitamin D Is Associated with Improved Survival in Early-Stage Non–Small Cell Lung Cancer Patients

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Abstract

Vitamin D may inhibit the development and progression of a wide spectrum of cancers. We investigated the associations of surgery season and vitamin D intake with recurrence-free survival (RFS) and overall survival in 456 early-stage non–small cell lung cancer patients. The data were analyzed using log-rank test and Cox proportional hazards models. The median (range) follow-up time was 71 (0.1-140) months, with 161 recurrence and 231 deaths. Patients who had surgery in summer had a better RFS than those who had surgery in winter (adjusted hazard ratio, 0.75; 95% confidence interval, 0.56-1.01), with 5-year RFS rates of 53% (45-61%) and 40% (32-49%), respectively (P = 0.10, log-rank test). Similar association between surgery season and RFS was found among the 321 patients with dietary information (P = 0.33, log-rank test). There was no statistically significant association between vitamin D intake and RFS. Because both season and vitamin D intake are important predictors for vitamin D levels, we investigated the joint effects of surgery season and vitamin D intake. Patients who had surgery during summer with the highest vitamin D intake had better RFS (adjusted hazard ratio, 0.33; 95% confidence interval, 0.15-0.74) than patients who had surgery during winter with the lowest vitamin D intake, with the 5-year RFS rates of 56% (34-78%) and 23% (4-42%), respectively. Similar associations of surgery season and vitamin D intake with overall survival were also observed. In conclusion, the joint effects of surgery season and recent vitamin D intake seem to be associated with the survival of early-stage non–small cell lung cancer patients. (Cancer Epidemiol Biomarkers Prev 2005;14(10):2303–9)

Introduction

Lung cancer is the leading cause of cancer death among both men and women in the United States. Disease stage at diagnosis and performance status are known prognostic factors for lung cancer. Besides quitting or preventing smoking, means to reduce mortality from lung cancer are limited. Recently, the possible beneficial role of vitamin D in cancer prognosis has generated increased interest. Vitamin D may be converted from 7-dehydrocholesterol by UV radiation (UVB) in the skin or ingested through natural food sources, fortified foods, or supplements. Vitamin D is hydroxylated in the liver to produce 25(OH)D, the primary circulating form of vitamin D, and is then converted into the active form $1,25(OH)_2D$ in many cells, including lung cancer cells (1). $1,25(OH)_2D$ has potent antiproliferative and anti-invasive properties *in vitro* in cancer cells and may induce cancer cell apoptosis (2).

A surrogate of vitamin D levels used in epidemiologic studies has been based on the average UVB radiation in a geographic region of residence. Geographic regions with lower UVB radiation have been noted to have increased incidence and mortality rates of a wide range of cancers (3-7). One study based on 115,096 cases and 45,667 deaths from breast, colon, or prostate cancer diagnosed between 1964 and 1992 in Norway found a 30% reduction in cancer fatality rates when the cancers were diagnosed in summer and fall, when vitamin D levels are higher, compared with winter (8). Currently, the association between vitamin D intake, another predictor of vitamin D levels, and cancer mortality remains unknown (9).

No study has reported on the association between vitamin D and lung cancer prognosis. Exposure to winter sunlight in the Boston area is insufficient to promote vitamin D synthesis in human skin (10), and previous studies have shown that in the Boston area, the serum 25(OH)D levels are much higher in summer than in winter season because of the sunlight exposure differences (10-13). Therefore, lung cancer patients who received treatment in the winter and consumed relatively low vitamin D intake may have lower vitamin D levels and a worse prognosis compared with patients who received treatment in the summer and consumed more vitamin D. We tested these hypotheses in our cohort of early-stage non–small cell lung cancer (NSCLC) patients.

Materials and Methods

Study Population. This study began in 1992 and was approved by the Human Subjects Committee of Massachusetts General Hospital (MGH) and Harvard School of Public Health. Eligible subjects were histologically confirmed NSCLC patients who were ages >18 years. Before 1997, enrollment was restricted to individuals with operable NSCLC, and after 1997, all stages of NSCLC patients were recruited. More than 85% of eligible patients participated in this study, and 96% were Caucasians.

In this population, we first identified 472 early-stage (stages IA-IIB) NSCLC patients recruited between 1992 and 2000, ensuring a follow-up time of at least 4 years, which accounted for 58% of all stages of NSCLC patients enrolled in this study. Thirteen nonincident patients and three patients who did not

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have complete follow-up information were excluded from the analyses, leaving the subset of 456 incident patients with histologic diagnoses confirmed at MGH, who had their surgical resection at MGH, and who had complete outpatient records available. The demographics of the subjects not included in the analysis were similar to those of the included subjects.

Data Collection. A modified version of detailed American Thoracic Society health questionnaire and validated semi-quantitative food frequency questionnaire (Harvard-Willett) were completed for each patient at the time of recruitment (14, 15). The 126–food item food frequency questionnaire, developed by the Nutrition Department at the Harvard School of Public Health, has been validated in a group of female Caucasian nurses (15) and male health professionals (14) living in Boston. Because the subjects in our study are also mainly Caucasians and from a similar geographic area, the food frequency questionnaire is likely valid for our study population, although nurses and health professionals are mostly college graduates, whereas subjects in our population have various education levels and backgrounds. In the food frequency questionnaire, a commonly used unit or portion size was specified for each food item, and subjects were asked about their average consumption over the past year before enrollment. Estimated average intakes for each specific food were obtained and nutrient intake was computed using the Harvard database, which is a modification of the U.S. Department of Agriculture Nutrition Composition Laboratory's food composition database. Vitamin D was not available from the dietary records, but the predominant sources of dietary vitamin D in the Health Professional Follow-up Study have shown good correlations (skim/low fat milk, $r = 0.88$; whole milk, $r = 0.67$; dark fish, $r = 0.58$; ref. 16). The vitamin D intake has also been validated for plasma 25(OH)D levels in the Health Professional Follow-up Study (17, 18).

Survival Measurements. Recurrence-free survival (RFS) and overall survival (OS) were the end points in this study. RFS is defined as the time from the date of surgery to the first date of recurrence or to death from any cause. Data on recurrence were obtained by reviewing the hospital and outpatient records of all patients. This included physicians' notes, surgeons' notes, radiographic reports, and biopsy results if available. For those patients who had their primary follow-up outside of the MGH system, we contacted the primary physician to obtain follow-up information. Copies of the original signed consent were forwarded to the local physician's office and data regarding frequency of clinical and radiographic follow-up and date of recurrence were collected.

OS time was calculated from the date of surgery to the date of death from any cause or to last follow-up. Dates of death were obtained and cross-checked using at least one of the following four methods: (*a*) inpatient and outpatient medical records, (*b*) MGH tumor registry, (*c*) confirmation with the patient's primary care physician and/or family, and (*d*) Social Security Death Index. Patients who were not deceased were censored at the last date they were known to be alive based on date of last contact. This date was verified by methods (*a*) and/or (*c*) as described above.

Statistical Analysis. In the Boston area, minimal solar UVB radiation is detected between November and February; the intensity of all UVB wavelengths increases steeply between February and March, with the intensity continuing to increase through July before declining again to a nadir in the winter months (10). Therefore, we generated three categories of seasons for patients who received surgery in this population: winter (the low sun exposure months of November-February), spring/fall (the intermediate sun exposure months of March, April, September, and October), and summer (the high sun exposure months of May-August; refs. 10, 19).

The analysis of vitamin D intake and survival was limited to the 321 patients who had complete dietary information. Appropriate statistical testing was done to compare the distribution of demographic, histologic, and treatment characteristics between patients with and without dietary information. Energy-adjusted nutrient values were created by regressing each nutrient on total calories and obtaining the residual from this model (20). The residual value for each observation was then added to the mean nutrient value over all subjects to get the individual value. For vitamin D intake, we used four categories in our primary analysis as well as in the joint analyses with surgery seasons.

Demographic and clinical information was compared across seasons and vitamin D levels using Pearson χ^2 tests (for categorical variables), Wilcoxon rank sum test, and one-way ANOVA (for continuous variables), where appropriate. Median follow-up time was computed only among censored observations. The associations of surgery season and vitamin D intake with RFS and OS were estimated using the method of Kaplan-Meier and assessed using the log-rank test. Cox proportional hazards models were used as our primary analyses, controlling for multiple variables simultaneously, with age as a continuous variable and gender, smoking status, and clinical stage as categorical variables. All reported Ps are from two-sided tests. Ps < 0.05 were considered statistically significant. All statistical analyses used SAS software version 8 (SAS, Cary, NC).

Results

Patient, Stage, and Treatment Characteristics. Among the 456 NSCLC patients, median age was 69 (range, 31-89), 48% were females, and 40% were current smokers. Adenocarcinoma, squamous cell, large cell, and bronchioloalveolar carcinoma represented 49%, 30%, 5%, and 11% of the tumor histologies, respectively. Fifty-one percent were stage IA, 28% were stage IB, 5% stage IIA, and 16% stage IIB. All patients had surgical resection as the initial treatment, including wedge (24%), lobectomy (61%), bilobectomy (4%), pneumonectomy (6%), sleeve lobectomy (4%), and lobectomy plus wedge (1%). Additionally, 39 (9%) patients received postoperative radiation and 5 (1%) patients received adjuvant chemotherapy. There were 161 recurrences and 231 deaths, including 113 deaths occurred in the absence of reported recurrence and 43 recurrence without death. Five-year RFS rate was 46% overall [95% confidence interval (95% CI), 41-51%] and 56% (95% CI, 49-63%), 39% (95% CI, 30-48%), 24% (95% CI, 5-43%), and 32% (95% CI, 21-43%), respectively, for stages IA to IIB. Five-year OS rate was 56% overall (95% CI, 51-61%) and 65% (95% CI, 58-71%), 53% (95% CI, 44-62%), 34% (95% CI, 13-55%), and 38% (95% CI, 26-49%), respectively, for stages IA to IIB. The median follow-up time for the 225 patients still alive was 71 months (range, 0.1-140 months).

The seasons of surgery were uniformly distributed among patients: 140 (31%) in winter, 165 (36%) in spring/fall, and 151 (33%) in summer. There was no statistically significant difference for most of the important demographic and clinical information among patients who had surgery in different seasons, overall and in patients with and without diet information, respectively (Table 1). When compared with those who had surgery in the winter, patients who had surgery in summer had a nonsignificantly higher frequency of stage IA (56% in summer and 46% in winter) and a lower frequency of stage IIB (15% in summer and 23% in winter; $P = 0.19$, χ^2 test). Adjustment for clinical stage was included in all of the Cox proportional hazards models.

Table 1. Demographic, clinical, and treatment characteristics in NSCLC patients by surgery season

Characteristics	Overall				Patients with diet information				Patients without diet information			
	Winter (n = 140)	Spring/fall (n = 165)	Summer (n = 151)	P	Winter (n = 100)	Spring/fall (n = 119)	Summer (n = 102)	P	Winter (n = 40)	Spring/fall (n = 46)	Summer (n = 49)	P
Age (y), median*	68	68	70	0.08	67	68	70	0.01	72	71	70	0.92
Age (y), range	35-88	31-89	32-87		43-88	31-89	42-87		35-88	35-88	32-86	
Gender, female (%)†	46	49	48	0.83	45	50	46	0.77	48	48	53	0.83
Histologic cell type (%)†												
Adenocarcinoma	51	50	46		53	50	52		45	52	35	
Squamous	30	28	31		26	29	29		40	24	35	
Large cell	6	4	3	0.34	6	4	4	0.99	8	4	2	0.02
Bronchioloalveolar	8	10	16		9	11	11		5	9	27	
Others	5	7	3		6	6	4		2	11	2	
Clinical stage (%)†												
IA	46	52	56		48	52	58		40	50	51	
IB	27	32	25	0.19	25	33	24	0.28	33	30	27	0.82
IIA	4	4	5		5	4	4		3	4	6	
IIB	23	12	15		22	11	14		25	15	16	
Surgery type (%)†												
Wedge	27	23	23		28	24	24		23	22	22	
Lobectomy	54	65	64	0.25	52	63	67	0.18	60	70	59	0.69
Others	19	12	13		20	13	10		17	9	18	
Radiation/chemotherapy†	13	6	9	0.12	14	6	11	0.13	10	7	4	0.54
Smoking status (%)†												
Never smokers	5	10	7		5	9	5		5	13	10	
Ex-smokers	50	51	57	0.25	51	51	60	0.41	45	50	51	0.62
Current smokers	45	39	36		44	40	35		40	37	39	

*Wilcoxon rank sum test.
† Frequency, tested by χ^2 test.

Surgery Season and Lung Cancer RFS and OS. In the log-rank test, patients who had surgery in summer had a nonstatistically significantly longer RFS compared with those who had surgery in winter (P = 0.10, log-rank test; Fig. 1A), with 5-year RFS rates of 40% (95% CI, 32-49%), 44% (95% CI, 36-53%), and 53% (95% CI, 45-61%), respectively, for patients who had surgery in winter, spring/fall, and summer. Similar nonstatistically significant difference was observed for OS (P = 0.30, log-rank test; Fig. 1B) in different seasons, and the 5-year OS rates were 50% (95% CI, 41-58%), 57% (95% CI, 49-65%), and 59% (95% CI, 51-68%), respectively, for patients who had surgery in winter, spring/fall, and summer, respectively.

In the univariate analyses of Cox proportional hazards model, age, gender, current cigarette smoking status, and more advanced clinical stages were associated with worse RFS and OS and were adjusted in the final models for both RFS and OS. For RFS, the adjusted hazard ratios (AHR) for patients who had surgery in spring/fall and summer were 0.98 (95% CI, 0.73-1.31) and 0.75 (95% CI, 0.56-1.01), respectively (when compared with winter), which were similar to the corresponding crude HRs of 0.92 (95% CI, 0.69-1.22) and 0.73 (95% CI, 0.55-0.98), respectively. For OS, the AHRs for patients who had surgery in spring/fall and summer were 0.96 (95% CI, 0.70-1.31) and 0.77 (95% CI, 0.55-1.06), respectively, similar to the corresponding crude HRs of 0.94 (95% CI, 0.69-1.28) and 0.78 (95% CI, 0.57-1.08), respectively. Similar associations between surgery season and RFS (or OS) were found for patients with different age, gender, histologic subtypes, smoking status, clinical stages, treatment (surgery only versus other patients), and recruitment periods.

We then analyzed the associations between surgery season and RFS (or OS) among subjects with (n = 321) and without complete diet information (n = 135), respectively. Compared with patients without complete diet information (median age, 71 years; range, 32-88), patients who completed the diet questionnaire are slightly younger (median age, 69 years; range, 31-89; P = 0.02, Wilcoxon rank sum test) and had nonsignificantly improved RFS (log-rank test, P = 0.10) and OS (log-rank test, P = 0.07). There were 115 recurrences and 161 deaths among patients with diet information. Similar association between surgery season and RFS (or OS) was found among patients with and without diet information. For RFS, the AHRs for patients who had surgery in spring/fall and summer (when compared with winter) were 0.82 (95% CI, 0.48-1.41) and 0.57 (95% CI, 0.33-0.98), respectively, for patients without diet information (P = 0.16, log-rank test) and 1.04 (95% CI, 0.74-1. 47) and 0.80 (95% CI, 0.56-1.15), respectively, for patients with diet information (P = 0.33, log-rank test). For OS, the AHRs for patients who had surgery in spring/fall and summer (when compared with winter) were 0.72 (95% CI, 0.40-1.29) and 0.57 (95% CI, 0.32-1.02), respectively, for patients without diet information (P = 0.15, log-rank test) and 1.09 (95% CI, 0.75-1.60) and 0.88 (95% CI, 0.59-1.31), respectively, for patients with diet information (P = 0.59, log-rank test).

Vitamin D Intake and Lung Cancer RFS and OS. Among the 321 patients with complete diet information, there were no statistically significant differences by vitamin D intake levels in the distribution of important demographic and clinical information (Table 2). No vitamin D supplement was taken among patients who had vitamin D intake <239 IU/d (lowest quartile). We did not observe the statistically significant association between vitamin D intake and RFS (AHR of highest versus lowest quartile of intake, 0.85; 95% CI, 0.56-1.28; P_{trend} = 0.66) or OS (AHR of highest versus lowest quartile of intake, 0.77; 95% CI, 0.50-1.21; P_{trend} = 0.44) overall. Stratified by surgery season, higher vitamin D intake was associated with statistically significantly better RFS (AHR of highest versus lowest quartile of intake, 0.30; 95% CI, 0.13-0.70; P_{trend} <0.01) and OS (AHR of highest versus lowest quartile of intake, 0.29; 95% CI, 0.11-0.75; P_{trend} <0.01) among patients who received surgery in summer (Table 3). Similar associations for vitamin D and survival were found when tertiles or medians instead of quartiles of vitamin D intake were included in the model and for dietary vitamin D intake only (without supplement).

Joint Effects of Surgery Season and Vitamin D Intake. Because both season and vitamin D intake are important

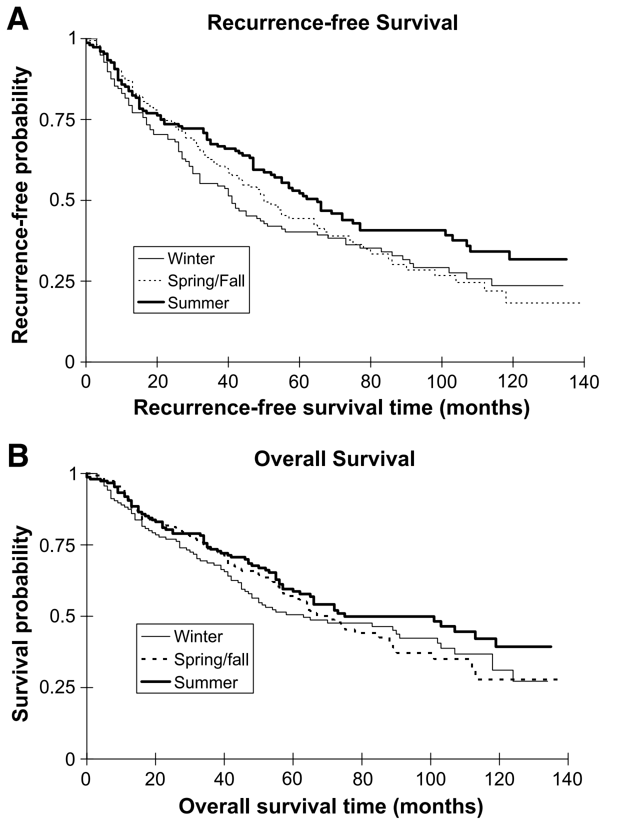

Figure 1. Kaplan-Meier curves of RFS (**A**; $P = 0.10$, log-rank test) and OS (**B**; $P = 0.30$, log-rank test) for NSCLC patients who received surgery in different seasons ($n = 456$). Log-rank test was based on the full data.

predictors for vitamin D levels, we investigated the joint effects of surgery season and vitamin D intake among the 321 patients with diet information. Patients who received surgery in winter with the lowest vitamin D intake (<239 IU/d, no vitamin D supplements) were treated as the ''reference group'' in comparisons. Table 4 shows all other patient groups had improved RFS (or OS) rates (some with statistically significant differences) when compared with the reference group. Specifically, patients who had surgery during summer with the highest vitamin D intake (''comparison group'') had statistically significantly better RFS (AHR, 0.33; 95% CI, 0.15-0.74) and OS (AHR, 0.25; 95% CI, 0.10-0.63) than patients who had surgery during winter with the lowest vitamin D intake, with 5-year RFS of 56% (95% CI, 34-78%) and 23% (95% CI, 4-42%) and 5-year OS rates of 72% (95% CI, 52-91%) and 30% (95% CI, 9-51%), respectively, for the comparison group and reference group (Table 4; Fig. 2). Similar associations were found when we classified vitamin D intake into three or two categories based on tertiles or median (data not shown).

Discussion

Several factors, including regional UVB levels, vitamin D intake, skin pigmentation, sunlight exposure behaviors, and adiposity may influence *in vivo* vitamin D levels (21). Seasonal variation in 25(OH)D concentrations have been observed for residents in Boston (10-13), with inadequate vitamin D intake and winter season being independent predictors of hypovitaminosis D (13). We investigated the effects of season and vitamin D intake on NSCLC survival and found that both higher UVB exposure (patients who had surgery in summer) and higher vitamin D intake (diet and supplement) improved lung cancer survival. Patients who had surgery in summer with high vitamin D intake had a 3-fold better RFS and a 4-fold better OS than those with surgery in winter and low vitamin D intake, with all of the other patient groups falling between the two groups (Table 4; Fig. 2). In Cox proportional hazards models, we adjusted for the most important predictors of NSCLC prognosis, including age, gender, smoking status, and clinical stage, which may affect on long-term survival, did subgroup analyses in different covariates, analyzed surgery season and vitamin D intake independently and jointly, and found consistent associations between vitamin D and RFS and OS in all of the analyses, suggesting that confounding by other factors is unlikely. We also analyzed the association between diagnosed season and RFS or OS in all of the 456 patients and among the 400 (88%) patients who were diagnosed and received surgery during the same season, all found very similar results (data not shown). Our results support the hypothesis that vitamin D levels at the time of surgery are associated with lung cancer survival.

The suggestion by our data that vitamin D may protect against lung cancer progression is supported by *in vitro* and animal studies. $1,25(OH)_2D$ inhibits metastatic growth of lung cancer cells, and mice fed manipulated diets display an apparent inverse relationship between the physiologic levels of serum $1,25(OH)_2D$ and tumorigenesis (22, 23). In a metastatic Lewis lung carcinoma tumor model, vitamin D3 treatment increases intratumoral T-cell immune reactivity and limits metastasis and locoregional tumor recurrence (24). $1,25(OH)_2D$ also inhibits proliferation and induces differentiation of lung cancer cell lines (25, 26), and increased expression of the 1α-hydroxylase gene has been found in alveolar macrophages of patients with lung cancer (27).

In humans, $1,25(OH)_2D$ may exert its anticarcinogenic effects through stimulating the secretion of protein glues, such as E-cadherin and catenin, which make cells more adherent to each other (28), reducing the likelihood of mobilization of many malignant cells into the lymphatic or blood circulation. Surgery could potentially be a time that tumor cells can escape and regional metastasis may occur. Higher $1,25(OH)_2D$ levels may up-regulate the secretion of protein glues, make cancer cells adherent more tightly and the tumor less friable, and decrease the probability of cancer cells dislodge from the tumor tissue during surgery, which may be the reason that higher vitamin D levels at the time of surgery are associated with improved NSCLC survival. Therefore, although $1,25(OH)_2D$ may have anti-invasion and antimetastasis effects at various stages before and during carcinogenesis, the $1,25(OH)_2D$ levels at the time of surgery may be particularly important. Reduced expression of E-cadherin and catenins has been associated with tumor cell dedifferentiation, local invasion, regional metastasis, and reduced survival in NSCLC (29, 30).

Among the 321 patients who had dietary information, 112 (35%) patients reported taking multivitamins, most of which contain vitamin D supplements. Thomas et al. investigated 290 consecutive patients on general medical wards at MGH in March and September 1994 and found that 65 (22%) were considered severely vitamin D deficient (13). The above rate of severe hypovitaminosis D may help to explain why we observed better RFS and OS for patients with >239 IU/d vitamin D intake (the combined three highest quartiles) when compared with patients <239 IU/d vitamin D intake (the lowest quartile, without vitamin D supplement intake; Table 3). For RFS, the AHRs were 0.79 (95% CI, 0.57-1.11) overall and 0.47 (95% CI, 0.26-0.87), 1.63 (95% CI, 0.90-2.94), and 0.43 (95% CI, 0.23-0.80) in winter, spring/fall, and summer, respectively, for patients with >239 IU/d versus patients <239 IU/d vitamin D intake. For OS, the AHRs were 0.73 (95% CI, 0.51-1.05) overall and 0.40 (95% CI, 0.21-0.77), 1.46 (95% CI, 0.77-2.77),

Table 2. Demographic, treatment, and dietary/supplement characteristics in different vitamin D intake levels among patients with diet information

Characteristics	Vitamin D intake (IU/d, n = 321)				P
	<239 (n = 79)	239-362 (n = 81)	363-595 (n = 80)	≥596 (n = 81)	
Age (y)*	68 (33-82)	69 (31-89)	69 (46-88)	68 (47-85)	0.45
Gender, female (%)†	47	49	49	43	0.86
Histologic cell type (%)†					
Adenocarcinoma	54	46	49	57	0.53
Squamous	26	41	27	20	
Large cell	5	2	5	6	
Bronchioloalveolar	10	7	13	11	
Others	5	4	6	6	
Clinical stage (%)†					
IA	58	57	51	44	0.50
IB	18	26	33	35	
IIA	5	4	4	5	
IIB	19	13	12	16	
Surgery type (%)†					
Wedge	24	31	25	20	0.69
Lobectomy	60	57	59	68	
Others	16	12	16	12	
Radiation/chemotherapy (%)†	11	10	8	11	0.84
Smoking status (%)†					
Never	3	4	15	5	0.02
Ex-smokers	49	55	50	60	
Current smokers	48	41	35	35	
Alcohol drinking (g/d)‡	1.1 (0.8)	1.1 (0.8)	1.0 (0.8)	0.8 (0.8)	0.33
Education levels (%)†,§					
High school, lower	55	55	65	53	0.45
College, higher	45	45	35	47	
Multivitamins use†	0	11	38	90	<0.001
Body mass index at 18 y†,‖	22 (3)	22 (4)	21 (3)	22 (3)	0.49
Energy intake (kcal/d)‡	2,123 (698)	2,044 (691)	2,313 (685)	2,023 (683)	0.03
Animal protein (g/d)‡	47 (15)	54 (13)	55 (15)	60 (18)	<0.001
Animal fat (g/d)‡	39 (14)	37 (14)	35 (13)	37 (14)	0.34
Retinol intake (IU/d)‡	1,558 (1,503)	2,493 (2,017)	3,285 (3,201)	6,601 (4,689)	<0.001
Calcium intake (mg/d)‡	665 (227)	870 (304)	1,118 (429)	1,267 (583)	<0.001
Vitamin D intake (IU/d)‡	165 (47)	296 (38)	468 (70)	813 (205)	<0.001

*Median (range), tested by Wilcoxon rank sum test.
† Frequency, tested by χ^2 test.
‡ Mean (SD), tested by one way ANOVA.
§Available for 310 patients.
‖Available for 309 patients.

and 0.52 (95% CI, 0.27-0.99) in winter, spring/fall, and summer, respectively. Because the population has less exposure to sunlight in winter and the amount of winter sunlight in Boston area may not be sufficient to promote vitamin D synthesis in human skin (10), dietary vitamin D supplementation may be advisable for lung cancer patients, especially in the winter season (11).

In our population, patients who had surgery in summer had a higher frequency of stage IA and a lower frequency of stage IIB compared with winter. We also compared the vitamin D

Table 3. AHRs for vitamin D intake on RFS and OS by surgery season (n = 321)

Surgery season	Vitamin D intake (IU/d)					
	n	<239	239-362	363-595	≥596	P_{trend}
RFS						
Overall	321	1	0.77 (0.52-1.15)	0.76 (0.50-1.17)	0.85 (0.56-1.28)	0.66
Winter	100	1	0.27 (0.12-0.62)	0.46 (0.21-1.01)	0.72 (0.37-1.43)	0.46
Spring + fall	119	1	1.70 (0.82-3.49)	1.66 (0.81-3.38)	1.54 (0.75-3.16)	0.37
Summer	102	1	0.63 (0.31-1.31)	0.37 (0.17-0.81)	0.30 (0.13-0.70)	<0.01
Summer*	102	1	0.79 (0.39-1.59)	0.35 (0.15-0.82)	0.28 (0.10-0.80)	<0.01
Summer†	102	1	1.20 (0.56-2.59)	0.47 (0.22-1.01)	0.37 (0.16-0.85)	<0.01
OS						
Overall	321	1	0.73 (0.47-1.13)	0.68 (0.43-1.10)	0.77 (0.50-1.21)	0.44
Winter	100	1	0.29 (0.13-0.70)	0.23 (0.08-0.64)	0.68 (0.33-1.41)	0.84
Spring + fall	119	1	1.34 (0.60-2.98)	1.78 (0.82-3.87)	1.34 (0.62-2.88)	0.42
Summer	102	1	0.75 (0.35-1.60)	0.50 (0.22-1.13)	0.29 (0.11-0.75)	<0.01
Summer*	102	1	0.92 (0.43-1.96)	0.50 (0.22-1.17)	0.26 (0.08-0.79)	<0.01
Summer†	102	1	1.67 (0.73-3.83)	0.40 (0.17-0.92)	0.34 (0.14-0.88)	<0.01

NOTE: Adjusted for age, gender, stages, smoking status, and surgery season in Cox proportional hazards model, with vitamin D intake <239 IU/d as the reference group, with the values expressed as AHRs (95% CI).
*Adjusted for age, gender, stages, and retinol intake in Cox proportional hazards model.
† Dietary vitamin D intake only, with the quartiles of <200, 200-273, 274-378, and ≥379 IU/d, respectively. Adjusted for age, gender, and stages in Cox proportional hazards model, with dietary vitamin D intake <200 IU/d as the reference group, and the values expressed as AHRs (95% CI).

Table 4. AHRs for the joint effect of surgery seasons and vitamin D intake on RFS and OS (n = 321)

Season	Vitamin D intake (IU/d)											
	<239			239-362			363-595			≥596		
	n	Adjusted HR	5-y RFS or OS rate (%)	n	Adjusted HR	5-y RFS or OS rate (%)	n	Adjusted HR	5-y RFS or OS rate (%)	n	Adjusted HR	5-y RFS or OS rate (%)
RFS												
Winter	23	1	23	25	0.37 (0.17-0.80)	66	21	0.54 (0.26-1.13)	47	31	0.86 (0.52-2.87)	35
Spring/fall	31	0.56 (0.28-1.12)	55	28	0.76 (0.38-1.50)	42	32	0.72 (0.37-1.40)	44	28	0.67 (0.33-1.37)	38
Summer	25	0.85 (0.42-1.71)	38	28	0.63 (0.32-1.24)	49	27	0.39 (0.19-0.83)	74	22	0.33 (0.15-0.74)	56
OS												
Winter	23	1	30	25	0.35 (0.16-0.79)	66	21	0.25 (0.10-0.65)	75	31	0.72 (0.37-1.42)	47
Spring/fall	31	0.51 (0.25-1.06)	66	28	0.58 (0.28-1.21)	55	32	0.71 (0.35-1.43)	51	28	0.60 (0.29-1.25)	52
Summer	25	0.72 (0.35-1.50)	42	28	0.57 (0.28-1.16)	56	27	0.42 (0.20-0.92)	73	22	0.25 (0.10-0.63)	72

NOTE: Adjusted for age, gender, and clinical stages in Cox proportional hazards model, with the values expressed as AHRs (95% CI).

intake between 482 early-stage and 338 advanced-stage (stage IIIA-IV) NSCLC patients from the same cohort and found that early-stage patients have statistically significantly higher dietary vitamin D intake (mean, 291 IU/d; SD, 142) than advanced-stage patients (mean, 268 IU/d; SD, 124; P = 0.02, one-way ANOVA) and nonsignificantly higher total vitamin D intake (mean, 470 IU/d; SD, 282) than advanced-stage patients (mean, 447 IU/d; SD, 292; P = 0.27, one-way ANOVA). Although the development and clinical stage of NSCLC depend on many clinical factors, higher vitamin D levels may be associated with less aggressive lung cancer development as defined by an earlier stage at the time of diagnosis.

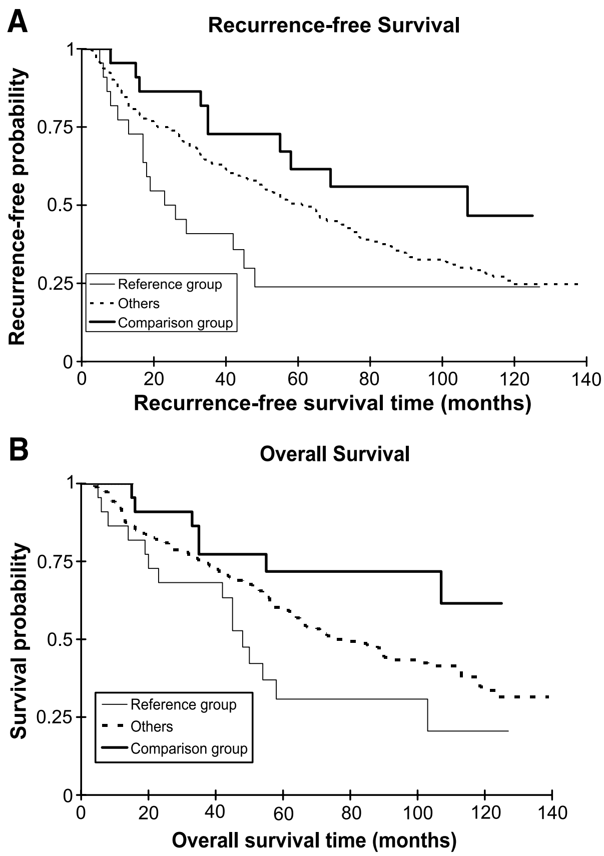

Figure 2. Kaplan-Meier curves of RFS (**A**; P = 0.02, log-rank test) and OS (**B**; P = 0.02; log-rank test) for early-stage NSCLC patients by the joint effects of surgery season and vitamin D intake (n = 321). The log-rank test was based on the full data. "Reference group" were patients who received surgery in winter with the lowest vitamin D intake (<239 IU/d), "comparison group" were patients who had surgery during summer with the highest vitamin D intake (≥596 IU/d), and "others" were all other patients combined.

We acknowledge several limitations in this study. (*a*) Sample size: This is a moderate sample size study, especially in the joint analyses of surgery season and vitamin D intake. (*b*) Missing data: In our population, 30% of patients were missing diet information. Patients who have diet data had nonstatistically significantly improved OS when compared with those without diet information, which may limit the generalizability of our results to all early-stage NSCLC patients. However, we did observe similar associations between surgery season and RFS (or OS) among the patients with and without diet information. (*c*) Change of diet: The food frequency questionnaire inquired diet for the period of 1 year before diagnosis. It is possible that dietary habits may have changed in the few months before diagnosis due to cancer-related symptoms. However, as all patients in our population are incident early-stage patients, it is less likely that the cancer-related symptoms occurred 1 year before the diagnosis. (*d*) Survival data collection: In this population, recurrence data were collected retrospectively and patients were not on a prescribed surveillance schedule. We attempted to contact local physicians whenever patients were followed outside of the MGH system. We collected the vital status data for each patient. However, we could not distinguish between death from lung cancer and death from other causes. Because the 5-year OS rates in this population were 65%, 53%, 34%, and 38%, respectively, for stages IA to IIB, the vast majority of these patients likely died from lung cancer. In addition, we found similar and consistent associations of surgery season and vitamin D intake with RFS and OS. (*e*) Incomplete adjustment of covariates: We did not take into account actual behaviors in seeking or avoiding sunlight, skin pigmentation, and other determinants. It is possible that some of the NSCLC patients stayed in other regions with higher UVB levels (e.g., Florida) before the time of surgery treatment. Because the majority of patients were diagnosed and followed up at MGH, the proportion of these patients is assumed to be very small. In addition, if some of these patients who stayed in Florida in winter and were misclassified in the season group with low vitamin D levels, the bias is toward the null and may actually attenuate associations of vitamin D-lung cancer prognosis. Because UVB radiation is the major source of vitamin D for the majority of people and only a few food sources contain vitamin D, it is not surprising that we did not observe the statistically significant associations between vitamin D intake and RFS (or OS). We did observe the statistically significant associations between the joint effects of surgery season and vitamin D intake and RFS (or OS).

In summary, for early-stage NSCLC patients, patients who had surgery in summer with "high" recent vitamin D intake have a statistically significantly improved RFS and OS than patients who had surgery in winter with ''low'' vitamin D intake. These results should be confirmed in a prospective study to assess the serum vitamin D levels at time of surgery. If the results are confirmed, our results, combined with findings in other studies, suggest that dietary vitamin D supplementation may be advisable for early stages of lung cancer patients, particularly during the winter season and in groups that tend to be deficient in vitamin D.

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