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 (OS) in 456 early stage non-small cell lung cancer (NSCLC) patients. The data were analyzed using logrank 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 ratios [AHR], 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, logrank test). Similar association between surgery season and RFS was found among the 321 patients with dietary information (P=0.33, logrank 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 (AHR, 0.33; 95%CI, 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 OS were also observed. In conclusion, the joint effects of surgery season and recent vitamin D intake appear to be associated with the survival of early stage NSCLC patients.

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 ultraviolet radiation (UV-B) 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 anti-proliferative 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 UV-B radiation in a geographical region of residence. Geographic regions with lower UV-B 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 to the 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 Boston area, the serum 25D 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 to patients who received treatment in the summer and consumed more vitamin D. We tested these hypotheses in our cohort of early stage 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 over 18 years old. 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 to IIB) NSCLC patients recruited between 1992 and 2000, ensuring a follow-up time of at least four years, which accounted for 58% of all stages of NSCLC patients enrolled in this study. Thirteen non-incident patients and three patients who did not 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 (FFQ, Harvard-Willett) were completed for each patient at the time of recruitment (14;15). The 126 food item FFQ, 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 geographical area, the FFQ is likely valid for our study population, although nurses and health professionals are mostly college graduates while subjects in our population have various education levels and backgrounds. In the FFQ, 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 prior to 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 USDA 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 (HPFS) have shown good correlations (skim/low fat milk, r=0.88; whole milk, r=0.67; dark fish, r=0.58) (16). The vitamin D intake has also been validated for plasma 25(OH)D levels in HPFS (17;18).

Survival Measurements. Recurrence-free survival (RFS) and overall survival (OS) were the endpoints in this study. RFS is defined as the time from the date of surgery to the first date of recurrence, or death from any cause. Data on recurrence was 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 clinic and radiographic follow-up and date of recurrence was collected.

OS time was calculated from the date of surgery to the date of death from any cause or last follow-up. Dates of death were obtained and cross-checked using at least one of the following four methods: (i) inpatient and outpatient medical records, (ii) MGH tumor registry, (iii) confirmation with the patient's primary care physician and/or family, and (iv) 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 (i) and/or (iii) as described above.

Statistical Analysis. In the Boston area, minimal solar UV-B 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) (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 performed to compare the distribution of demographic, histological, 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 chi-squared 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 and Meier and assessed using the logrank test. Cox proportional hazards models were used as our primary analyses, controlling for multiple variables simultaneously, with age as a continuous variable, gender, smoking status, and clinical stage as categorical variables. All reported p-values are from two-sided tests. P-values less than 0.05 were considered statistically significant. All statistical analyses utilized SAS software Version 8 (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 post-operative 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 [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 (ranged 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 non-significantly higher frequency of stage IA (56% in summer and 46% in winter) and a lower frequency of stage 2B (15% in summer and 23% in winter; P=.19, Chi-square test). Adjustment for clinical stage was included in all of the Cox proportional hazards models.

Surgery Season and Lung Cancer RFS and OS. In the logrank test, patients who had surgery in summer had a non-statistically significantly longer RFS compared to those who had surgery in winter (P=0.10, logrank test; Figure 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 non-statistically significant difference was observed for OS (P=0.30, logrank test; Figure 1B) in different seasons, 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 (AHRs) 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 to winter), which were similar to the corresponding crude hazard ratios of 0.92 (95% CI, 0.69-1.22) and 0.73 (95% CI, 0.55-0.98). 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 hazard ratios of 0.94 (95% CI, 0.69-1.28) and 0.78 (95% CI, 0.57-1.08), too. Similar associations between surgery season and RFS (or OS) were found for patients with different age, gender, histological subtypes, smoking status, clinical stages, treatment (surgery only *vs*. 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=.02 by Wilcoxon Rank Sum Test), had non-significantly improved RFS (logrank test, P=.10) and OS (logrank test, P=.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 to 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, logrank 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, logrank test). For OS, the AHRs for patients who had surgery in spring/fall and summer (when compared to 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, logrank 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, logrank 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/day (lowest quartile). We did not observe the statistically significant association between vitamin D intake and RFS (AHR of highest *vs.* lowest quartile of intake, 0.85; 95% CI, 0.56-1.28; P_{trend}=0.66) or OS (AHR of highest *vs.* 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 *vs.* lowest quartile of intake, 0.30; 95% CI, 0.13-0.70; P_{trend} <0.01) and OS (AHR of highest *vs.* 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 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/day, no vitamin D supplements) were treated as the "reference group" in comparisons. Table 4 demonstrates all other patient groups had improved RFS (or OS) rates (some with statistically significant differences) when compared to 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, Figure 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

A number of factors, including regional UV-B 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 UV-B 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 three-fold better RFS and a four-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, Figure 2). In Cox proportional hazard models, we adjusted for the most important predictors of NSCLC prognosis including age, gender, smoking status, and clinical stage which may impact on lung term survival, performed 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 patients (88%) 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$(OH)_2$D inhibits metastatic growth of lung cancer cells, and mice fed manipulated diets display an apparent inverse relationship between the physiological levels of serum 1,25$(OH)_2$D 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$(OH)_2$D also inhibits proliferation and induces differentiation of lung cancer cell lines (25;26), and increased expression of the 1-alpha-hydroxylase gene has been found in alveolar macrophages of patients with lung cancer (27).

In humans, 1,25$(OH)_2$D may exert its anticarcinogenic effects through stimulating the secretion of protein glues such as E-cadherin and catenin that make cells more adherent to each other (28), making cells in the tumor mass more adherent to each other, reducing the likelihood of mobilization of large numbers of 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$(OH)_2$D 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 anti-metastasis 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 MK *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 more than 239 IU/day vitamin D intake (the combined three highest quartiles) when compared with patients <239 IU/day 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 more than 239 IU/day versus patients <239 IU/day 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), 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 intake between 482 early and 338 advanced stage (stage IIIA to IV) NSCLCs patients from the same cohort, and found that early stage patients have statistically significantly higher dietary vitamin D intake (mean=291 IU/day, standard deviation, SD=142) than advanced stage patients (mean=268 IU/day, SD=124; P=0.02 by one-way ANOVA), and non-significantly higher total vitamin D intake (mean=470 IU/day, SD=282) than advanced stage patients (mean=447 IU/day, SD=292; P=0.27 by 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.

We acknowledge several limitations in this study. (i) Sample size: This is a moderate sample size study, especially in the joint analyses of surgery season and vitamin D intake. (ii): Missing data: In our population, 30% of patients were missing diet information. Patients who have diet data had non-statistically significantly improved OS when compared with those without diet information, which may limit the generalizibility 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. (iii) Change of diet: The FFQ inquired diet for the time period of one year prior to diagnosis. It is possible that dietary habits may have changed in the few months prior to 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 one year before the diagnosis. (iv) 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 the death from lung cancer and 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. (v). 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 UV-B 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 towards the null and may actually attenuate associations of vitamin D-lung cancer prognosis. Because UV-B 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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References

1. Jones G, Ramshaw H, Zhang A, et al. Expression and activity of vitamin D-metabolizing cytochrome P450s (CYP1alpha and CYP24) in human nonsmall cell lung carcinomas. Endocrinology 1999;140:3303-10.
2. Jiang F, Bao J, Li P, Nicosia SV, Bai W. Induction of ovarian cancer cell apoptosis by 1,25-dihydroxyvitamin D3 through the down-regulation of telomerase. J Biol Chem 2004;279:53213-21.
3. Garland FC, Garland CF, Gorham ED, Young JF. Geographic variation in breast cancer mortality in the United States: a hypothesis involving exposure to solar radiation. Prev Med 1990;19:614-22.
4. Schwartz GG, Hulka BS. Is vitamin D deficiency a risk factor for prostate cancer? (Hypothesis). Anticancer Res 1990;10:1307-11.
5. Hanchette CL, Schwartz GG. Geographic patterns of prostate cancer mortality. Evidence for a protective effect of ultraviolet radiation. Cancer 1992;70:2861-9.
6. Lefkowitz ES, Garland CF. Sunlight, vitamin D, and ovarian cancer mortality rates in US women. Int J Epidemiol 1994;23:1133-6.
7. Grant WB. An estimate of premature cancer mortality in the U.S. due to inadequate doses of solar ultraviolet-B radiation. Cancer 2002;94:1867-75.
8. Robsahm TE, Tretli S, Dahlback A, Moan J. Vitamin D3 from sunlight may improve the prognosis of breast-, colon- and prostate cancer (Norway). Cancer Causes Control 2004;15:149-58.

9. Brustad M, Alsaker E, Engelsen O, Aksnes L, Lund E. Vitamin D status of middle-aged women at 65-71 degrees N in relation to dietary intake and exposure to ultraviolet radiation. Public Health Nutr 2004;7:327-35.
10. Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. J Clin Endocrinol Metab 1988;67:373-8.
11. Webb AR, Pilbeam C, Hanafin N, Holick MF. An evaluation of the relative contributions of exposure to sunlight and of diet to the circulating concentrations of 25-hydroxyvitamin D in an elderly nursing home population in Boston. Am J Clin Nutr 1990;51:1075-81.
12. Harris SS, Dawson-Hughes B. Seasonal changes in plasma 25-hydroxyvitamin D concentrations of young American black and white women. Am J Clin Nutr 1998;67:1232-6.
13. Thomas MK, Lloyd-Jones DM, Thadhani RI, et al. Hypovitaminosis D in medical inpatients. N Engl J Med 1998;338:777-83.
14. Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol 1992;135:1114-26.
15. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51-65.
16. Feskanich D, Rimm EB, Giovannucci EL, et al. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. J Am Diet Assoc 1993;93:790-6.

17. Platz EA, Rimm EB, Willett WC, Kantoff PW, Giovannucci E. Racial variation in prostate cancer incidence and in hormonal system markers among male health professionals. J Natl Cancer Inst 2000;92:2009-17.

18. Platz EA, Leitzmann MF, Hollis BW, Willett WC, Giovannucci E. Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and subsequent risk of prostate cancer. Cancer Causes Control 2004;15:255-65.

19. Oliveira S, Aro A, Sparrow D, Hu H. Season modifies the relationship between bone and blood lead levels: the Normative Aging Study. Arch Environ Health 2002;57:466-72.

20. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986;124:17-27.

21. Giovannucci E. The epidemiology of vitamin D and cancer incidence and mortality: A review (United States). Cancer Causes Control 2005;16:83-95.

22. Nakagawa K, Kawaura A, Kato S, Takeda E, Okano T. Metastatic growth of lung cancer cells is extremely reduced in Vitamin D receptor knockout mice. J Steroid Biochem Mol Biol 2004;89-90:545-7.

23. Nakagawa K, Kawaura A, Kato S, Takeda E, Okano T. 1{alpha},25-Dihydroxyvitamin D3 is a preventive factor in the metastasis of lung cancer. Carcinogenesis 2005;26:429-40.

24. Wiers KM, Lathers DM, Wright MA, Young MR. Vitamin D3 treatment to diminish the levels of immune suppressive CD34+ cells increases the effectiveness of adoptive immunotherapy. J Immunother 2000;23:115-24.

25. Higashimoto Y, Ohata M, Nishio K, et al. 1 alpha, 25-dihydroxyvitamin D3 and all-trans-retinoic acid inhibit the growth of a lung cancer cell line. Anticancer Res 1996;16:2653-9.

26. Guzey M, Sattler C, DeLuca HF. Combinational effects of vitamin D3 and retinoic acid (all trans and 9 cis) on proliferation, differentiation, and programmed cell death in two small cell lung carcinoma cell lines. Biochem Biophys Res Commun 1998;249:735-44.

27. Yokomura K, Suda T, Sasaki S, Inui N, Chida K, Nakamura H. Increased expression of the 25-hydroxyvitamin D(3)-1alpha-hydroxylase gene in alveolar macrophages of patients with lung cancer. J Clin Endocrinol Metab 2003;88:5704-9.

28. Palmer HG, Gonzalez-Sancho JM, Espada J, et al. Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling. J Cell Biol 2001;154:369-87.

29. Kalogeraki A, Bouros D, Zoras O, et al. E-cadherin expression on fine-needle aspiration biopsies in primary lung adenocarcinomas is related to tumor differentiation and invasion. Anticancer Res 2003;23:3367-71.

30. Choi YS, Shim YM, Kim SH, et al. Prognostic significance of E-cadherin and beta-catenin in resected stage I non-small cell lung cancer. Eur J Cardiothorac Surg 2003;24:441-9.

Table 1 Demographic, clinical, and treatment characteristics in non-small cell lung cancer patients by surgery season

	Overall				Patients with diet information				Patients without diet information			
Characteristic	Winter n=140	Spring/Fall n=165	Summer n=151	*P* Value	Winter n=100	Spring/Fall n=119	Summer n=102	*P* Value	Winter n=40	Spring/Fall n=46	Summer n=49	*P* Value
Age, median [a]	68	68	70	.08	67	68	70	.01	72	71	70	.92
range	35-88	31-89	32-87		43-88	31-89	42-87		35-88	35-88	32-86	
Gender, female [b]	46%	49%	48%	.83	45%	50%	46%	.77	48%	48%	53%	.83
Histological cell type [b]												
Adenocarcinoma	51%	50%	46%	.34	53%	50%	52%	.99	45%	52%	35%	.02
Squamous	30%	28%	31%		26%	29%	29%		40%	24%	35%	
Large cell	6%	4%	3%		6%	4%	4%		8%	4%	2%	
Bronchioloalveolar	8%	10%	16%		9%	11%	11%		5%	9%	27%	
Others	5%	7%	3%		6%	6%	4%		2%	11%	2%	
Clinical stage [b]												
IA	46%	52%	56%	.19	48%	52%	58%	.28	40%	50%	51%	.82
IB	27%	32%	25%		25%	33%	24%		33%	30%	27%	
IIA	4%	4%	5%		5%	4%	4%		3%	4%	6%	
IIB	23%	12%	15%		22%	11%	14%		25%	15%	16%	
Surgery type [b]												
Wedge	27%	23%	23%	.25	28%	24%	24%	.18	23%	22%	22%	.69
Lobectomy	54%	65%	64%		52%	63%	67%		60%	70%	59%	
Others	19%	12%	13%		20%	13%	10%		17%	9%	18%	
Radiation/ chemotherapy [b]	13%	6%	9%	.12	14%	6%	11%	.13	10%	7%	4%	.54
Smoking status [b]												
Never smokers	5%	10%	7%	.25	5%	9%	5%	.41	5%	13%	10%	.62
Ex-smokers	50%	51%	57%		51%	51%	60%		45%	50%	51%	
Current-smokers	45%	39%	36%		44%	40%	35%		40%	37%	39%	

[a]: Wilcoxon Rank Sum test.

[b]: Frequency, tested by Chi-square test.

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

	Vitamin D intake (IU/day, n=321)				
Characteristic	<239 n=79	239-362 n=81	363-595 n=80	=596 n=81	*P* Value
Age, years [a]	68 (33-82)	69 (31-89)	69 (46-88)	68 (47-85)	.45
Gender, female [b]	47%	49%	49%	43%	.86
Histological cell type [b]					
Adenocarcinoma	54%	46%	49%	57%	
Squamous	26%	41%	27%	20%	
Large cell	5%	2%	5%	6%	.53
Bronchioloalveolar	10%	7%	13%	11%	
Others	5%	4%	6%	6%	
Clinical stage [b]					
IA	58%	57%	51%	44%	
IB	18%	26%	33%	35%	.50
IIA	5%	4%	4%	5%	
IIB	19%	13%	12%	16%	
Surgery type [b]					
Wedge	24%	31%	25%	20%	
Lobectomy	60%	57%	59%	68%	.69
Others	16%	12%	16%	12%	
Radiation/chemotherapy [b]	11%	10%	8%	11%	.84
Smoking status [b]					
Never	3%	4%	15%	5%	
Ex-smokers	49%	55%	50%	60%	.02
Current-smokers	48%	41%	35%	35%	
Alcohol drinking (gm/day) [c]	1.1 (0.8)	1.1 (0.8)	1.0 (0.8)	0.8 (0.8)	.33
Education levels [b,d]					
High school, lower	55%	55%	65%	53%	.45
College, higher	45%	45%	35%	47%	
Multivitamins use [b]	0	11%	38%	90%	<.001
BMI at 18 years [c, e]	22 (3)	22 (4)	21 (3)	22 (3)	.49
Energy intake (kcal/day) [c]	2123 (698)	2044 (691)	2313 (685)	2023 (683)	.03
Animal protein (gm/day) [c]	47 (15)	54 (13)	55 (15)	60 (18)	<.001
Animal fat (gm/day) [c]	39 (14)	37 (14)	35 (13)	37 (14)	.34
Retinol intake (IU/day) [c]	1558 (1503)	2493 (2017)	3285 (3201)	6601(4689)	<.001
Calcium intake (mg/day) [c]	665 (227)	870 (304)	1118 (429)	1267 (583)	<.001
Vitamin D intake (IU/day) [c]	165 (47)	296 (38)	468 (70)	813 (205)	<.001

[a]: Median (range), tested by Wilcoxon Rank Sum test.

[b]: Frequency, tested by Chi-square test.

[c]: Mean (standard deviation), tested by one way ANOVA.

[d]: Available for 310 patients.

[e]: Available for 309 patients.

Table 3 Adjusted hazard ratios for vitamin D intake on recurrence-free survival and overall survival by surgery season (n=321) [a]

		Vitamin D intake (IU/day)				
Surgery Season	N	<239	239-362	363-595	=596	P_{trend}
Recurrence-free Survival						
Overall	321	1	0.77 (0.52-1.15)	0.76 (0.50-1.17)	0.85 (0.56-1.28)	.66
Winter	100	1	0.27 (0.12-0.62)	0.46 (0.21-1.01)	0.72(0.37-1.43)	.46
Spring + Fall	119	1	1.70 (0.82-3.49)	1.66 (0.81-3.38)	1.54 (0.75-3.16)	.37
Summer	102	1	0.63 (0.31-1.31)	0.37 (0.17-0.81)	0.30 (0.13-0.70)	<.01
Summer [b]	102	1	0.79 (0.39-1.59)	0.35 (0.15-0.82)	0.28 (0.10-0.80)	<.01
Summer [c]	102	1	1.20 (0.56-2.59)	0.47 (0.22-1.01)	0.37 (0.16-0.85)	<.01
Overall Survival						
Overall	321	1	0.73 (0.47-1.13)	0.68 (0.43-1.10)	0.77 (0.50-1.21)	.44
Winter	100	1	0.29 (0.13-0.70)	0.23 (0.08-0.64)	0.68 (0.33-1.41)	.84
Spring + Fall	119	1	1.34 (0.60-2.98)	1.78 (0.82-3.87)	1.34 (0.62-2.88)	.42
Summer	102	1	0.75 (0.35-1.60)	0.50 (0.22-1.13)	0.29 (0.11-0.75)	<.01
Summer [b]	102	1	0.92 (0.43-1.96)	0.50 (0.22-1.17)	0.26 (0.08-0.79)	<.01
Summer [c]	102	1	1.67 (0.73-3.83)	0.40 (0.17-0.92)	0.34 (0.14-0.88)	<.01

[a]: Adjusted for age, gender, stages, smoking status, and surgery season in Cox proportional hazard model, with vitamin D intake <239 IU/day as the reference group, with the values expressed as adjusted hazard ratios (95% confidence interval).
[b]: Adjusted for age, gender, stages, and retinol intake in Cox proportional hazard model.
[c]: Dietary vitamin D intake only, with the quartiles of <200, 200-273, 274-378, and =379 IU/day, respectively. Adjusted for age, gender, and stages in Cox proportional hazard model, with dietary vitamin D intake <200 IU/day as the reference group, and the values expressed as adjusted hazard ratios (95% confidence interval).

Table 4 Adjusted hazard ratios for the joint effect of surgery seasons and vitamin D intake on recurrence-free survival and overall survival (n=321) [a]

	Vitamin D intake (IU/day)											
	<239			239-362			363-595			=596		
Season	N	Adjusted HR	5-yr	N	Adjusted HR	5-yr	N	Adjusted HR	5-yr	N	Adjusted HR	5-yr
Recurrence-free Survival												
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%
Overall Survival												
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%

Abbreviations: HR: hazard ratio, 5-yr: 5-year recurrence-free survival or overall survival rate.

[a]: Adjusted for age, gender, and clinical stages in Cox proportional hazard model, with the values expressed as adjusted hazard ratios (95% confidence interval).

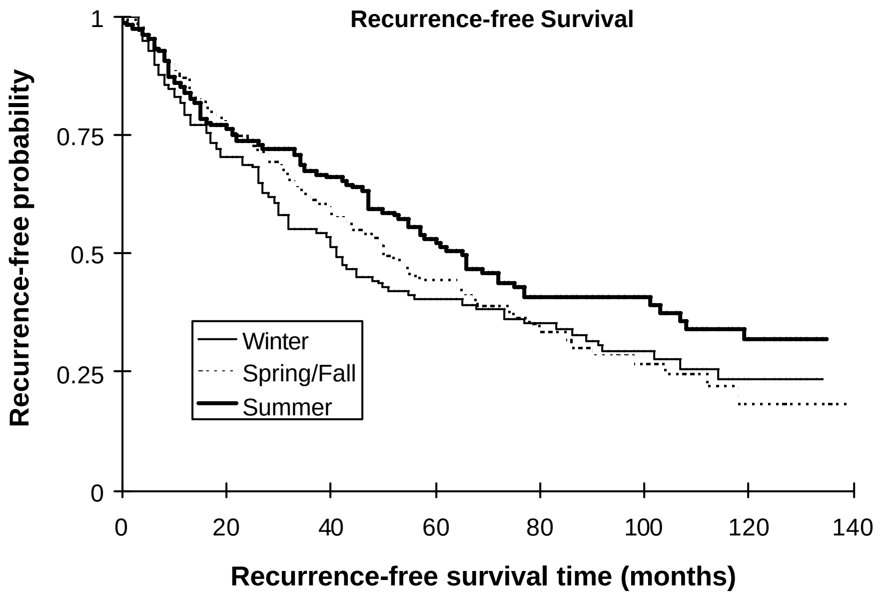


Figure 1A

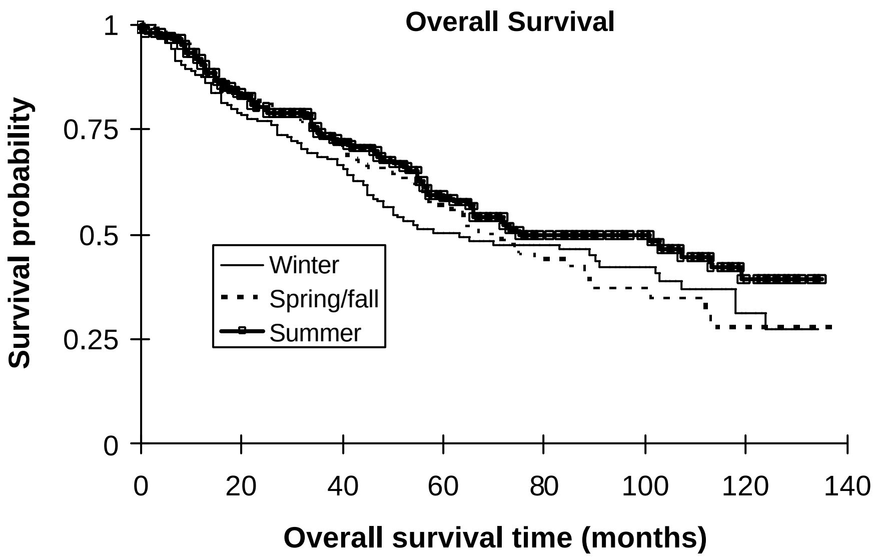
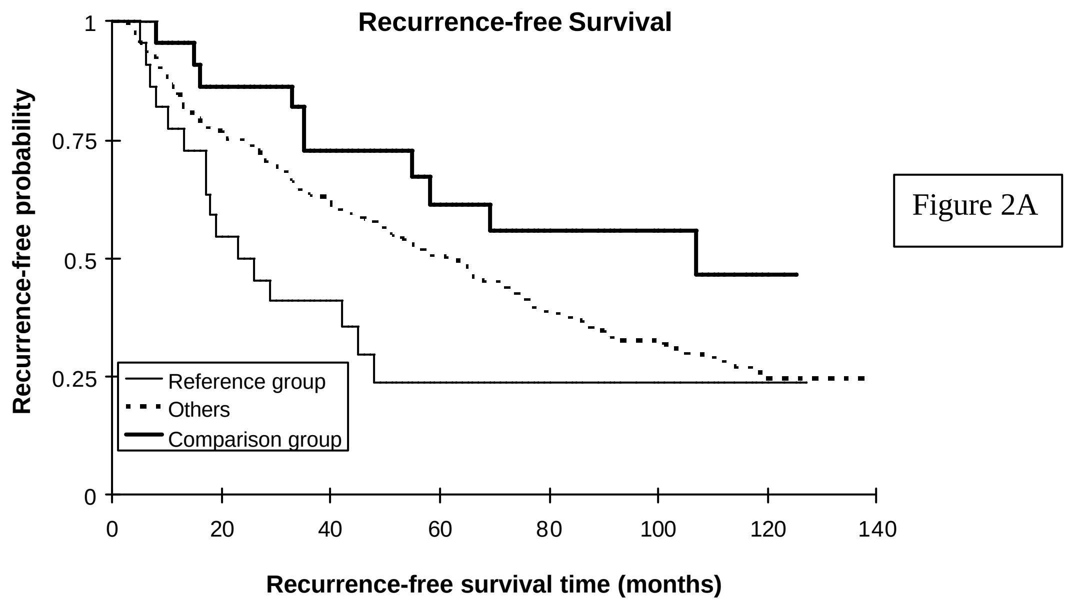


Figure 1B

Figure 1 Kaplan-Meier curves of recurrence-free survival (A, P=0.10, logrank test) and overall survival (B, P=0.30, logrank test) for non-small cell lung cancer patients who received surgery in different seasons (n=456). Logrank test was based on the full data.



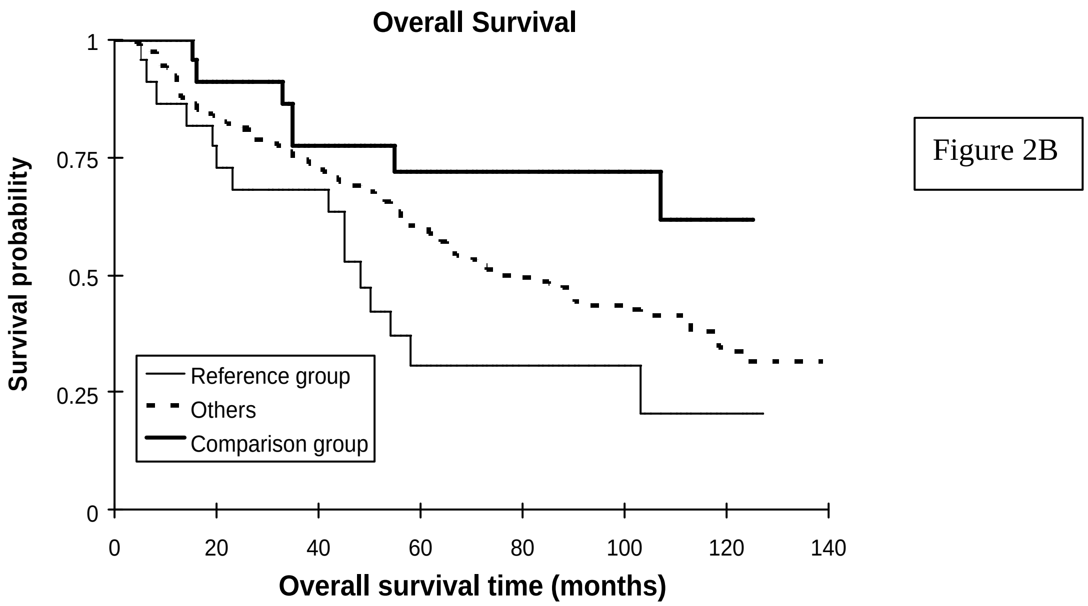


Figure 2 Kaplan-Meier curves of recurrence-free survival (A, p=0.02, logrank) and overall survival (B, P=0.02; logrank test) for early stages NSCLC patients by the joint effects of surgery season and vitamin D intake (n=321). The logrank test was based on the full data. “Reference group” were patients who received surgery in winter with the lowest vitamin D intake (<239 IU/day), “comparison group” were patients who had surgery during summer with the highest vitamin D intake (=596 IU/day), “others” were all other patients combined.