# Vitamin D, Interaction with Vitamin A and Lung Cancer

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## Abstract

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Vitamin D inhibits several pathways of lung cancer carcinogenesis and cells in the respiratory tract produce and utilize vitamin D. Vitamin D's functions rely on vitamin D receptor together with retinoid X receptor, which ligands with 9-*cis*-retinoic acid, a vitamin A (retinol) metabolite. The objectives of this dissertation are to investigate 1) whether high versus low vitamin D intake is associated with lower lung cancer incidence, 2) whether high/excess vitamin A intake attenuates the inverse association of vitamin D intake with lung cancer, and 3) whether vitamin D intake is associated with vitamin D status, represented by serum 25-hydroxyvitamin D concentrations. Data sources were the Women's Health Initiative Clinical Trials and Observational Study (WHI-CT and OS), recruiting postmenopausal women mostly former/never smokers, and the Carotene and Retinol Efficacy Trial (CARET), recruiting male and female current/former heavy

smokers and workers with occupational exposure to asbestos. Vitamin D exposure included total vitamin D intake from food and dietary supplements and 1 g calcium+400 IU vitamin $D_3$ daily supplementation from the WHI Calcium/Vitamin D Trial. Vitamin A exposure included total vitamin A intake from food and dietary supplements and CARET's intervention—30 mg β-carotene+25,000 IU retinyl palmitate daily supplementation (22,500 μg/day Retinal Activity Equivalent [RAE]). Results from the WHI-OS showed that total vitamin D intake was strongly associated with serum 25-hydroxyvitamin D concentrations after adjusting for available covariates and sun exposure variables. The vitamin D intake-lung cancer associations were examined separately in the WHI CT+OS and CARET. High (≥400 IU/day in WHI and ≥600 IU/day in CARET) versus low total vitamin D intake was associated with a lower risk of lung cancer, particularly for non-small cell lung cancer and adenocarcinoma, among never smokers in WHI and former smokers in CARET. The patterns of effect modification of vitamin A intake were heterogeneous according to participants' smoking status. Among current smokers (and CARET former smokers, who generally were heavy smokers before quitting), an inverse association of total vitamin D intake with lung cancer was only observed among those with high total vitamin A intake (≥3,000 μg/day RAE in WHI [*P*-interaction=0.26] and ≥1,500 μg/day RAE in CARET [*P*-interaction=0.08]) or receiving the CARET intervention (*P*-interaction=0.24). However, among WHI participants as a whole, high vitamin A intake (≥1,000 μg/day RAE) may attenuate a protective association of 1 g calcium+400 IU vitamin $D_3$ supplementation with lung cancer (*P*-interaction=0.09). The difference in smoking between WHI and CARET may contribute to the discrepant findings on vitamin A effect modification. The findings need further

confirmation by biomarkers of vitamin A that reflects internal dose and have less measurement error compared to dietary data. This work demonstrates that vitamin D is an important determinant for postmenopausal women and provides important fundamentals for vitamin D and vitamin A in lung cancer prevention.

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# Chapter 1

## Background

## 1.1 Epidemiology of lung cancer

Lung cancer is a major disease burden in the United States. It is estimated that 226,160 new cases of lung cancer (including bronchus) occurred in 2012.(1) Lung cancer ranks second of the most common cancers (14% in both males and females). Due to its high case-fatality rate, lung cancer is the number one cause of cancer deaths. In 2012, 160,340 people died from lung cancer. Lung cancer mortality accounts for over a quarter of all cancer deaths (29% in males and 26% in females).(1)

Chronologically, the epidemic of lung cancer in the U.S. aligns with the use of tobacco. The cigarette consumption per capita ≥18 years increased by 80 times (54 to 4,000) from 1880 to 1970s.(2) The lung cancer mortality in men increased 18-fold (5 to 90 per 100,000, age-standardized to the 2000 U.S. standard population) between 1930 and 1990.(1) Although technologies to diagnose lung cancer have improved over time, overwhelming evidence indicates that the major reason of this steep increase in incidence was the upsurge in cigarette smoking.(3) Men's lung cancer incidence and mortality rate has started to decline since 1990s mainly due to a decrease in smoking prevalence (from 56.9% in 1955 to 21.6 in 2011).(4-6) The latest incidence data in men was 75 per 100,000 in 2009.(1) For women, the lung cancer incidence rate doubled from 25 per 100,000 in 1975 to 50 per 100,000 in 2009. The female lung cancer mortality has flattened and shown a sign of decline since 2005.(1)

**Survival.** Compared to other cancers with high survival rate (e.g., 99% for prostate cancer and 89% for breast cancer, 5 years after diagnosis), lung cancer is devastating because its overall 5-year survival rate is only 16%.(7) The dismal survival rate is related to the stage of lung cancer diagnosis. Patients with lung cancer diagnosed in stages I and II (American Joint Committee on Cancer [AJCC] staging system) have modest survival rates (approximately 65% and 40%, respectively).(8) However, over half (54%) of non-small cell lung cancers, the major type of lung cancer representing 85% of all lung cancers, are detected with distant metastases (stage IV). Stage IV lung cancer has a substantially lower 5-year survival rate (13%).(8)

**Histopathology.** Lung cancer can be classified into two major cell types according to its histopathology: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC).(9) NSCLC represents 85% of all lung cancers (**Table 1**). Among NSCLCs, there are three main subtypes: squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. Squamous cell carcinoma is a malignant epithelial tumor arising from bronchial epithelium. The majority of squamous cell carcinomas originate in the mainstem, lobar or segmental bronchi.(10) Adenocarcinoma is a malignant epithelial tumor with glandular differentiation or mucin production, i.e., glands and gland-like elements. The subtypes include acinar adenocarcinoma (malignant cells in a small saclike dilation composing a compound gland), papillary adenocarcinoma (malignant papillary structure replacing normal tissue), bronchioloalveolar carcinoma and other variants.(11) Large cell carcinoma is an undifferentiated non-small cell carcinoma. Large cell

carcinoma lacks the cytologic features of small cell carcinoma and glandular or squamous differentiation.(9) SCLC is a malignant epithelial tumor consisting of small cells, which have scant cytoplasm, ill-defined cell borders, finely granular nuclear chromatin, and absent nucleoli. Ninety percent of SCLC contains only small cells; the remaining cases contain large-cell components.(12)

The distribution of lung cancer histology is associated with gender, smoking behavior and cigarette design. Although smoking is associated with all histological types of lung cancer, the effect is stronger for squamous cell carcinoma and SCLC compared to adenocarcinoma and large cell carcinoma.(13-15) Globally, the ratios of adenocarcinoma to squamous cell carcinoma are 0.4:1 among smokers and 3.4:1 among never smokers.(16) The reason that non-tobacco-related carcinogens favor adenocarcinoma histology, particularly in women, is poorly understood. A U.S. local cancer surveillance program in 1984 showed that the adenocarcinoma-to-squamous cell carcinoma ratio was 0.3:1 among male never smokers; however, the ratio was 3.6:1 among female never smokers.(17) The proportion of adenocarcinoma among NSCLCs has been steeply increasing over time. In the U.S., the adenocarcinoma-to-squamous cell carcinoma ratios in males were 1:18 in 1950, 1:1.4 in 1983–87,(18) and 1.4:1 in 1988–2001 (Table 1). The change in the ratio may be due to changes in cigarette design (adoption of filtertips) and smoking behavior rather than advances in diagnosis.(19, 20) Compared to using unfiltered cigarettes, smokers who consume filtered cigarettes need to inhale deeply to achieve a comparable effect from nicotine. Thus, more smoke reaches peripheral airways and alveoli where glands locate. Also, to enhance the combustion, the levels of nitrate in

cigarettes have been increased, resulting higher concentrations of nitrosamine in tobacco smoke which causes more adenocarcinomas than other types of lung cancer [reviewed in (21, 22)].

Table 1. The distribution of lung cancer histology by gender, 12 SEER areas, 1988–2001(23)

| Cell type | Males (%) | Females (%) |
|---|---|---|
| **Non-small cell lung cancer** | | |
| Squamous cell carcinoma | 24.2 | 15.2 |
| Adenocarcinoma | 33.5 | 40.9 |
| Large Cell carcinoma | 7.7 | 7.1 |
| Others | 19.4 | 18.6 |
| **Small cell lung cancer** | 15.2 | 18.2 |

**Lung cancer in never smokers**. In the U.S., approximately 10% of lung cancers are not smoking-related.(24) The mortality rates were 17.1 for male and 14.7 per 100,000 for female never smokers based on estimates from two large American Cancer Society Cancer Prevention Study cohorts (1959–1972 and 1982–2000). The rates were 25%–35% lower when age-standardized to the U.S. population in year 2000.(25) The mortality rates were constant over time. If lung cancer among never smokers can be considered as a separate disease, the mortality rate is similar to several top 10 cancer causes of death such as non-Hudgkin lymphoma and endometrial cancer.(1)

Globally, the incidence and mortality of lung cancer among never smokers vary by gender and race. A pooled analysis of never smokers showed that in men, lung cancer incidence rates were similar among individuals of European descent, African Americans,

and Asians residing in Asia (11.2–12.9 per 100,000, age-standardized to the IARC World Standard Population for 2000). However, in women, African Americans had 60% higher lung cancer incidence compared to European Descent individuals (19.4 versus 12.4 per 100,000; rate ratio= 1.6, 95% CI=1.2–2.1). For lung cancer mortality, male Asians had a 2-fold higher mortality rate compare to male European descents (26.0 versus 12.0 per 100,000; rate ratio= 2.0, 95% CI=1.7–2.3). The difference was smaller between Asian women and women of European descent (16.1 versus 9.5 per 100,000; rate ratio= 1.7, 95% CI=1.51.8).(26) Due to the relatively higher mortality rate and low smoking prevalence among Asian women (e.g., 2.4 % in China),(27) it is estimated that over half of female lung cancers worldwide are not attributable to tobacco use.(28)

**Risk factors. Table 2** lists risk factors for lung cancer. Cigarette smoking or tobacco use is the most important risk factor for lung cancer. In the U.S., Smoking causes 90% of lung cancer deaths.(29, 30) In tobacco smoke, at least 20 carcinogens convincingly cause lung tumors in animals or humans. These carcinogens (e.g., polycyclic aromatic hydrocarbons [PAH] and N-Nitrosamines) lead to DNA adducts, which result in persistent miscoding. Miscoding leads to mutation in oncogenes including *RAS, MYC, p52, p16, RB*, and *FHIT* in the lung tumorigenesis pathways.(31) Secondhand or environmental tobacco smoke increases lung cancer risks in never smokers.(32, 33) In addition, numerous environmental and occupational exposure or toxins have been identified as risk factors for lung cancer. Also, genetic variants in several oncogenes and metabolic enzymes are linked to lung cancer. Genome-wide association (GWA) studies

have identified several DNA regions and genetic variants associated with lung cancer; more research is required to understand their biological significance.(34) For dietary factors, many have been studied, but most evidence remains controversial except for carotenoids.

Table 2. Lung cancer risk factors

| Risk factor | Important statistics and evidence[1] |
|---|---|
| *Demographic* | |
| Age | In 2009 SEER data, 0% of lung cancers were diagnosed under age 20; 0.2% between 20 and 34; 1.5% between 35 and 44; 8.8% between 45 and 54; 21.3% between 55 and 64; 31.3% between 65 and 74; 28.3% between 75 and 84; and 8.4% 85+ years of age.(7) |
| Race/ethnicity | A multi-nation, pooled analysis of never smokers showed that in women, African Americans had 60% higher lung cancer incidence compared to European descent individuals (19.4 versus 12.4 per 100,000; rate ratio= 1.6, 95% CI=1.2–2.1), although the difference was not observed in men.(26) |
| Socio-economic status (SES) | A meta-analysis reported that among studies of SES adjusted for smoking and other confounders, the relative risks for lung cancer risk associated with SES based on education attainment (lowest versus highest) was 1.46 (95% CI=1.27-1.68); occupation: 1.33 (95% CI=1.14-1.55); income: 1.25 (95% CI=0.93-1.70).(35) |
| *Behavioral* | |
| Smoking | Tobacco smoke is IARC Group 1 agent for lung cancer[2].(36) A landmark, large cohort study in the U.S. showed that the relative risks for lung cancer (current versus never smoking) were 22.3 for men and 11.9 for women.(3) |
| Postmenopausal hormone therapy | A randomized, double-blind, placebo-controlled trial shows that estrogen plus progestin in postmenopausal women increases lung cancer mortality (HR 1.71, 95% CI=1.16-2.52), but not incidence (HR=1.23, 95% CI 0.92-1.63).(37) Use of conjugated equine estrogen alone (among who had a previous hysterectomy) does not increase incidence or mortality.(38) |
| Physical inactivity | A meta-analysis including 14 prospective studies reported that both high (RR=0.77, 95% CI=0.73-0.81) and medium (RR=0.87, 95% CI=0.83-0.90) levels of physical activity are associated with a lower risk of lung |

| | |
|---|---|
| | cancer compared to low level of physical activity.(39) Another meta-analysis including earlier (1966-2003) studies also observed similar risks.(40) |
| *Environmental* | |
| Outdoor air pollution | Large cohort studies overall and in never smokers showed that fine particulate matter diameter ≤2.5 μm ($PM_{2.5}$) is positively associated with lung cancer risk.(41, 42) |
| Indoor air pollutant (combustion of coal and biomass and cooking fumes) | Indoor coal smoke is IARC Group 1 agent for lung cancer. Biomass burning and high-temperature frying indoor emissions are IARC Group 2A agents.[3] Meta- and pooled analyses suggest that in-home burning of both coal and biomass is consistently associated with an increased risk of lung cancer.(43-45) Evidence from case-control studies among Asian, never-smoking women show consistent positive association of cooking fumes with lung cancer [reviewed in (22)]. |
| Radon | Radon-222 and its decay products are IARC Group 1 agent for lung cancer. Pooled analyses of case-control studies show a clear dose-response relationship between residential radon exposure and lung cancer.(46) |
| Secondhand or environmental tobacco smoke (ETS) | ETS is IARC Group 1 agent for lung cancer. Consistent evidence from meta-analyses shows ETS from spouse and workplace increases lung cancer risk.(32, 33) |
| Occupational agents | IARC Group 1 agents include aluminum production, arsenic, asbestos, beryllium, bis(chloromethyl)ether and chloromethyl methyl ether, cadmium, chromium (VI), coal gasification, coal-tar pitch, coke production, diesel exhaust, hematite mining (underground), iron and steel founding, MOPP (vincristine-prednisone-nitrogen mustard-procarbazine mixture), nickel compounds, painting, plutonium, rubber production industry, silica, soot, and sulfur mustard. In addition, a recent pooled analysis of case-control studies shows that organic dust exposure is associated with increased risk.(47) |
| *Host factors* | |
| Preexisting lung diseases | A pooled analysis (ILCC) showed elevated risk of lung cancer associated with a history of emphysema, chronic bronchitis, tuberculosis, and pneumonia, independently of smoking.(48) |
| Asthma | A pooled analysis (ILCC) showed increases in lung cancer risk only in sub-groups (squamous cell and small-cell carcinomas and lung cancer occurred 2 years after asthma diagnosis).(49) |
| Inflammation | A meta-analysis of 24 prospective studies shows that C-reactive protein (CRP) is positively associated with mortality of respiratory/intrathoracic cancer (RR=2.32; 95% CI=1.96-2.74 per 1.11 higher $\log_e$ CRP).(50) A pooled analysis observed ever- versus never-use of non-steroid anti-inflammatory drugs (NSAIDs) is inversely associated with lung cancer risk among sub-groups (males and ever-smokers).(51) |

| | |
|---|---|
| Family history | Pooled analyses of case-control studies and a meta-analysis consistently have observed that positive family history of lung cancer is associated with lung cancer risk (OR=1.63; 95% CI=1.31-2.01).(52, 53) |
| Genetics | Genetic variants in several cancer-related pathways (e.g., apoptosis,(54) nucleotide excision repair,(55) and DNA repair and cell cycle (56)) and metabolic enzymes (e.g., cytochrome P450 1A1 [CYP1A1] and glutathione S-transferase M1 [GSTM1]) have been linked to lung cancer risk.(57-59) GWA studies and meta-analyses also revealed and replicated that several DNA regions (e.g., 5p15.33, 6p21-6p22, and 15q25) are associated with lung cancer risk.(60, 61) |
| *Dietary* | |
| Low fruit & vegetables intake | The expert panel in WCRF concluded that the evidence that fruit and foods containing carotenoids can decrease lung cancer risk is "probable"[4].(62) A subsequent meta-analysis of prospective cohort studies also observed an inverse association of carotenoids with lung cancer.(63) |
| Beta-carotene supplement | Two major trials found that beta-carotene supplementation increases lung cancer risk in current smokers.(64, 65) |
| Arsenic in drinking water | Evidence from 9 ecological studies, 2 case-control studies, and 6 cohort studies provides support for a causal association of arsenic in drinking water with lung cancer.(66) |

GWA: genome-wide association; IARC: International Agency for Research on Cancer; ILCC: International Lung Cancer Consortium; OR: odds ratio; RR: relative risk; SEER: Surveillance Epidemiology and End Results; WCRF: World Cancer Research Fund.

[1] Latest meta-analysis, pooled analysis, and clinical trials if available.

[2] Carcinogenic agent with sufficient evidence in humans, i.e., the agent is carcinogenic to humans.

[3] Agent with limited evidence in humans, i.e., the agent is probably carcinogenic to humans.

[4] The order of evidence is: convincing, probable, limited-suggestive, limited-no conclusion, and substantial effect on risk unlikely.

## 1.2 Overview of vitamin D: its sources, metabolism, and physiological functions

Vitamin D is a group of fat-soluble secosteroids, i.e., steroids in which one of the bonds in the steroid rings is broken. Vitamin D's function was noticed as early as 1650 in relation to searching for the cause of rickets, defective bone mineralization in children.(67) In humans, two major forms of vitamin D are ingested: vitamin $D_2$ (ergocalciferol) and vitamin $D_3$ (cholecalciferol) (**Figure 1**). Compared to vitamin $D_3$, vitamin $D_2$ contains a double bond between carbons 22 and 23, and a methyl group on carbon 24. Dietary sources of vitamin $D_2$ are plant-based foods, such as mushrooms, while dietary sources of vitamin $D_3$ are animal-based foods, such as fatty fish. In addition, human skin produces vitamin $D_3$ from its precursor 7-dehydrocholesterol when exposed to ultraviolet irradiation. Both vitamin $D_3$ and vitamin $D_2$ are used in dietary supplements and food fortification. Both forms are equally metabolized in the 25- and 1α-hydroxylation steps.(68, 69) As well, for the biological functions in the bone and gene transcriptions, two forms are equipotent.(70, 71) Nevertheless, animal data suggest that vitamin $D_2$ is less toxic at high doses compared to vitamin $D_3$.(72, 73)

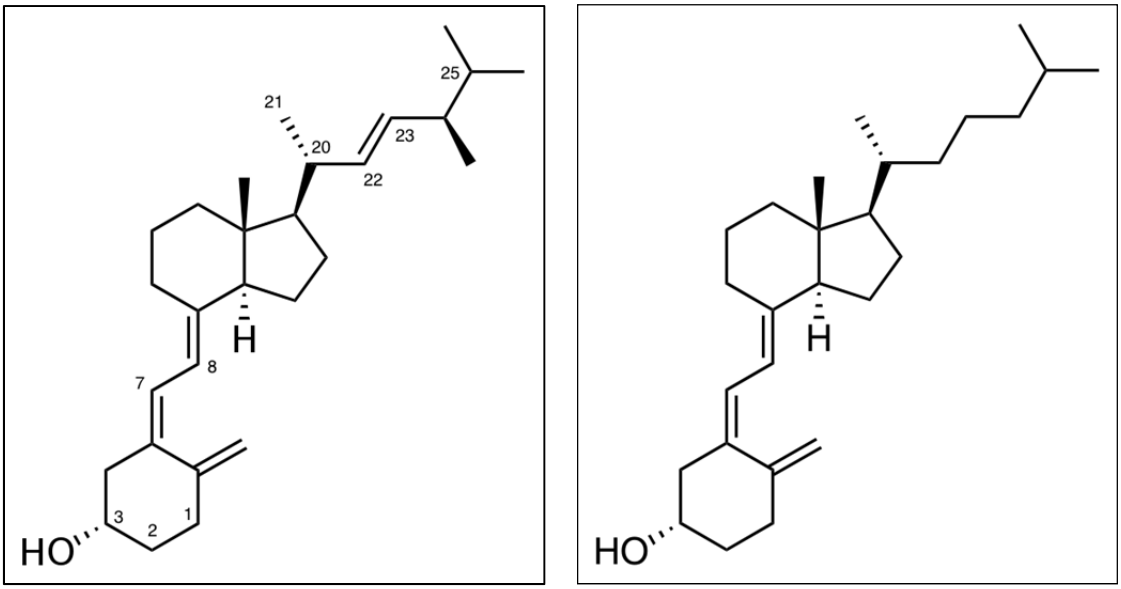


Figure 1. Vitamin $D_2$ (ergocalciferol, left panel) and vitamin $D_3$ (cholecalciferol, right panel). The carbon numbers are provided for vitamin $D_2$. Solid lines are bond in plane of paper; dashed triangles are bonds going back into paper; solid triangles are bond out of paper toward the front (modified from Wikimedia Commons file "Images: Ergocalciferol.svg; Cholecalciferol.svg. http://en.wikipedia.org/wiki/Vitamin_D).

**Major food sources of dietary vitamin D.** The major food sources of dietary vitamin D include fatty fish, mushrooms, egg yolk, and fortified foods [reviewed in (74)]. A complete list of foods containing vitamin D can be found at USDA National Nutrient Database for Standard Reference, Release 24.(75) Vitamin $D_3$-rich fatty fish foods include salmon (600–1,000 IU per 3.5 oz. in fresh-wild; 100–250 IU in fresh-farmed; 300–600 IU in canned varieties; conversion: 40 IU=1 μg), canned sardines (300 IU per 3.5 oz.), canned mackerel (250 IU per 3.5 oz.), canned tuna (330 IU per 3.6 oz.), and cod liver oil (400–1,000 IU per teaspoon). Mushrooms contain a large amount of ergosterol, which converts vitamin $D_2$ when exposed to UV light.(76) Fresh shiitake contains 100 IU of vitamin $D_2$ per 3.5 oz., and the vitamin $D_2$ content increases to 1,600 IU after the sun-

drying process. Compared to fatty fish and mushrooms, egg yolk contains less vitamin D (36 IU/17g, 1 large egg yolk).(75)

In terms of fortified foods, dairy products are fortified with vitamin $D_3$ on a voluntary basis in the U.S.(77) The fortified amount is approximately 100 IU per 8 oz. in milk and yogurt, 100 IU per 3 oz. in cheese, 50 IU per 3.5 oz. in butter, and 430 IU per 3.5 oz. in margarine. Orange juice (8 oz.) and breakfast cereals (1 serving), if fortified, typically contain 100 IU of vitamin $D_3$. These fortified foods provide 65–86% of total daily vitamin D intake from foods, since adults often do not consume the foods with natural source of vitamin D, such as fatty fish, on a regular basis.(77, 78) For infant formulas, the minimum amount of vitamin D is required to be 40–100 IU per 100 kcal or about 5 oz., according to the Food and Drug Administration (FDA).(79)

In addition to Vitamin $D_3$, animal-based foods also contain 25-hydroxyvitamin D [25(OH)D], which is absorbed faster and has higher bioavailability compared to vitamin D. The amount of 25(OH)D is typically very low in milk and fish (<0.1 μg/100 g), somewhat higher in meat and liver (0.2–0.4 μg/100 g) and up to 1 μg/100 g in egg yolk.(80)

**Vitamin D supplements.** Vitamin D is commonly prepared as an ingredient in commercial or over-the-counter dietary supplements. The manufacturing process is through the UV irradiation of ergosterol in yeast (for vitamin $D_2$) or 7-hydrocholesterol in

lanolin, i.e., wool grease (for vitamin $D_3$).(74) The form of vitamin D in dietary supplements is usually vitamin $D_3$. A nationwide survey in 1986 showed that one-third (33%) of all single and multivitamin supplements that were used by U.S. adults and children contained vitamin D. Single vitamin D products were only 1% of total number of products. The most common potency per tablet was 200 IU for adults and 400 IU for children (both 100% of Recommended Dietary Allowances [RDA] at the time of the survey) in multivitamin supplements.(81) Since RDAs have been raised (600 IU for 1-70 years; 800 IU for ≥70 years),(82) vitamin D potencies in the current market may be higher compared to the 1986 data. The most updated data in 2007–2010 showed that a total of 36.8% of U.S. adults (≥20 years) consumed multivitamin supplements (31.9%), which might contained vitamin D, and single vitamin D supplements (4.9%).(83) In the Women's Health Initiative Observational Study, the median level of potency of vitamin D supplements used by postmenopausal women ranged from 400 to less than 800 IU.(84)

**Dietary vitamin D and vitamin D status.** The standard biomarker of vitamin D status is serum 25(OH)D. Dietary source of vitamin D alone may not be able to maintain serum 25(OH)D concentrations at the sufficiency level (>=50 nmol/L) suggested by the Institute of Medicine. A study in Denmark (54–58 ºN, where there is negligible skin vitamin D photosynthesis in winter) found that among middle-aged female non-supplement users with a mean dietary vitamin D intake at 120 IU/d, their mean serum 25(OH)D concentrations was 37 nmol/L during winter and spring.(85) Beyond dietary intake, vitamin D supplementation can effectively elevate serum 25(OH)D contributions in a rate

of 1 to 2 nmol/L per 100 IU of intake.(86, 87) Vitamin D supplement users who used 200 IU or higher per day had 17% to 25% higher serum 25(OH)D concentrations compared to non-supplement users, with a larger increase among those with a lower baseline serum 25(OH)D concentrations.(85)

**Vitamin D intake in the U.S.** Data from the National Health and Nutrition Examination Survey (NHANES), 2005–2006, showed that average intake levels from foods alone ranged from 204 to 288 IU/d for males depending on life stage group; for females the range was 144 to 276 IU/d.(88) Because 37% of the U.S. population used a dietary supplement containing vitamin D, the mean values of total intake (foods plus supplements) were substantially higher: 264–428 IU/d for males and 200–404 for females. The most marked difference was seen among older women. For women aged 51–70 years, mean intake of vitamin D from foods alone was 156 IU/d, but 404 IU/d with supplements (40% used supplements containing vitamin D). For women >70 years, the corresponding figures were 180 IU/d and 400 IU/d (49% used supplements containing vitamin D).

**Reference intakes.** In 2011, the Institute of Medicine revised the Dietary Reference Intake for vitamin D. To achieve sufficient vitamin D status (serum 25(OH)D concentrations of 50 nmol/L), one would need to consume vitamin D from foods and dietary supplements at the RDA levels (600 IU for 1-70 y; 800 IU for ≥70 y).(79) The current RDA was developed based on maintaining bone health and assumed minimal sun

exposure. The Institute of Medicine concluded insufficient evidence for vitamin D associated with other health outcomes such as cancer and cardiovascular diseases after systematic reviews.(79)

Although high vitamin D intake is recommended, very high levels of vitamin D intake (>10,000 IU per day) can cause kidney damage and hypercalcemia, which can be fatal.(89) The current Tolerable Upper Levels of vitamin D intake is 4,000 IU/d.(82)

**Photosynthesis of vitamin D.** Human skin synthesizes vitamin $D_3$ from 7-dehydrocholesterol after exposure to ultraviolet B radiation (UVB, wavelength 290–320 nm). 7-dehydrocholesterol is formed in the skin from cholesterol after being catalyzed by $\Delta^7$-reductase. 7-dehydrocholesterol firstly forms 9,10-seco-sterol, or previtamin $D_3$, which then undergoes a non-enzymatic reaction to form vitamin $D_3$. Sunlight exposure provides a significant contribution to vitamin D status.(89) For example, expose to a daily dose of 20 mJ/cm$^2$ of UVB (equivalent to 15 minutes of sun exposure in Omaha, NE, [41 degree N] in July at noon) three times per week for 4 weeks on 90% of skin surface area can lead to a 25 nmol/L increase in serum 25(OH)D concentrations among people with lighter pigmentation.(90) The effect is equivalent to taking 2,000 IU of vitamin D supplements per day for 5 months.(82, 86) However, the rapid synthesis of vitamin D in skin is confined to the initial exposure. A cross-sectional study in Norway (65–71 degrees N) showed that each additional 15 minutes of active sunbathing was

associated with an increase of 1.5 nmol/L of 25(OH)D concentrations, but the concentrations plateaued after 2 hours of exposure.(91)

Although photosynthesis of vitamin D in skin is a efficient method to acquire vitamin D, many factors can affect the results of vitamin D production [reviewed in (92)]. First, various factors influence the amount of UVB radiation reaching the earth surface: time of the day, season, thickness of ozone layer, latitudes, condition of earth surface (e.g., sand or snow covered), meteorology, and air quality. Taking latitudes for example, the annual mean solar radiation (including UV and visible light) is 500 gm-cal/$cm^2$ in New Mexico and Arizona (34 degrees N).(93) In these regions, skin still effectively photosynthesizes previtamin $D_3$ in the middle of winter.(94) However, the annual mean solar radiation is substantially lower (300 gm-cal/$cm^2$) in the New England area (42 degrees N).(93) At this latitude, human skin produces no previtamin $D_3$.

Second, the levels of UVB-induced synthesis of vitamin $D_3$ are additionally dependent on many personal factors including time spent outdoors, hours of the day of outdoor activities, skin pigmentation, skin reaction to the sun, age that affects skin synthesis capacity, clothing, sunscreen use, genetics, and baseline blood cholesterol and 25(OH)D concentrations.(79, 95-97) For example, sunscreen with a sun protection factor (SPF) of 8 can decrease vitamin D synthetic capacity by 95%, whereas sunscreen of a SPF 15 can reduce the capacity by 98%.(98) Moreover, since sun exposure is the main environmental cause of cutaneous melanoma and non-melanocytic skin cancer,(99)

preventing sun exposure is recommended,(100) which in turn largely decreases the opportunity of vitamin D production in skin.(96, 101, 102)

**Vitamin D metabolism.** The metabolism of vitamin D can be described in four steps: absorption, metabolism to the active form, storage, and excretion [reviewed in (74, 82)]. The human body absorbs dietary vitamin $D_2$ and $D_3$ into chylomicrons in the small intestine with the help of bile acids and pancreatic lipase. Chylomicrons reach the liver via venous circulation and the portal system. During the transportation, vitamin D can be taken by adipose tissue and skeletal muscle. Adipose tissue has been considered as the major non-specific storage of vitamin D. Vitamin $D_3$ from skin photosynthesis is mainly carried by vitamin D binding protein (DBP).(103)

To be biologically active, vitamin D needs to be metabolized by two enzymatic hydroxylation reactions. First, in the liver, vitamin D is converted into 25(OH)D by 25-hydroxylase (likely CYP2R1). 25(OH)D carried by DBP (104, 105) enters blood circulation with a half-life of 15 days.(103) No feedback regulation exists at this point. Second, when there is a lack of calcium or phosphate, 25(OH)D is converted to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$ or calcitriol) in the kidney by 1$\alpha$-hydroxylase (CYP27B1). $1,25(OH)_2D$ is the active form of vitamin D and binds to vitamin D receptors (VDR) in cells primarily epithelium in the intestine and osteoblast in bones.(106) $1,25(OH)_2D$ has a lifetime of only 10–20 hours.(103) This metabolic step is tightly regulated, where $1,25(OH)_2D$ is upregulated via parathyroid hormone (PTH) and

low phosphate concentrations, but downregulated by fibroblast-like growthfactor-23 (FGF23), a phosphaturic hormone. 1,25$(OH)_2$D also downregulates CYP27B1, a process which forms a negative feedback loop to control its own concentrations. In addition, 1,25$(OH)_2$D stimulates its own destructive enzyme 24-hydroxyase (CYP24A1), which also degrades 25(OH)D. CYP24A1 converts 1,25$(OH)_2$D to calcitroic acid (1α-OH-23-carboxy-24,25,26,27-tetranorvitamin D) and 25(OH)D to 24,25$(OH)_2$D and then 1-deoxycalcitroic acid. Calcitroic acid and 1-deoxycalcitroic acid are excreted to bile and eliminated mainly through feces.

**Physiological functions.** The major physiological function of vitamin D is calcium and phosphate homeostasis, which is essential for bone mineralization [reviewed in (74)]. 1,25$(OH)_2$D elevates blood calcium concentrations through three mechanisms. First, 1,25$(OH)_2$D binds vitamin D receptor–retinoid X receptor complex (VDR-RXR) in epithelial cells of the intestine, particularly duodenum, and stimulates the absorption of calcium. Second, 1,25$(OH)_2$D stimulates RANK (receptor activator for nuclear factor k B) ligand and binds to VDR-RXR to facilitate the formation of osteoclasts, bone cells responsible for bone resorption. Bone resorption is the process by which osteoclasts break down bone and release calcium from bone fluid to the blood. Third, 1,25$(OH)_2$D stimulates the reabsorption of calcium in renal distal tubule. This is made by PTH singling CYP27B1 to produce more 1,25$(OH)_2$D. Serum calcium concentrations are tightly regulated between 8.5 and 10.5 mg/dL. If calcium concentrations exceed this range, calcitonin, secreted by the parafollicular cells of the thyroid, blocks bone

resorption. Also, 1,25$(OH)_2$D binds to VDR-RXR to initiate a feedback loop that suppresses parathyroid gene expression and parathyroid cell proliferation, which in turn reduces PTH. Two mechanisms in which 1,25$(OH)_2$D regulates serum phosphate concentrations have been discovered. First, when serum phosphate concentrations are low, CYP27B1 is signaled to produce more 1,25$(OH)_2$D, which leads to more phosphate absorption in the small intestine. Second, 1,25$(OH)_2$D signals osteocytes in the bone to secrete fibroblast growth factor (FGF) 23, a phosphaturic hormone that upregulates phosphate excretion in the kidney. When serum phosphate concentrations are high, the negative feedback loop is initiated where FGF23 downregulates 1,25$(OH)_2$D and inhibits renal phosphate reabsorption.(107)

Other extra-skeletal functions of vitamin D involve the regulation of immune system, endocrine system, cell cycles, and gene expression [reviewed in (108)]. VDR-RXR locates in activated T cells, cytotoxic T cells, antigen-presenting calls, macrophages, and monocytes.(109, 110) 1,25$(OH)_2$D binds to VDR-RXR in monocytes and macrophages, and stimulates the production of cathelicidin, an anti-microbial peptide.(111) 1,25$(OH)_2$D also promotes the gathering of immunosuppressive regulatory T cells at the sites of inflammation.(112) As a hormone, 1,25$(OH)_2$D inhibits the synthesis of renin,(113) an angiotensinogenase that regulates arterial blood pressure, increases insulin production,(114) and promotes myocardial contractility.(115) Moreover, 1,25$(OH)_2$D has anti-neoplastic properties because it regulates cell cycles and gene expression [reviewed in (116, 117)]. 1,25$(OH)_2$D induces cell cycle G1 cyclin-dependent kinase inhibitor (CKI) p21 and p27.(118-121) p21 and p27 “arrest” cell cycle in G1 phase

so cell proliferation is inhibited. Both p21 and p27 have been suggested as therapeutic targets for several cancers, including lung cancer.(122-124) In addition, $1,25(OH)_2D$ unfolds differentiation with cofactors including DRIP205 and SRC3.(125, 126) Also, through binding to VDR-RXR complex, $1,25(OH)_2D$ regulates genes responsible for anti-proliferation and differentiation.(127-132)

**Vitamin D as exposure in epidemiology**

Various components in vitamin D metabolism have been measured as exposure in epidemiological studies: sun exposure, vitamin D intake, serum 25(OH)D concentrations, serum $1,25(OH)_2D$ concentrations, serum DBP concentrations, and genetic variation in enzymes in the vitamin D axis including VDR. Measuring sun exposure using residential latitudes and questionnaires has limited validity. Correlation between diaries or self-report habitual sun exposure and personal UV dosimetry is approximately 0.3.(133) In addition, self-report sun exposure has limited correlation with serum 25(OH)D concentrations ($r = 0.16–0.39$).(134) Vitamin D intake assessed by food frequency questionnaires (FFQ) has fairly good accuracy ($r = 0.7$ with 8 days of dietary intake in the WHI).(135) However, correlations between vitamin D intake from FFQ and serum 25(OH)D concentrations are also relatively low ($r = 0.11–0.35$),(136, 137) unless study participants have low sun exposure ($r = 0.55$).(138) Studies using dietary intake as vitamin D exposure need to consider sun exposure or vice versa.

Currently, serum 25(OH)D is regarded as the standard biomarker of vitamin D status because it reflects both sources of vitamin D. Serum 25(OH)D concentrations have acceptable reproducibility representing long-term exposure (5-year intraclass correlation coefficient = 0.59 in the WHI).(139) Serum $1,25(OH)_2D$ concentrations may have important implications to health outcomes because of its bioactivity. However, $1,25(OH)_2D$ has less utility compared to 25(OH)D in indicating nutritional vitamin D status because $1,25(OH)_2D$ is highly regulated in circulation and has short half-life (10–20 hours). DBP is unlikely to reflect nutritional vitamin D status either. DBP can be assessed as an anti-inflammatory factor because DBP inhibits inflammation and modulate immune response independent of carrying vitamin D.(140, 141) Studies have suggested that the importance of DBP may lie in its influence on the concentrations of free, unbounded 25(OH)D, which may be more relevant to cancer etiology compared to total 25(OH)D.(142-144) Genetic variation in vitamin D metabolism is important to mechanistic investigations of vitamin D and health outcomes (145) although it cannot be independent of vitamin D exposure (diet, sunlight, and serum 25(OH)D) because the bioactive active metabolite is $1,25(OH)_2D$. However, recent research suggests that the genetic variants are important modulators of serum 25(OH)D concentrations and disease associations.(146-148) In sum, serum 25(OH)D as exposure of nutritional vitamin D status is well characterized. DBP and genetic variation in vitamin D axis are potentially important effect modifiers and should be considered whenever possible in epidemiological studies.

### 1.3 Vitamin D and lung cancer prevention

Data from preclinical studies and epidemiological investigations have shed light on the potential of vitamin D in lung cancer prevention. Respiratory cells produce and utilize vitamin D.(117) Vitamin D modulates pathways of carcinogenesis of lung cancer through two mechanisms—direct effects from $1,25(OH)_2D$ and the action of $1,25(OH)_2D$-VDR binding.

**Preclinical studies.** *In vitro* and *in vivo* studies have demonstrated that vitamin D modulates the immune function, inhibits proliferation and angiogenesis, and promotes differentiation of pulmonary cells.(120, 149-151) By binding to VDR, $1,25(OH)_2D$ enhances host defense by signaling cathelicidin, an antimicrobial peptide, and CD14, a co-receptor for detecting bacterial lipopolysaccharide. These two peptides function crucially on innate immunity in the lung.(151) In human squamous cell carcinoma and lung cancer cell lines and mouse models, $1,25(OH)_2D$ inhibits tumor growth.(120, 149, 150) Potential mechanisms may involve inducing G0/G1 cell arrest (152) and downregulating epidermal growth factor receptor (EGFR)–Ras,(153, 154) an important proliferation signaling pathway of non-small cell lung cancer if mutated.(13) These anti-proliferative functions of $1,25(OH)_2D$ may require binding with VDR.(155) Also, $1,25(OH)_2D$ prohibits Wnt-β-catenin-TCF4 signaling pathway that promotes lung tumorigenesis.(156, 157) Additionally, $1,25(OH)_2D$ inhibits vascular endothelial growth factor (VEGF) (158) that stimulates angiogenesis of lung cancer.(13) Moreover,

$1,25(OH)_2D$ promotes the secretion of E-cadherin and β-catenin,(159, 160) which are adhesion molecules that decrease lung tumor cell dedifferentiation and invasion.(161)

**Expression of vitamin D metabolic molecules in the lung.** Observational studies at human cellular and histological levels have shown evidence for the extra-renal production and utilization of vitamin D in the lung. Respiratory cells (alveoli type II cell) have megalin receptor that uptakes bonded 25(OH)D.(162, 163) Respiratory epithelial cells and alveolar macrophages have high levels of CYP27B1, which converts 25(OH)D to the active form $1,25(OH)_2D$.(151, 164, 165) $1,25(OH)_2D$ produced in this manner are not regulated in a tight manner.(92, 108) In addition to vitamin D metabolic enzymes, normal respiratory epithelial cells have high levels of VDR.(166-168) This supports vitamin D's anti-neoplastic properties, which heavily involves VDR.

The expression of vitamin D metabolic enzymes and VDR between normal and lung cancer cells is substantially different. In NSCLC tissues, CYP27B1 is suppressed (169). On the other hand, CYP24A1, the enzyme degrading $1,25(OH)_2D$, is over-expressed in both NSCLC and SCLC tissues, compared to normal bronchial epithelium.(167, 169-172) Lung adenocarcinomas that have higher CYP24A1 expression are more poorly differentiated compared to those that have lower CYP24A1 expression.(173) The mechanism for the upregulation of CYP24A1 in lung cancer may involve activation of aryl hydrocarbon receptor (AhR), which induces the expression of cytochrome P450 family, by benzo[a]pyrene.(174) Taken together, the decreased

expression in CYP27B1 and increased expression in CYP24A1 lead to a decrease in $1,25(OH)_2D$, deterring vitamin D's anti-proliferative function. Furthermore, VDR expression levels are lower in malignant human lung tissues compared to normal tissues.(166-168) It is plausible that lack of expression of VDR in lung cancer tissues prevents transcriptional activation of the vitamin D. These observations that lower $1,25(OH)_2D$ levels and VDR expression in lung cancer tissues compared to normal tissues suggest a potential role of vitamin D in lung cancer carcinogenesis.

The level of VDR expression may be different by lung cancer histology. Kaiser et al. reported that among 154 human lung cancer tissue samples, VDR expression in SCLC was significantly lower compared to that in squamous cell carcinoma and adenocarcinoma combined (26% vs. 66% samples found positive VDR expression).(168) VDR expression was also low in large-cell carcinoma (25%). There was no difference in VDR expression between squamous cell carcinoma (67%) and adenocarcinoma (64%). Similarly, Menezes et al. reported no difference in VDR expression between squamous cell carcinoma and adenocarcinoma; however, the VDR expression in adenocarcinoma was higher in the cell nucleus than cytoplasma, compared to that in squamous cell carcinoma.(166) The VDR expression in nucleus may indicate the actual vitamin D pathway activity, because the receptor must be translocated to nucleus for the pathway to function. The study did not examine samples from large-cell carcinoma or SCLC. These observations suggest that squamous cell carcinoma and adenocarcinoma tissues preserve more VDR and may be more responsive to $1,25(OH)_2D$ compared to large-cell lung

cancer or SCLC. In addition, adenocarcinoma may be more responsive to $1,25(OH)_2D$ compared to squamous cell carcinoma.

In addition to histology, the responsiveness of lung tumors to $1,25(OH)_2D$ may be different by the mutation profile of lung cancer, which is very important clinically for treatment selection. Zhang et al. observed that from a panel of NSCLC cell lines, K-ras mutant lines are more likely to display a low-VDR/high-CYP24A1 phenotype, whereas EGFR mutant lines, high-VDR/low-CYP24A1.(153) In clinical specimens (147 primary lung adenocarcinoma cases), adenocarcinomas with EGFR mutation also have much lower CYP24A1 mRNA expression compared to those with K-ras mutation. These observations suggest that EGFR mutation-positive NSCLC, particularly adenocarcinoma, respond preferentially to $1,25(OH)_2D$ or treatments (erlotinib) combined with $1,25(OH)_2D$, which was later confirmed by the study.(153)

**Epidemiological studies for lung cancer risk.** Several epidemiological studies, including observational studies and a clinical trial, have examined the association of vitamin D with lung cancer. Observational studies investigating serum 25(OH)D concentrations with lung cancer risk were conducted in Finnish (latitudes=60–70 ºN) and the U.S. (latitudes=25–45 ºN) populations. The Mini-Finland Heath Survey recruited 6,937 men and women in 40 areas of Finland; 77% of the participants were current smokers. With a follow-up period of 24 years, the study showed that serum 25(OH)D concentrations were not associated with lung cancer risk overall (124 lung cancer cases);

however, inverse associations were seen in two subgroups. Among women (27 cases), serum 25(OH)D concentrations ≥47 (tertile 3) versus <31 (tertile 1) nmol/L were associated with a 84% lower risk of lung cancer (OR=0.16, 95% CI=0.04–0.59). Also, among younger participants (≤50 y), serum 25(OH)D concentrations approximately ≥50 (tertile 3) verses <50 (tertiles 2 & 1) nmol/L were associated with a 66% lower risk of lung cancer (OR=0.34, 95% CI=0.13–0.90).(175) The other Finnish study, namely, the Alpha-Tocopherol, Beta Carotene Cancer Prevention Study (ATBC), recruiting solely male smokers (500 lung cancer cases) observed no association of serum 25(OH)D with lung cancer overall. Similarly, a linear, inverse association was observed only among subgroups including participants whose blood was drawn during darker months from November–April (OR=0.89, 95% CI=0.81–0.98 for every 10 nmol/L increase), and who had higher (approximately ≥200 IU/d) total vitamin D intake (OR=0.67, 95% CI=0.47–0.97; approximately ≥40 versus <40 nmol/L).(176)

Data from U.S. populations are inconsistent and have some limitations in exposure and outcome measurements. In the Third National Health and Nutrition Examination Survey (NHANES III) Follow-up, high serum 25(OH)D concentrations were associated with higher lung cancer mortality in men (HR=1.87, 95% CI=1.04–3.34 [175 deaths]; ≥100 vs. <50 nmol/L).(177) However, in a separate analysis of the same study population, lower lung cancer mortality was observed in non-smoking men and women (HR=0.53, 95% CI=0.31–0.92 for former/never smokers [127 deaths] and HR=0.31, 95% CI=0.13–0.77 for distant-former [quit ≥20 y]/never smokers [51 deaths]; ≥44 versus <44 nmol/L).(178) A limitation of using lung cancer mortality instead of

incidence is that the prognosis of lung cancer after diagnosis may have influenced the risk estimates although the 5-year survival rate of lung cancer is low. However, the analysis did provide important data for regions in relative lower latitudes (25–45 ºN). In another study of U.S. males, the Health Professionals Follow-Up Study showed that there was a non-significant, inverse association (418 cases; RR=0.8, 95% CI=0.6–1.2 for each of 25 nmol/L increase) between lung cancer incidence and plasma 25(OH)D concentrations predicted from vitamin D intake, demographic and lifestyle factors.(179) Because these factors were unable to explain all variation in plasma 25(OH)D concentrations, the use of predicted 25(OH)D concentrations might have led to a wide confidence interval, but the risk estimate should approximate the one from measured plasma 25(OH)D concentrations if available.(180) Finally, in the Women's Health Initiative (WHI) randomized, placebo-controlled, clinical trial, supplementation with daily calcium carbonate (1 g) and vitamin $D_3$ (400 IU) in otherwise healthy postmenopausal women resulted in fewer lung cancers in the supplement group (109 [0.09% annualized rate] versus 126 [0.10%], hazard ratio [HR]= 0.86, 95% confidence interval [CI]= 0.67-1.12), but the difference was not statistically significant (P=0.26) after 7 years of follow-up.(181) The limitation of the WHI trial included a relatively low dose of vitamin D supplementation and limited compliance (70% participants took at least 50% of the still pills through Year 6).(182)

Although genetic factors play a role in vitamin D metabolism,(183) there are currently no published studies examining the association of genetic variation in the components of vitamin D metabolism, such as CYP27B1 and VDR, with lung cancer risk. However, a GWA study identified a SNP in *CYP24A1* (rs4809957 in 20q13.2 region)

was associated with lung cancer risk in a Chinese population (OR per variant allele [T] = 1.11, 95% CI=1.05–1.19 for squamous cell carcinoma, 1.13, 95% CI=1.07–1.18 for adenocarcinoma, and 1.17, 95% CI=1.05–1.29 for SCLC compared to wild type [CC]).(184) In addition, the SNP also interacted multiplicatively with smoking dose for lung cancer risk. Smokers consuming >24 cigarettes/d with the TT genotype had a 4.77-fold (95% CI=4.06–5.61) higher risk of lung cancer compared to never smokers with the CC genotype. This observation is plausible because high expression of CYP24A1, which catabolizes 1,25$(OH)_2$D, is associated with lung cancer cell proliferation.(152) Also, benzo[a]pyrene, a smoking-generated lung carcinogen, enhances the expression of CYP24A1.(174) The observation on the genetic variant in *CYP24A1* needs to be replicated in confirmatory studies.

**Risk by histology.** Only one epidemiological study reported the vitamin D-lung cancer association by lung cancer histology. In the ATBC, serum 25(OH)D concentrations of approximately ≥40 versus <40 nmol/L were suggestively associated with a reduction in risk for squamous cell carcinoma (OR=0.65, 95% CI=0.42–1.02, 179 cases) and for adenocarcinoma (OR=0.68, 95% CI=0.34–1.39, 72 cases). However, the association was opposite for SCLC (OR=1.33, 95% CI=0.72–2.46, 100 cases).(176) These findings suggest that vitamin D status may be more associated with NSCLC, which includes squamous cell carcinoma and adenocarcinoma, compared to SCLC. Nevertheless, since these odds ratios are either non-significant or at borderline significance, the findings warrant further research with a larger sample size.

Taken together, although biological mechanisms exist, whether there is an association of vitamin D exposure, measured as serum 25(OH)D concentrations, with lung cancer risk remain uncertain. Specific analyses among non- or never smokers are important because smoking is the most significant risk factor of lung cancer and smoking affects vitamin D metabolism.(185) The NHANES III analysis among nonsmokers relied on a small number of lung cancer deaths. A larger study with a large number of lung cancer cases among non- or never smokers is warranted to verify the association. Future studies should also provide risk estimates by histology if the data are available because lung tumors response to vitamin D differently depending on their histology and histology-associated mutations.

### 1.4 Interaction between vitamin D and vitamin A for lung cancer prevention

**Vitamin A and primary prevention of lung cancer**. Observational studies have extensively investigated the relationship between vitamin A and lung cancer although a causal relationship has not been completely established. Vitamin A (retinol) is a crucial micronutrient for the development and cell differentiation in the lung [reviewed in (186)]. The association of vitamin A with lung cancer risk was first investigated in 1975.(187) During 1980s and 1990s, a large amount of observational data supported that blood retinol and carotenoids and intake of fruits and vegetables that are rich in carotenoids were inversely associated with lung cancer risk [reviewed in (188)]. Carotenoids were studied because many of the compounds, e.g., β-carotene, can be converted to retinol through enzymatic cleavage reaction and quench singlet oxygen in the body.(189, 190) Subsequently, three large randomized trials were conducted to answer the question about whether β-carotene and/or retinol supplementation could reduce lung cancer. Among the three trials, two studies recruited heavy smokers. Alpha-Tocopherol Beta-Carotene Cancer Prevention Study (ATBC) gave 20 mg β-carotene or placebo daily to heavy smoking men in Finland. In addition, in the U.S., Carotene and Retinol Efficacy Trial (CARET) gave 30 mg β-carotene plus 25,000 IU retinyl palmitate or placebo daily to heavy smokers (current/former, male and female) and men exposed occupationally to asbestos. Unexpectedly, both studies found that participants receiving the active interventions had a significantly increase in lung cancer risk.(64, 65) This harmful effect was not observed among ATBC participants who smoked less (<20 cigarettes/day) and CARET participants who had quit smoking prior to the randomizations.(191) The third

trial was conducted among participants with majority of nonsmokers (89%). Physicians' Health Study (PHS) gave 50 mg β-carotene or placebo on alternate days to U.S. physicians for 12 years. The trial result showed lack of effect on lung cancer risk.(192) *Ad hoc* experimental data revealed that in free radical-rich environment created by smoking, high-dose β-carotene produces transient oxidative metabolites that destructs retinoic acid, diminishes retinoid signaling, and enhances cell proliferation.(193-195) High doses of β-carotene also enhance carcinogen-metabolizing enzymes, converting benzo[a]pyrene to ultimate carcinogens.(196, 197) The field of chemoprevention of vitamin A on lung cancer has progressed little since the alarming findings from those two trials.(198)

**Vitamin A intake from dietary supplements.** The average daily dietary vitamin A intake levels are 649 mcg RAE and 580 mcg RAE in U.S. adult men and women, respectively, according to 2007-2008 National Health and Nutrition Examination Survey.(199) The intake levels are considered to be adequate. However, a substantial proportion of U.S. adults consume vitamin A in a level that exceeds the Dietary Reference Intake. Dietary supplemental intake is likely the main contributor to this excess intake. One-third of adults consume multivitamin/multimineral or single supplements containing vitamin A, with the potency often at 200% the Recommended Dietary Allowance (RDA; 900 μg RAE for man and 700 μg RAE for women).(83, 200-202) In addition, it is often neglected that fish/cod liver oil, another popular dietary supplement which is used by 10% of U.S. adults,(83) also contains high doses of retinol

(1,200–9,000 μg/tablespoon).(203) The Hawaii-Los Angeles Multiethnic Cohort (MEC) study showed that 16% men and women who use multivitamin supplement have a total vitamin A intake level exceeding the Tolerable Upper Intake Level (UL; 3,000 μg RAE).(204) While the vitamin A deficiency is uncommon in U.S. adults,(205) potential adverse effect in health attributable to high-dose supplementary intake of vitamin A has been warned but not yet extensively evaluated.(203)

**Vitamin D and vitamin A interaction.** Vitamin D-related gene transcriptions require the assistance of vitamin A, but excess vitamin A may disrupt the function of vitamin D. The mechanisms involve VDR and RXR. VDR and RXR must form a heterodimer complex that binds to vitamin D response element (VDRE) in order to regulate gene transcription (Figure 2, A).(206-208) 1,25$(OH)_2$D and 9-*cis*-retinoic acid, a biologically active metabolite of retinol, are ligands of VDR and RXR, respectively. However, excessively high levels of 9-*cis*-retinoic acid can lead to the formation of RXR-RXR homodimers, resulting in the interruption of VDR-RXR heterodimers. Consequently, 1,25$(OH)_2$D cannot bind VDR and thus will be degraded by CYP24A1 (Figure 2, B).(209-211) Therefore, no VDR-related transcription can be initiated. Both *in vitro* (209, 212, 213) and animal (214-216) studies have repeatedly shown this molecular mechanism of how 9-*cis*-retinoic acid, along with RXR, interacts with vitamin D and VDR. Excess all-trans-RA, another major metabolite of retinol, also antagonizes vitamin D function but to a lesser extent.(213, 215)

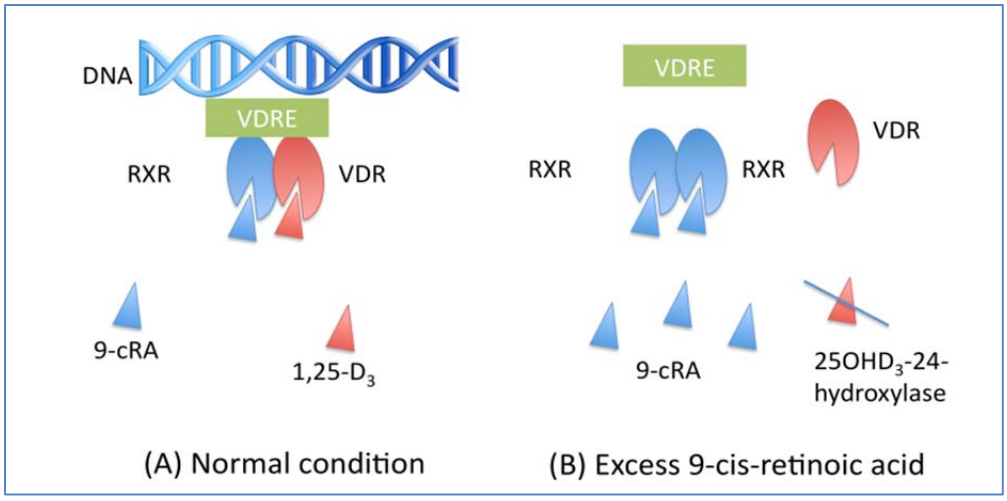


Figure 2. Model for transcriptional activity of RXR-VDR heterodimer (A); the transcription stops when excess 9-*cis*-retinoic acid (9-cRA) compete RXR with VDR and signal CYP24A1 or 25-hydroxyvitamin $D_3$-24-hydroxylase (B). (VDRE: Vitamin D response element; VDR: vitamin D receptor; RXR: retinoid X receptor; 1,25-$D_3$: 1,25-dihydroxyvitamin $D_3$)

Both direct and indirect evidence has suggested that vitamin A intake is associated with circulating and cellular levels of 9-*cis*-retinoic acid. Liver consumption leads to elevated concentrations of serum 9-*cis*-retinoic acid in healthy human.(217) In animal models, restricting vitamin A intake directly affects 9-*cis*-retinoic acid-related cellular functions (insulin secretion in β-cells).(218, 219) Therefore, based on these human and animal data, one can reasonably assume that dietary vitamin A intake alters the cellular levels of 9-*cis*-retinoic acid, a condition that influence vitamin D-related gene transcription.

**Epidemiological studies.** Observational studies investigating the association between vitamin A with outcomes related to vitamin D provide indirect evidence that excess

vitamin A may influence vitamin D's functions (e.g., maintaining bone health). Large, prospective studies with long-term follow-up consistently observed an association of higher vitamin A intake with higher incidence of fracture. A nested case-control study recruiting Swedish women in 40–76 years of age observed that women who consumed retinol from diet >1,500 μg/d had an increase risk in hip fracture (OR=2.05, 95% CI=1.05–3.98) compared to those consumed ≤500 μg/d.(220) A limitation of the study was no assessment of retinol intake from dietary supplements. Nevertheless, a subsequent study recruiting Swedish men 49–51 years of age showed a very similar result when intake from dietary supplements containing vitamin A was included in the exposure calculation (RR=1.99, 95% CI=0.98–4.01 for any fracture, total retinol intake >1,500 versus ≤500 μg/d).(221) Results from Nurses' Health Study recruiting U.S. women aged 34–77 years also observed that total vitamin A intake (food plus supplements) ≥3,000 versus <1,250 μg Retinol Equivalent (RE)/d was associated with an increased risk of hip fracture (RR=1.48, 95% CI=1.05–2.07). The association was strengthened when the analysis was restricted to total retinol intake (RR=1.89, 95% CI=1.33–2.68; ≥2,000 versus <500 μg/d).(222) All of the above studies did not observe an association of β-carotene intake with hip fracture risk. Moreover, the Iowa Women's Health Study recruiting postmenopausal women aged 55–69 years observed suggestive evidence that the use of supplements containing vitamin A (mean= approximately 7,000 IU or 2,100 μg), was associated with 1.18-fold increased risk of hip fracture (95% CI=0.99–1.41).(223) It is noteworthy that the study did not calculate the intake of β-carotene and other carotenoids as retinol-equivalent values, and thus the vitamin A intake level was likely over-estimated. Studies investigating the association of serum retinol with fracture

risk have also showed consistent data.(221, 224) Collectively, as far as fracture risk is concerned in adult females, retinol intake should be restricted to lower than about twice the RDA or ≤1,500 μg/d.(225, 226) The evidence suggests potential influencing of high vitamin A on vitamin D's protective functions.

Few studies have explored the potential effect modification of high vitamin A on the association of vitamin D with cancers in the digestive tract, which has been linked to vitamin D.(92, 227) In the Nurses' Health Study, there was a tendency that lower risk of distal colorectal adenoma (2,747 cases) was observed among women with high vitamin D (≥400 IU/d) plus low retinol (<2,646 IU or approximately 800 μg/d) intake compared to those with low vitamin D (<240 IU/d) plus high retinol (4,784 IU or approximately 1,500 μg/d) intake.(228) The difference in risk of distal colorectal adenoma between the intake groups was statistically significant (*P* for interaction=0.02). A consistent pattern of associations with colorectal cancer was also observed in the European Prospective Investigation into Cancer and Nutrition (EPIC). The study showed that lower colorectal cancer risk (1,220 cases) was observed among men and women with high serum 25(OH)D concentrations (≥75 nmol/L) plus low dietary retinol intake (approximately <500 μg/d) compared to those with low serum 25(OH)D concentrations (<50 nmol/L) plus high retinol intake (approximately ≥1,000 μg/d; *P* for interaction=0.03).(229) In addition, an analysis combining Nurses' Health Study and Health Professionals Follow-up study showed that an inverse association of predicted vitamin D status with pancreatic cancer risk (575 cases) was only observed among participants with total retinol intake <2,669 IU/d (890 μg/d) and those without using multivitamin supplements and but not

among their counterparts although the interactions were not statistically significant.(230) The above observations demonstrate that retinol intake above the RDA may interrupt the protective association of vitamin D with these cancers.

Two studies have investigated the potential effect modification of vitamin A on the serum 25(OH)D-lung cancer association. In these studies, three different exposures of vitamin A were investigated: serum retinol ester concentrations (a biomarker for excess circulating vitamin A), commercial vitamin A/β-carotene supplement use, and high-dose β-carotene supplement. In the NHANES III Mortality Follow-up study, among former/never smokers, ≥44 versus <44 nmol/L of serum 25(OH)D was associated with a decreased risk of lung cancer mortality (HR=0.53, 95% CI=0.31–0.92). The associations were attenuated among participants with excess circulating vitamin A (serum retinyl esters ≥7.0 μg/dL or the ratio of retinyl esters to retinol ≥0.08) and among personal vitamin A/β-carotene supplement users (all *P* for interaction >0.05).(178) The study did not observe an association of serum 25(OH)D concentrations with lung cancer mortality or effect modification of vitamin A among current smokers. The analysis investigating potential effect modification of high-dose β-carotene supplement was conducted in the Alpha-Tocopherol, Beta Carotene Cancer Prevention Study (ATBC), which recruited only male current smokers. In a stratification analysis, lung cancer risk associated with serum 25(OH)D concentrations (quintiles 4–5 versus 1–3) was lower among participants receiving 20 mg β-carotene supplement daily (OR=0.76, 95% CI=0.46–1.27) and among those receiving 20 mg β-carotene plus 50 mg α-tocopherol supplements daily (OR=0.69, 95% CI=0.40–1.17 for), compared to that among those receiving placebo (OR=1.34, 95%

CI=0.78–2.32; *P* for intervention >0.05).(176) The dose of β-carotene supplementation in the ATBC was substantially higher compared to commercial supplements (median dose=0.3 mg; data from a 1998 survey).(231) The β-carotene supplementation in the ATBC might have led to dual effects: free radical scavenging, which was later recognized harmful among current smokers,(194) and retinol-equivalent activity, which a certain proportion of β-carotene is converted to retinol. High-dose β-carotene supplementation can increase serum retinol and retinyl ester concentrations.(232, 233) Although the actual concentrations of retinol and 9-*cis*-retinoic acid from the β-carotene supplementation influenced the VDR function is unknown in the ATBC, from the odds ratios that favored the protective association of serum 25(OH)D concentrations (ORs=0.69 & 0.76), we can reasonably hypothesize that the levels of retinol from the β-carotene supplementation might assist, rather than antagonize, VDR functions. From the observations of these two studies, excess vitamin A may counteract the potentially protective association of vitamin D with lung cancer among nonsmokers, a finding similar to those with colorectal and pancreatic cancers.(228-230) However, it is unclear that excess vitamin A has the same function among current smokers from the data on the null finding of excess circulating vitamin A interacting with serum 25(OH)D in the NHANES III analysis and the potentially protective association from retinol-equivalent activity of β-carotene supplementation plus high serum 25(OH)D concentrations in the ATBC. Neither study investigated the effect modification of vitamin A in relation to specific lung cancer histology.

In summary, there is sufficient biological evidence for high vitamin A antagonizing the function of vitamin D. High retinol intake and serum retinol concentrations are associated with fracture, which is an important health outcome related to vitamin D. High/excess vitamin A may attenuate the inverse associations of vitamin D with colorectal and pancreatic cancer risks and with lung cancer mortality in nonsmokers. The role of vitamin A on the vitamin D-lung cancer association in smokers remains unclear. The vitamin D and vitamin A interaction with lung cancer risk warrants further study, and future analyses should be conducted for nonsmokers and smokers separately. There is essentially no study to examine the interaction of vitamin D with vitamin A in relation to specific lung cancer histology.

## 1.5 Specific aims

The overall goal of this dissertation is to test whether vitamin D intake is associated with lung cancer risk and whether vitamin A intake modifies any association of vitamin D with lung cancer. Since vitamin D intake is used as the main exposure, its relationship with serum 25(OH)D concentrations, the standard biomarker of vitamin D status, is also investigated. Cigarette smoking, influencing both lung cancer risk and vitamin D metabolism, is considered as a confounder *a priori*. The Primary Specific Aims are:

1. To test whether high vs. low total vitamin D intake is associated with lower lung cancer incidence in postmenopausal women.
2. To test whether high vs. low total vitamin D intake is associated with lower lung cancer incidence in men and women at high risk of lung cancer, i.e., heavy smokers and/or asbestos exposure workers.
3. To evaluate whether vitamin D intake is associated with serum 25(OH)D concentrations in postmenopausal women.

The Secondary Specific Aims are:

1. To test whether a low lung cancer risk associated with high total vitamin D intake is stronger among postmenopausal women with normal/low total vitamin A intake, compared to that among those with high total vitamin A intake.
2. To test whether a low lung cancer risk associated with high total vitamin D intake is stronger among men and women at high risk of lung cancer who had normal/low total vitamin A intake from food and commercial supplements and

who did not receive high-dose retinol plus β-carotene supplementation, compared to that among their counterparts.

The study populations are participants in the Women's Health Initiative (WHI) Clinical Trials and Observational Study for Aims 1, the Carotene and Retinol Efficacy Trial (CARET) for Aim 2, and the WHI Observational Study for Aim 3. The WHI provides an excellent environment in which to examine lung cancer risk factors other than smoking since the smoking prevalence was low (7%).(234) The CARET gives a unique opportunity to test the hypothesized vitamin D/vitamin A interaction because this randomized trial administered high-dose retinol plus β-carotene supplements (22,500 μg RAE/d).(65) If data support this vitamin D/vitamin A interaction hypothesis, our understanding of lung cancer etiology will largely advance. Findings from this study may provide important information on the association of vitamin D with lung cancer risk in postmenopausal women, including smokers and nonsmokers, and men and women at high risk of lung cancer.

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# Chapter 2

## Vitamin D intake and lung cancer risk in the Women's Health Initiative (WHI)

## 2.1 Abstract

**Background**: Prior research suggests that vitamin D protects against lung cancer with evidence only among certain subgroups.

**Objective**: We investigated whether vitamin D intake was associated with lung cancer and explored whether vitamin A intake modified the association.

**Design**: Prospective cohort data from 128,779 postmenopausal women including 1,771 incident lung cancers (527 current, 896 former, and 278 never smokers) in the Women's Health Initiative Clinical Trials and Observational Study, 1993-2010, were analyzed. Twelve percent of women received active intervention (1 g calcium+400 IU vitamin $D_3$ daily) in the Calcium/Vitamin D Trial. Baseline total intake included both dietary intake (from food frequency questionnaire) and supplement intake (from bottle labels). Hazard ratios (HR) were estimated by Cox proportional hazard models.

**Results**: No association was observed overall. Among never smokers, vitamin D intake was inversely associated with total lung cancer (HR=0.37, 95% confidence interval [CI]=0.18-0.77, ≥800 vs. <100 IU/d; *P*-trend=0.01). The Calcium/Vitamin D Trial active intervention was associated with a lower lung cancer risk only among women with vitamin A intake <1,000 µg/d Retinol Activity Equivalent (RAE); (HR=0.69, 95% CI=0.50-0.96; *P*-interaction=0.09). Among current smokers, the intervention was associated with a higher lung cancer risk among women with excess vitamin A intake (≥3,000 µg/d RAE); (HR=2.26, 95% CI=1.02-5.01), but not among women with vitamin A intake <3,000 µg/d RAE (*P*-interaction=0.01).

**Conclusions**: Vitamin D intake was associated with a lower lung cancer risk in never-smoking, postmenopausal women. Lower vitamin A intake may be important for a beneficial association of 1 g calcium+400 IU vitamin $D_3$ supplementation with lung cancer.

## 2.2 **Introduction**

Lung cancer is the leading cause of cancer death in women in the United States (1). In addition to smoking cessation, novel prevention strategies are needed as half of lung cancer cases in women are not attributed to smoking (2).

While associations of vitamin D intake or status with several cancer sites have been proposed (3), current evidence is controversial (4), and few studies have examined the lung cancer and vitamin D association. In two Finnish studies, serum concentrations of 25-hydroxyvitamin D, the standard biomarker for assessing vitamin D status, were not associated with lung cancer risk overall although inverse associations were seen in subsets including those whose blood was drawn during darker months (5) and in women (6). In U.S. populations, high serum 25-hydroxyvitamin D concentrations were associated with higher lung cancer mortality in men (7), but with lower lung cancer mortality in non-smoking men and women (8). Another U.S. study showed no association between lung cancer incidence and plasma 25-hydroxyvitamin D levels predicted from vitamin D intake, demographic and lifestyle factors in men (9). Finally, in the Women's Health Initiative (WHI) randomized, placebo-controlled, clinical trial, supplementation with daily calcium carbonate (1 g) and vitamin $D_3$ (400 IU) in otherwise healthy postmenopausal women resulted in fewer lung cancers in the supplement group (109 [0.09% annualized rate] versus 126 [0.10%], hazard ratio [HR]= 0.86, 95% confidence interval [CI]= 0.67-1.12), but the difference was not statistically significant (P=0.26) (10).

In addition to the 36,282 postmenopausal women participating in the WHI

Calcium/Vitamin D supplementation trial, 125,526 other postmenopausal women participated in the WHI observational study or the two other WHI clinical trials. In this paper, we use the entire WHI population to determine whether total vitamin D intake (diet plus supplements) was associated with lung cancer risk. In addition, we investigate a recent hypothesis that a beneficial association of 25-hydroxyvitamin D concentration with lung cancer mortality may be attenuated by excess circulating vitamin A (8) by examining effect modification of vitamin A intake on this association.

## 2.3 Methods

*WHI overview*

Eligible, interested, and consenting women aged 50-79 years at baseline joined the WHI between 1993 and 1998, either in one of the Clinical Trials (n=68,132) or the Observational Study (n=93,676) (**Figure 1**). The three Clinical Trial components included trials of Hormone Therapy (HT) for women with or without a uterus (without a uterus- estrogen only vs. placebo, n=10,739; with a uterus- estrogen plus progesterone vs. placebo, n=16,608), and Dietary Modification (DM) behavioral intervention vs. comparison (n=48,835). The third trial was offered only to women participating in one of the HT trials or the DM trial, and was of Calcium/Vitamin D supplementation vs. placebo (n=36,282) (11). A partial factorial design was used for the Clinical Trial Program whereby participants could be randomized to one, two, or all three of the components, thus providing a cost-efficient model.

*Study participants*

The current study included all WHI participants in the Clinical Trials and Observational Study. We excluded participants who had (1) a history of conditions that affect vitamin D status by impairing fat malabsorption or vitamin D metabolism, including ulcerative colitis, Crohn's disease, part of intestines removed, high blood calcium, liver diseases, dialysis for kidney failure, and a malignancy other than nonmelanoma skin carcinoma (n=22,955); (2) implausible body mass index (BMI, <15.0 or >50.0 $kg/m^2$ [n=854]) and/or estimated energy intake from a baseline food frequency questionnaire (<600 or >4,000 kcal/d [n=4,598]); (3) missing data on baseline intake from dietary (n=299), supplement use (n=2), follow-up time (n=697), or covariates for multivariate analyses (n=4,698). As a result, 128,799 participants entered statistical analyses.

*Outcome ascertainment*

Participants reported lung cancer diagnoses at each follow-up semiannually in the Clinical Trials and annually in the Observational Study. Trained study physicians, blinded to WHI study components and randomization allocations, at local clinics confirmed and adjudicated cases by reviewing medical records (12). Tumor histology was coded using the Surveillance, Epidemiology, and End Results guidelines (13). As of September 30, 2010, the present study included 1,701 incident cases of lung cancer; 99.5% (1,693) cases had tumor histology data. Median follow-up was 12.7 years and

6.7% of women were lost to follow-up.

*Assessment of dietary and supplemental intake*

Dietary intake at baseline was assessed by a self-administered food frequency questionnaire (FFQ) developed specifically for the WHI (14). Among the subgroup of women who also completed an additional dietary intake assessment, correlation coefficients between the FFQ and 8 days of dietary intake (four 24-hr recalls and a 4-day food record) were 0.70 for vitamin D, 0.30 for retinol, and 0.52 for β-carotene. Nutrient values were calculated based on the Nutrition Data Systems for Research version 2006, University of Minnesota Nutrition Coordinating Center food and nutrient database augmented with manufacturers' data. Information on usual use of vitamin and mineral supplements was collected by a simplified inventory system (15). Participants were asked to bring their supplement bottles to the baseline clinic visit, and trained staff entered doses of vitamins and minerals based on the bottle labels. Only supplements used once per week or more were transcribed. The frequency (pills per week) and duration (months taken last year and total years taken) of use were also queried. The average duration of vitamin D supplement use was 8.7 years (standard deviation=9.7) among the users.

For both Clinical Trials and Observational Study participants, the average daily intake of total vitamin D and vitamin A were calculated by summing food and supplement sources together. Vitamin A was expressed as μg Retinol Activity Equivalent

(RAE), since it consists of a wide range of compounds including retinol and carotenoids. The calculations of RAE for dietary and supplemental intake were:

Dietary intake = μg retinol + (μg β-carotene equivalent/12), where β-carotene equivalent = μg β-carotene + ½ (μg α-carotene + μg β-cryptoxanthin);

Supplemental intake = μg retinol + (μg β-carotene equivalent/2) (16).

For participants in the active intervention arm of the Calcium/Vitamin D Trial, we did not combine their vitamin D intake from the intervention supplementation (400 IU/d) with the estimated average daily intake from food and supplements because the intervention began 12-24 months after the baseline and continued for 8 years with close monitoring. We therefore treated the Calcium/Vitamin D Trial as a separate indicator variable in regression models. Calcium/Vitamin D Trial participants were allowed to continue their own personal use of calcium and vitamin supplements as long as the dose of vitamin D did not exceed 600 IU (1,000 IU from 1999 onward). Adherence to intervention was assessed by weighing returned pill bottles; at least 50% participants adhered to 80% or more of the study medication throughout the trial (17).

*Covariate assessment*

Covariates including age, race/ethnicity, education level, hormone use, smoking habits, and physical activity at baseline were collected by standardized, self-administered questionnaires. Participants were asked if they had smoked at least 100 cigarettes in their

lifetime and if they smoked currently to identify current and former smokers; an individual was a former smoker if she did not smoke currently but had smoked in the past. Number of cigarettes smoked per day and years as a regular smoker were also queried. Never smokers were defined as those who had not smoked more than 100 cigarettes in their lifetime (18). Weight and height at baseline were measured by trained staff. Baseline data on environmental tobacco smoke (ETS) exposure at home and at work since age 18, number of months and average duration working in the yard, were collected only in the Observational Study. Information on sun exposure history including time spent outdoors and current usual sunscreen use was collected from the follow-up Year 4 questionnaire in the Observational Study (19). Time spent outdoors in summer and other seasons was queried for the current year, i.e., the time of WHI assessment, as well as for the ages of 30-40 years.

*Statistical analysis*

Lung cancer risks were estimated for categorical (<100, 100 - <200, 200 - <400, 400 - <600, 600 - <800, and ≥800 IU/d) and linear (per 100 IU/d increment) total vitamin D intake in separate models. We chose these cutoffs because they are relevant to population intake levels and maintaining desirable vitamin D status for bone health: 200 IU/d, approximately the median level of U.S. women in all ages; 400 IU/d, the Estimated Average Requirement for all ages; 600 and 800 IU/d, the Recommended Dietary Allowance for ≤70 years and 71 years or older, respectively (20). The categories were collapsed (<100, 100 - <400, and ≥400 IU/d and <400 and ≥400 IU/d) for subgroup and

effect modification analyses, respectively, to maintain sufficient numbers of lung cancer in each stratum. HRs and 95% CIs for lung cancer were estimated by Cox's Proportional Hazards models. Participants contributed follow-up time from the enrollment to the date of lung cancer diagnosis, date of death from causes other than lung cancer, the last documented follow-up contact, or September 30, 2010, whichever came first. The proportionality assumption was examined by testing whether scaled Schoenfeld residuals for total vitamin D intake were associated with survival time (21); the assumption was fulfilled ($P$=0.81). Multivariate models for assessing the association of total vitamin D intake included baseline covariates that were chosen *a priori*: age (continuous), region (Northeast, South, Midwest, West), race/ethnicity (non-Hispanic White, Black, Hispanic, others), education level (high school or less, school after high school, college degree or higher, unknown), treatment assignments of the Hormone Therapy Trials (22), Calcium/Vitamin D Trial active intervention, BMI (<25.0, 25.0 - <30.0, ≥30.0 kg/m²), frequency of walking outside ≥10 minutes (rarely/never, 1–3 times/month, 1 time/week, 2–3 times/week, 4–6 times/week, ≥7 times/week), smoking status (current, former, never), number of cigarettes smoked per day (<1, 1–4, 5–14, 15–24, 25–34, 35–44, ≥45), total years of smoking (<5, 5–9, 10–19, 20–29, 30–39, 40–49, ≥50), total vitamin A intake (<700, 700 - <2,000, ≥2000 μg/d RAE), total calcium intake (<800, 800 - <1,500, 1500 mg/d), and energy intake (continuous). Additional baseline variables, including WHI study components, treatment assignments of the Dietary Modification Trial, use of oral contraceptives, use of hormone replacement therapy, history of non-melanoma skin cancer, and alcohol use, made no meaningful contribution to models or changes to risk estimates in all women or never smokers. Thus, only the *a priori* set of covariates was

included in final models. Linear trends of risk estimates were examined by Wald tests (1 df) of an ordinal variable of total vitamin D intake categories. Cox models were performed for all women and by *a priori* smoking status subgroups (current, former, and never smokers) whenever possible. Calcium/Vitamin D Trial active intervention was modeled as a time-dependent variable, allowing the hazard of lung cancer to vary before, during, and after (2005 onward) the trial (21). Risks for a histological subtype of lung cancer were estimated by competing risk models, censoring the other subtypes in addition to deaths and the end of follow-up (21). We evaluated whether pre-clinical lung cancer affected vitamin D intake by excluding women who were diagnosed with lung cancer within 2 years of study entry. To evaluate potential healthy user effect of multivitamin supplement use (23), lung cancer risks were estimated among participants who did not use multivitamin supplements.

To evaluate effect modification, we stratified the associations of total vitamin D intake and Calcium/Vitamin D Trial active intervention by total vitamin A intake category (≥3,000, 2,999-1,000, <1,000 μg/d RAE). We considered total vitamin A intake ≥3,000 μg/d RAE as excess intake because it is the Tolerable Upper Intake Level for preventing liver toxicity in adults (16). Also, vitamin A intake ≥3,000 μg/d RAE can lead to excess circulating vitamin A (i.e., serum retinyl esters concentrations ≥7.0 μg/dL) in elderly (24-26). The lower cutoff of 1,000 μg/d RAE was chosen because the level is close to the Dietary Reference Intake (700 μg/d RAE for females) (16). Interaction was examined by likelihood ratio tests (1 df) on separate models before and after entering a

cross-product term of total vitamin D intake or Calcium/Vitamin D Trial active intervention and total vitamin A intake (all ordinal variables).

Sensitivity analyses were conducted by further considering ETS and sun exposure as confounders in Observational Study participants. First, we additionally included ever ETS exposure at home (yes or no) and at work (yes or no) (27), months of yard work a year (<1, 1-6, ≥7 months), and time per week of yard work (<0.5, 0.5-2, >2 hrs) in main effect models. Second, the main effect was stratified by time spent outdoors (<0.5, 0.5 to 2, or >2 hours a day), additionally adjusting usual sunscreen use (no use, sun protective factor 2-24, sun protective factor ≥25). Participants in this analysis contributed person-years from the date when the Year 4 questionnaire was returned. All statistical tests were two-sided; statistical significance was defined as $P$ <0.05. Statistical analyses were conducted using STATA (12.0, College Station, Texas).

**2.4 Results**

**Table 1** shows selected baseline characteristics of participants by their total vitamin D intake level. Higher vitamin D intake was more likely to be observed among those of older age, with a lower BMI, living in Northeast or Midwest regions, non-Hispanic whites, with high education attainment, not participating in the Hormone Therapy Trials or Calcium/Vitamin D Trial, walking outside more frequently, and never smoking (all p<0.001).

**Table 2** shows multivariate-adjusted associations of total vitamin D intake with lung cancer risk. No associations were observed among all women, current smokers, or former smokers. Among never smokers, however, total vitamin D intake was inversely associated with lung cancer risk (HR=0.37, 95% CI=0.18-0.77, ≥800 vs. <100 IU/d; *P*-trend=0.01). The observed associations did not materially change after further adjusting ETS exposure and time doing yard work (**Supplemental Table 1**), among multivitamin non-users (**Supplemental Table 2**), or excluding lung cancer cases diagnosed within the first 2 years of follow-up (data not shown). In the analysis of histological subtype (**Table 3**), total vitamin D intake ≥400 versus <100 IU/d was associated with lower risks for non-small cell lung cancer (HR=0.37, 95% =0.22-0.64, *P*-trend<0.001) and for adenocarcinoma (HR=0.34, 95% =0.19-0.60, *P*-trend<0.001) among never smokers.

No effect modification by total vitamin A intake was observed for the association of vitamin D intake with lung cancer (**Table 4**). Among women with total vitamin A intake <1,000 μg/d RAE, those who received 1 g calcium and 400 IU Vitamin $D_3$ daily during the Calcium/Vitamin D Trial had a lower risk of lung cancer (HR=0.69, 95% CI=0.50-0.96), compared to those who received the placebo and the rest of Clinical Trials and Observational Study participants. The risk did not significantly differ from those in women with total vitamin A intake ≥1,000 μg/d RAE (*P*-interaction=0.09). However, effect modification by total vitamin A intake was observed among current smokers. Among current smokers with total vitamin A intake ≥3,000 μg/d RAE, the Tolerable Upper Intake Level specified by the Institutes of Medicine, the Calcium/Vitamin D Trial active intervention was associated with a higher lung cancer risk (HR=2.26, 95%

CI=1.02-5.01), while there was no association among those with total vitamin A intake below 3,000 μg/d RAE (*P*-interaction=0.01).

In **Table 5**, the vitamin D intake-lung cancer association was strengthened among Observational Study participants with less time spent outdoors (<0.5 hr/d) in summer this year, compared to those with more time spent outdoors (*P*-interaction=0.02). Total vitamin D intake ≥100 vs. <100 IU/d was associated with lower lung cancer risks among those who spent <0.5 hr/d outdoors in summer between the ages of 30-40 years.

## 2.5 Discussion

In the Women's Health Initiative, we found that among never-smoking, postmenopausal women, total vitamin D intake at 400 IU/d or greater was associated with a significantly lower risk of lung cancer. To our knowledge, this is the first study reporting dietary vitamin D intake and lung cancer risk in postmenopausal women.

The anticarcinogenic functions of vitamin D in regulating cell proliferation and angiogenesis are relevant to lung tumorigenesis. Lung cancer signaling pathways including mutations in epidermal growth factor receptor and Wnt-β-catenin dysregulation (28, 29) can be prohibited by vitamin D (30). Additionally, vitamin D inhibits vascular endothelial growth factor that stimulates angiogenesis of lung cancer (29).

Our observation that vitamin D intake was inversely associated with adenocarcinoma among never smokers but not with other histological types of lung cancer or among smokers provides important biological implications. A major anticarcinogenic function of vitamin D involves G1 cell-cycle arrest through signaling cyclin-dependent kinase inhibitor p21 and p27 (31). Both p21 and p27 proteins cooperate closely with the ras oncogene family (32, 33), and K-ras, a member of ras, often mutates in adenocarcinoma (29). Adenocarcinoma occurs in never smokers more often than in smokers, compared to other types of histology including squamous cell carcinoma and small-cell lung cancer (2). Thus, one could hypothesize that vitamin D may be more effective in preventing or reversing the mutations that are not tobacco-related in adenocarcinoma compared to those tobacco-induced mutations.

In the WHI, sun exposure, another contributor to vitamin D status, was measured as several variables. Regions of study center and time of walking outdoor for exercise and yard work, which all influence vitamin D status (34, 35), can represent the vitamin D synthesis effect of sun exposure. We also stratified the main effects by time spent outdoors considering seasonality and life stage. The analysis showed that vitamin D-associated lung cancer risk was more protective among women with shorter time spent outdoors in summer, compared to those with longer time. This is reasonable because the contribution of diet to vitamin D status increases when sun exposure decreases (36). Further, the inverse association of vitamin D intake was significant among those who spent less time outdoors during the fourth decade of life, suggesting that vitamin D intake in early adulthood may be important in lung cancer etiology. Sun exposure that

contributes vitamin D status can vary due to many factors including age, latitude, season, and skin pigment (37). Thus, the importance of vitamin D intake should be emphasized for both skeletal and non-skeletal benefits.

Our analyses show some evidence for the hypothesized effect modification of vitamin A intake. We observed a tendency that the HRs for lung cancer associated with the Calcium/Vitamin D Trial active intervention was protective among women with total vitamin A intake <1,000 μg/d RAE, and the direction of the association was opposite among those with total vitamin A intake ≥3,000 μg/d RAE. However, the effect modification was only statistically significant in a subgroup (current smokers). The reason for hypothesizing that vitamin A modifies vitamin D actions is that the vitamin D receptor (VDR) must form a heterodimer complex with retinoid X receptor (RXR) in order to regulate gene transcription (38). However, excessively high levels of 9-cis-retinoic acid, the ligand of RXR, can lead to the formation of RXR homodimers, which blunt VDR transcription (39, 40). High vitamin A intake can lead to a surge of plasma 9-cis-retinoic acid concentrations (41) although whether the level of excess vitamin A intake (≥3,000 μg RAE or approximately 10,000 IU) results in excess cytosol 9-cis-retinoic acid remains uncertain. We were unable to isolate the effect of vitamin D from that of calcium in the Calcium/Vitamin D Trial supplementation. This limits our inferential ability because calcium intake is associated with a lower risk of lung cancer (42, 43). Our finding that the Calcium/Vitamin D Trial active intervention was associated with a significantly higher risk of lung cancer among current smokers with excess vitamin A intake is unexpected. This observation was based on 9 lung cancer cases in the

Calcium/Vitamin D Trial active intervention group so the risk estimate was imprecise; the finding may be due to chance and further replication is needed. To test whether β-carotene supplementation, which increases lung cancer risk in current smokers (44), played a role in this observation, we additionally included intake of β-carotene from supplements (μg/d, continuous scale) in the regression model, but the risk estimate was not materially altered (HR=2.23, 95% CI=1.00-4.95; data not shown). Potential interactions between excess vitamin A intake and calcium/vitamin D supplementation among current smokers on lung cancer risk warrant further research.

Major strengths of our study include the prospective design, detailed exposure measurement on vitamin D intake from both food and supplements, using clinically relevant cutoffs of intake levels, and a large number of incident lung cancer cases for stratified analyses by smoking status and histological subtype. Nevertheless, several limitations should be noted. First, self-report dietary data from FFQ are subject to measurement error (45). Also, the accuracy of the potencies on supplement labels is of concern (46). In addition, we were unable to address potential effects from long-term systematic or random variations in nutrient intake. Second, non-differential misclassification of the outcome is possible. Lung cancer was not a predefined study outcome in the WHI. Chest radiology imaging was not specified by the protocol at study entry or serially. Third, we were unable to eliminate possible residual confounding. Lastly, the generalizability of WHI might be limited, since the study enrolled postmenopausal women who volunteered rather than selecting from a random sample of the population.

In conclusion, vitamin D intake of 400 IU/d or higher from food and supplements was associated with a lower risk of lung cancer risk among never-smoking, postmenopausal women. Lower vitamin A intake (<1,000 μg/d RAE for all women and <3,000 μg/d RAE for current smokers) may be important to achieve a lower lung cancer risk associated with 1 g calcium+400 IU vitamin $D_3$ supplementation.

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Table 1. Baseline characteristics of participants in the Women's Health Initiative Clinical Trials and Observational Study, 1993-2010

| | Total vitamin D intake (IU/d) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Total | <100 | 100- <200 | 200- <400 | 400- <600 | 600- <800 | ≥800 |
| No. of participants | 128,779 | 20,003 | 28,484 | 24,042 | 31,567 | 16,651 | 8,032 |
| **Total vitamin D intake (IU/d)[1]** | 370.9 (277.0) | 66.8 (21.9) | 146.3 (28.4) | 281.6 (56.9) | 508.5 (51.4) | 682.5 (56.3) | 1,004.9 (298.1) |
| **Dietary vitamin D intake (IU/d)[1]** | 175.0 (120.8) | 66.6 (21.9) | 142.2 (32.1) | 228.6 (85.1) | 157.1 (115.1) | 243.7 (117.6) | 329.2 (224.3) |
| **Supplemental vitamin D intake (IU/d)[1]** | 195.9 (246.7) | 0.2 (3.0) | 4.2 (20.5) | 53.0 (85.4) | 351.4 (122.4) | 438.8 (115.3) | 675.7 (362.4) |
| **Vitamin D supplement use (%)** | 48.1 | 0.5 | 4.6 | 31.4 | 91.7 | 97.4 | 97.4 |
| **Total vitamin A intake (µg/d RAE)[1]** | 1,711.7 (1,705.6) | 692.3 (953.4) | 907.7 (1,054.5) | 1,241.0 (1,432.9) | 2,434.6 (1,682.8) | 2,770.7 (1,570.0) | 3,473.3 (2,242.2) |
| **Total retinol intake (µg/d)[1]** | 962.5 (1,056.4) | 345.6 (638.5) | 519.5 (771.8) | 736.0 (775.3) | 1,334.2 (1,064.2) | 1,556.2 (972.3) | 2,057.0 (1,512.5) |
| **Vitamin A supplement use (%)** | 47.9 | 9.1 | 10.9 | 23.6 | 87.5 | 95.2 | 94.4 |
| **Multivitamin supplement use (%)** | 39.2 | 0.2 | 1.4 | 11.2 | 79.9 | 90.1 | 88.0 |
| **Age (years)[1]** | 63.0 (7.2) | 62.1 (7.3) | 62.7 (7.2) | 63.0 (7.1) | 63.3 (7.2) | 63.9 (7.0) | 63.9 (7.1) |
| **Body mass index (kg/m$^2$)[1]** | 27.8 (5.6) | 28.0 (5.6) | 28.3 (5.7) | 28.1 (5.7) | 27.4 (5.4) | 27.5 (5.4) | 27.2 (5.4) |
| **Region (%)** | | | | | | | |
| Northeast | 23.5 | 20.2 | 23.9 | 24.6 | 23.5 | 25.6 | 22.9 |
| South | 25.0 | 29.6 | 27.6 | 24.2 | 22.9 | 21.5 | 22.0 |
| Midwest | 22.2 | 20.0 | 20.9 | 24.3 | 21.7 | 24.1 | 23.6 |
| West | 29.3 | 30.2 | 27.6 | 26.9 | 31.9 | 28.8 | 31.5 |
| **Race/ethnicity (%)** | | | | | | | |
| Black/African-American | 8.4 | 16.1 | 10.2 | 7.3 | 6.6 | 3.7 | 3.8 |
| Hispanic/Latino | 3.8 | 6.4 | 4.4 | 3.6 | 3.1 | 2.3 | 2.1 |
| Non-Hispanic White | 83.3 | 71.6 | 80.8 | 84.9 | 86.0 | 90.6 | 90.2 |
| Other[2] | 4.5 | 5.8 | 4.6 | 4.3 | 4.3 | 3.4 | 3.9 |
| **Education (%)** | | | | | | | |
| High school or less | 21.9 | 26.6 | 24.1 | 21.0 | 20.6 | 18.4 | 17.3 |
| School after high school | 37.4 | 39.2 | 37.4 | 36.7 | 37.5 | 36.7 | 36.7 |
| College degree or higher | 40.0 | 33.4 | 37.8 | 41.6 | 41.3 | 44.4 | 45.4 |
| Unknown | 0.7 | 0.8 | 0.7 | 0.7 | 0.7 | 0.6 | 0.6 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Randomized to Hormone Therapy Trial (%)** | 18.0 | 30.7 | 19.9 | 18.2 | 16.6 | 15.5 | 14.8 |
| **Randomized to Calcium/Vitamin D Trial (%)** | 24.4 | 25.1 | 26.5 | 26.3 | 23.3 | 22.0 | 19.4 |
| **Frequency of walking outside >10 minutes (%)** | | | | | | | |
| Rarely/never | 17.0 | 20.6 | 18.2 | 16.5 | 16.0 | 14.8 | 14.5 |
| 1-3 times/month | 15.1 | 16.2 | 15.7 | 15.1 | 14.9 | 13.8 | 13.8 |
| 1 time/week | 10.9 | 10.4 | 11.2 | 11.5 | 10.4 | 11.0 | 10.3 |
| 2-3 times/week | 27.3 | 25.3 | 26.9 | 27.6 | 27.9 | 28.1 | 28.5 |
| 4-6 times/week | 21.6 | 19.8 | 20.3 | 21.3 | 22.5 | 23.5 | 23.8 |
| 7+/week | 8.1 | 7.7 | 7.7 | 8.0 | 8.2 | 8.7 | 9.2 |
| **Smoking status (%)** | | | | | | | |
| Current smoker | 7.3 | 10.1 | 8.5 | 6.7 | 6.7 | 5.3 | 5.4 |
| Former smoker | 40.3 | 39.1 | 40.0 | 38.9 | 41.5 | 41.8 | 40.6 |
| Never smoker | 52.4 | 50.8 | 51.6 | 54.4 | 51.9 | 52.9 | 54.0 |

Note: Numbers are all column percentages unless otherwise noted. All characteristics were significantly different by vitamin D intake level (all $p<0.001$, Chi-square tests for categorical variables and F tests for continuous variables).

[1] Mean (standard deviation)

[2] Including American Indian, Alaska Native, Asian, Pacific Islander, other races, and unknown.

Table 2. Multivariate-adjusted[1] lung cancer risk by total vitamin D intake category, Women's Health Initiative Clinical Trials and Observational Study, 1993-2010 (N=128,779)

| | Total vitamin D intake (IU/d)[2] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Per 100 IU | <100 | 100 – <200 | 200- <400 | 400- <600 | 600-<800 | ≥800 | *P*-trend |
| All women, n | 1,701 | 291 | 385 | 283 | 418 | 218 | 106 | |
| HR (95% CI) | 1.00 (0.97–1.02) | 1.00 (Ref) | 0.94 (0.80–1.10) | 0.90 (0.74–1.09) | 0.88 (0.73-1.07) | 0.90 (0.71-1.14) | 0.92 (0.69-1.21) | 0.39 |
| Current smokers, n | 527 | 99 | 130 | 87 | 119 | 65 | 27 | |
| HR (95% CI) | 0.99 (0.94–1.04) | 1.00 (Ref) | 1.15 (0.88–1.52) | 1.29 (0.91–1.81) | 1.04 (0.73-1.49) | 1.40 (0.91-2.17) | 1.03 (0.60-1.76) | 0.53 |
| Former smokers, n | 896 | 147 | 201 | 135 | 236 | 112 | 62 | |
| HR (95% CI) | 1.01 (0.98–1.04) | 1.00 (Ref) | 0.91 (0.73–1.14) | 0.77 (0.59–1.01) | 0.91 (0.70-1.19) | 0.81 (0.59-1.11) | 1.07 (0.74-1.55) | 0.76 |
| Never smokers, n | 278 | 45 | 54 | 61 | 63 | 41 | 14 | |
| HR (95% CI) | 0.94 (0.88–1.01) | 1.00 (Ref) | 0.68 (0.45–1.03) | 0.71 (0.45–1.12) | 0.55 (0.31-0.83) | 0.55 (0.31-0.96) | 0.37 (0.18-0.77) | 0.01 |

Abbreviations: n, number of lung cancer cases; HR, hazard ratio; CI, confidence interval; Ref, referent.

[1] Adjusted for age, region, race/ethnicity, education, Hormone Therapy Trials treatment assignment, Calcium/Vitamin D Trial active intervention (time-dependent), body mass index, smoking status (for all women only), number of cigarettes per day (for all women and current and former smokers), duration of regular smoking in years (for all women and current and former smokers), frequency of walking outside for >10 minutes, total vitamin A intake, total calcium intake, and energy intake.

[2] Baseline total vitamin D intake was assessed for all WHI participants. The intake level did not include the active intervention (1g calcium and 400 IU vitamin $D_3$ daily) of the Calcium/Vitamin D Trial.

Table 3. Multivariate-adjusted[1] lung cancer risk within histological subtypes[2] by total vitamin D intake category, Women's Health Initiative Clinical Trials and Observational Study, 1993-2010 (N=128,779)

| | Total vitamin D intake (IU/d)[4] | | | | |
|---|---|---|---|---|---|
| | Per 100 IU | <100 | 100 – <400 | ≥400 | *P*-trend |
| **Non-small cell lung cancer** | | | | | |
| All women, n | 1,104 | 198 | 419 | 487 | |
| HR (95% CI) | 0.98 (0.95-1.01) | 1.00 (Ref) | 0.85 (0.71-1.03) | 0.80 (0.63-1.01) | 0.06 |
| Current smokers, n | 306 | 56 | 123 | 127 | |
| HR (95% CI) | 0.99 (0.93-1.05) | 1.00 (Ref) | 1.25 (0.88-1.77) | 1.19 (0.76-1.86) | 0.43 |
| Former smokers, n | 604 | 108 | 216 | 280 | |
| HR (95% CI) | 0.99 (0.95-1.03) | 1.00 (Ref) | 0.76 (0.59-0.98) | 0.80 (0.59-1.09) | 0.21 |
| Never smokers[1], n | 194 | 34 | 80 | 80 | |
| HR (95% CI) | 0.92 (0.85-1.00) | 1.00 (Ref) | 0.63 (0.40-0.99) | 0.37 (0.22-0.64) | <0.001 |
| **Adenocarcinoma** | | | | | |
| All women, n | 785 | 139 | 306 | 340 | |
| HR (95% CI) | 0.97 (0.93-1.00) | 1.00 (Ref) | 0.88 (0.70-1.10) | 0.77 (0.59-1.02) | 0.07 |
| Current smokers, n | 176 | 33 | 75 | 68 | |
| HR (95% CI) | 0.98 (0.90–1.07) | 1.00 (Ref) | 1.48 (0.95–2.30) | 1.34 (0.74–2.45) | 0.27 |
| Former smokers, n | 437 | 75 | 159 | 203 | |
| HR (95% CI) | 0.98 (0.93–1.03) | 1.00 (Ref) | 0.79 (0.58–1.07) | 0.83 (0.57–1.19) | 0.36 |
| Never smokers[1], n | 172 | 31 | 72 | 69 | |
| HR (95% CI) | 0.91 (0.83–0.99) | 1.00 (Ref) | 0.62 (0.39–1.00) | 0.34 (0.19–0.60) | <0.001 |
| **Squamous cell carcinoma**[3] | | | | | |
| All women, n | 236 | 45 | 77 | 114 | |
| HR (95% CI) | 1.03 (0.97-1.09) | 1.00 (Ref) | 0.69 (0.45-1.04) | 0.86 (0.52-1.44) | 0.64 |
| Current smokers, n | 105 | 17 | 37 | 51 | |
| HR (95% CI) | 1.03 (0.94–1.13) | 1.00 (Ref) | 1.00 (0.52–1.90) | 1.27 (0.57–2.79) | 0.54 |
| Former smokers, n | 120 | 26 | 37 | 57 | |
| HR (95% CI) | 1.02 (0.94–1.11) | 1.00 (Ref) | 0.51 (0.29–0.90) | 0.57 (0.29–1.15) | 0.13 |
| **Small cell lung cancer**[3] | | | | | |
| All women, n | 176 | 35 | 69 | 72 | |
| HR (95% CI) | 1.00 (0.93-1.09) | 1.00 (Ref) | 0.87 (0.55-1.37) | 0.79 (0.44-1.40) | 0.42 |
| Current smokers, n | 93 | 21 | 40 | 32 | |
| HR (95% CI) | 0.91 (0.81–1.03) | 1.00 (Ref) | 0.80 (0.44–1.46) | 0.49 (0.23–1.06) | 0.07 |
| Former smokers, n | 77 | 14 | 28 | 35 | |
| HR (95% CI) | 1.07 (0.98–1.16) | 1.00 (Ref) | 0.97 (0.48–1.96) | 1.30 (0.53–3.22) | 0.57 |

Abbreviations: n, number of lung cancer cases; HR, hazard ratio; CI, confidence interval; Ref, referent.

[1] Adjusted for age, region, race/ethnicity, education, Hormone Therapy Trials treatment assignment, Calcium/Vitamin D Trial active intervention (time-dependent), body mass index, smoking status (for all women only), number of cigarettes per day (for all women and current and former smokers), duration of regular smoking in years (for all women and current and former smokers), frequency of walking outside for >10 minutes, total vitamin A intake, total calcium intake, and energy intake.

[2] Histological subtypes were based on the *WHO Classification of Tumors* for tumors of the lung. Non-small cell lung cancer included squamous cell carcinoma, adenocarcinoma, large cell carcinoma, sarcomatoid carcinoma, and pleomorphic carcinoma (Reference #14).

[3] Data for never smokers were not shown due to small number of cases in the histological subtype (n<30).

[4] The intake level did not include the active intervention (1g calcium and 400 IU vitamin $D_3$ daily) of the Calcium/Vitamin D Trial.

Table 4. Effect modification of total vitamin A intake on the associations of total vitamin D intake and Calcium/Vitamin D Trial active intervention with lung cancer risk[1] in the Women's Health Initiative Clinical Trials and Observational Study, 1993-2010 (N=128,779)

| | Total vitamin D intake (IU/d)[2] | | | | Calcium/Vitamin D Trial active intervention[3] | | | |
|---|---|---|---|---|---|---|---|---|
| | <400 | | ≥400 | | No | | Yes | |
| | n | HR (95% CI) | n | HR (95% CI) | n | HR (95% CI) | n | HR (95% CI) |
| **All women** | | | | | | | | |
| Main effect | 959 | 1.00 (Ref) | 742 | 0.95 (0.83-1.10) | 1,599 | 1.00 (Ref) | 102 | 0.87 (0.70-1.07) |
| Vitamin A intake (μg/d RAE) | | | | | | | | |
| ≥3,000 | 50 | 1.00 (Ref) | 165 | 0.73 (0.53-1.02) | 201 | 1.00 (Ref) | 14 | 1.22 (0.69-2.15) |
| 2,999-1,000 | 196 | 1.00 (Ref) | 544 | 1.05 (0.86-1.27) | 693 | 1.00 (Ref) | 47 | 1.02 (0.75-1.39) |
| <1,000 | 713 | 1.00 (Ref) | 33 | 0.96 (0.66-1.38) | 705 | 1.00 (Ref) | 41 | 0.69 (0.50-0.96) |
| P-interaction[4] | | | | 0.25 | | | | 0.09 |
| **Current smokers** | | | | | | | | |
| Main effect | 316 | 1.00 (Ref) | 211 | 0.94 (0.72-1.23) | 485 | 1.00 (Ref) | 42 | 1.00 (0.72-1.40) |
| Vitamin A intake (μg/d RAE) | | | | | | | | |
| ≥3,000 | 14 | 1.00 (Ref) | 41 | 0.50 (0.26-0.98) | 46 | 1.00 (Ref) | 9 | 2.26 (1.02-5.01) |
| 2,999-1,000 | 55 | 1.00 (Ref) | 159 | 1.05 (0.73-1.51) | 197 | 1.00 (Ref) | 17 | 1.05 (0.62-1.78) |
| <1,000 | 247 | 1.00 (Ref) | 11 | 1.04 (0.64-1.70) | 242 | 1.00 (Ref) | 16 | 0.72 (0.41-1.22) |
| P-interaction | | | | 0.26 | | | | 0.01 |
| **Former smokers** | | | | | | | | |
| Main effect | 483 | 1.00 (Ref) | 413 | 1.05 (0.86-1.27) | 852 | 1.00 (Ref) | 44 | 0.76 (0.55-1.04) |
| Vitamin A intake (μg/d RAE) | | | | | | | | |
| ≥3,000 | 27 | 1.00 (Ref) | 100 | 0.86 (0.56-1.33) | 123 | 1.00 (Ref) | 4 | 0.65 (0.23-1.78) |
| 2,999-1,000 | 107 | 1.00 (Ref) | 294 | 1.07 (0.82-1.39) | 380 | 1.00 (Ref) | 21 | 0.92 (0.58-1.46) |
| <1,000 | 349 | 1.00 (Ref) | 19 | 1.04 (0.64-1.70) | 349 | 1.00 (Ref) | 19 | 0.67 (0.41-1.07) |
| P-interaction | | | | 0.48 | | | | 0.98 |
| **Never smokers** | | | | | | | | |
| Main effect | 160 | 1.00 (Ref) | 118 | 0.69 (0.49-0.97) | 262 | 1.00 (Ref) | 16 | 0.91 (0.54-1.54) |
| Vitamin A intake (μg/d RAE) | | | | | | | | |
| ≥3,000 | 9 | 1.00 (Ref) | 24 | 0.53 (0.24-1.16) | 32 | 1.00 (Ref) | 1 | 0.75 (0.10-5.80) |
| 2,999-1,000 | 34 | 1.00 (Ref) | 91 | 0.86 (0.53-1.39) | 116 | 1.00 (Ref) | 9 | 1.20 (0.59-2.45) |
| <1,000 | 117 | 1.00 (Ref) | 3 | 0.46 (0.14-1.49) | 114 | 1.00 (Ref) | 6 | 0.68 (0.29-1.59) |
| P-interaction | | | | 0.94 | | | | 0.70 |

Abbreviations: n, number of lung cancer cases; HR, hazard ratio; CI, confidence interval; RAE, Retinol Activity Equivalent; Ref, referent.

[1] Total vitamin D intake and Calcium/Vitamin D Trial active intervention (time-dependent) were mutually adjusted. Regression models additionally included age, region, race/ethnicity, education, Hormone Therapy Trials treatment assignment, body mass index, smoking status (for all women only), number of cigarettes

per day (for all women and current and former smokers), duration of regular smoking in years (for all women and current and former smokers), frequency of walking outside for >10 minutes, total vitamin A intake, total calcium intake, and energy intake.

[2] The intake level did not include the active intervention (1g calcium and 400 IU vitamin $D_3$ daily) of the Calcium/Vitamin D Trial.

[3] Women with the exposure of Calcium/Vitamin D active intervention was those randomized to the calcium and vitamin D arm; women without the exposure was the rest of Observational Study and Clinical Trial individuals, including those randomized to the Calcium/Vitamin D placebo arm. Women in the active intervention arm received 1 g calcium plus 400 IU vitamin $D_3$ daily during the Calcium/Vitamin D Trial, 1994-2005.

[4] Likelihood ratio tests for the cross-product term of total vitamin D intake or Calcium/Vitamin D Trial active intervention and total vitamin A intake (all ordinal variables; df=1).

Table 5. Association of total vitamin D intake with lung cancer risk[1] stratified by time spent outdoors in summer and other seasons this year and at the age of thirties among Women's Health Initiative Observational Study participants who completed Year 4 follow-up questionnaire (N=56,003)

| | | Total vitamin D intake (IU/d) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | <100 | | 100– <400 | | ≥400 | | | |
| | N | n | HR (95% CI) | n | HR (95% CI) | n | HR (95% CI) | *P*-trend | *P*-interaction[2] |
| Time outdoors/ summer/this year | | | | | | | | | |
| <0.5 hr/d | 17,166 | 32 | 1.00 (Ref) | 84 | 0.87 (0.56–1.36) | 92 | 0.63 (0.37–1.06) | 0.063 | 0.02 |
| 0.5 to 2 hrs/d | 28,121 | 47 | 1.00 (Ref) | 117 | 0.93 (0.65–1.34) | 145 | 0.71 (0.46–1.09) | 0.094 | |
| >2 hrs/d | 10,716 | 16 | 1.00 (Ref) | 39 | 0.99 (0.53–1.87) | 49 | 1.22 (0.59–2.54) | 0.553 | |
| Time outdoors/ other/this year | | | | | | | | | |
| <0.5 hr/d | 20,964 | 39 | 1.00 (Ref) | 96 | 0.91 (0.61–1.36) | 110 | 0.67 (0.41–1.09) | 0.085 | 0.046 |
| 0.5 to 2 hrs/d | 28,623 | 47 | 1.00 (Ref) | 123 | 0.92 (0.64–1.32) | 150 | 0.73 (0.48–1.11) | 0.111 | |
| >2 hrs/d | 6,416 | 9 | 1.00 (Ref) | 21 | 0.94 (0.41–2.17) | 26 | 1.19 (0.45–3.11) | 0.695 | |
| Time outdoors/ summer/age of thirties | | | | | | | | | |
| <0.5 hr/d | 7,388 | 16 | 1.00 (Ref) | 16 | 0.26 (0.12–0.56) | 33 | 0.32 (0.14–0.71) | 0.012 | 0.54 |
| 0.5 to 2 hrs/d | 31,254 | 47 | 1.00 (Ref) | 138 | 1.14 (0.80–1.63) | 160 | 0.90 (0.59–1.36) | 0.470 | |
| >2 hrs/d | 17,361 | 32 | 1.00 (Ref) | 86 | 0.98 (0.64–1.51) | 93 | 0.75 (0.45–1.27) | 0.255 | |
| Time outdoors/ other/age of thirties | | | | | | | | | |
| <0.5 hr/d | 13,268 | 21 | 1.00 (Ref) | 47 | 0.69 (0.40–1.21) | 68 | 0.68 (0.36–1.29) | 0.299 | 0.49 |
| 0.5 to 2 hrs/d | 33,145 | 54 | 1.00 (Ref) | 148 | 1.06 (0.76–1.48) | 164 | 0.76 (0.51–1.14) | 0.125 | |
| >2 hrs/d | 9,590 | 20 | 1.00 (Ref) | 45 | 0.83 (0.47–1.47) | 54 | 0.74 (0.37–1.45) | 0.378 | |

N: number of participants; n: number of lung cancer cases; HR: hazard ratio; CI: confidence interval; Ref, referent.

[1] Adjusted for age, region, race/ethnicity, education, body mass index, smoking status, number of cigarettes per day, duration of regular smoking in years, frequency of walking outside for >10 minutes, total vitamin A intake, total calcium intake, energy intake, and usual use of sun screening.

[2] Likelihood ratio tests for the cross-product term of total vitamin D intake and time spent outdoors, both as ordinal variables (df=1).

Supplemental Table 1. Multivariate-adjusted hazard ratios (HR) for lung cancer incidence associated with total vitamin D intake, with additional adjustment for environmental tobacco smoke (ETS) exposure and duration of yard work at baseline among WHI Observational Study participants, 1993-2010

| | Total vitamin D intake (IU/d) | | | | |
|---|---|---|---|---|---|
| | <100 | 100 – <400 | 400 - <800 | ≥800 | *P*-trend |
| **All women (N=68,990)** | | | | | |
| No. cases/at risk | 136/10,345 | 329/26,230 | 350/27,493 | 56/4,922 | |
| HR (95% CI) | | | | | |
| Original model[1] | 1.00 (Ref) | 0.95 (0.77–1.19) | 0.86 (0.66–1.11) | 0.77 (0.53-1.13) | 0.14 |
| Original model + additional variables[2] | 1.00 (Ref) | 0.96 (0.77–1.19) | 0.86 (0.66–1.12) | 0.78 (0.54-1.13) | 0.15 |
| **Never smokers (N=36,025)** | | | | | |
| No. cases/at risk | 23/5,280 | 60/13,823 | 65/14,259 | 9/2,663 | |
| HR (95% CI) | | | | | |
| Original model[1] | 1.00 (Ref) | 0.82 (0.49–1.40) | 0.63 (0.34–1.18) | 0.43 (0.17-1.08) | 0.06 |
| Original model + additional variables[2] | 1.00 (Ref) | 0.83 (0.49–1.40) | 0.64 (0.34–1.19) | 0.43 (0.17-1.08) | 0.06 |

[1] Adjusted for age, region, race/ethnicity, education, body mass index, smoking status (for all women only), number of cigarettes per day (for all women only), duration of regular smoking in years (for all women only), frequency of walking outside for >10 minutes, total vitamin A intake, total calcium intake, and energy intake.

[2] Additional variables included living with smokers (including a parent, husband, or other adult person) after age 18 (yes, no), ever worked in a space where people smoked (yes, no), months of yard work a year (<1, 1 to 6, ≥7 months), and time per week of physical activity from yard work (<30 minutes, 30 minutes to 2 hours, >2 hours).

Supplemental Table 2. Multivariate-adjusted hazard ratios (HR) for lung cancer incidence associated with total vitamin D intake among women who did not use multivitamin supplements in the WHI Clinical Trials and Observational Study, 1993-2010

| | Total vitamin D intake (IU/d) | | | |
|---|---|---|---|---|
| Multivitamin non-users | <100 | 100 – <400 | ≥400 | *P*-trend |
| **All women (N= 78,366)** | | | | |
| No. cases/at risk | 289/19,973 | 631/49,447 | 128/8,946 | |
| HR (95% CI)[1] | 1.00 (Ref) | 0.92 (0.79–1.08) | 1.00 (0.78–1.28) | 0.70 |
| **Never smokers (N=41,074)** | | | | |
| No. cases/at risk | 45/10,154 | 110/26,137 | 19/4,783 | |
| HR (95% CI)[1] | 1.00 (Ref) | 0.69 (0.46–1.02) | 0.51 (0.27–0.96) | 0.03 |

[1] Adjusted for age, region, race/ethnicity, education, Hormone Therapy Trials treatment assignment, Calcium/Vitamin D Trial active intervention (time-dependent), body mass index, smoking status (for all women only), number of cigarettes per day (for all women only), duration of regular smoking in years (for all women only), frequency of walking outside for >10 minutes, total vitamin A intake, total calcium intake, and energy intake.

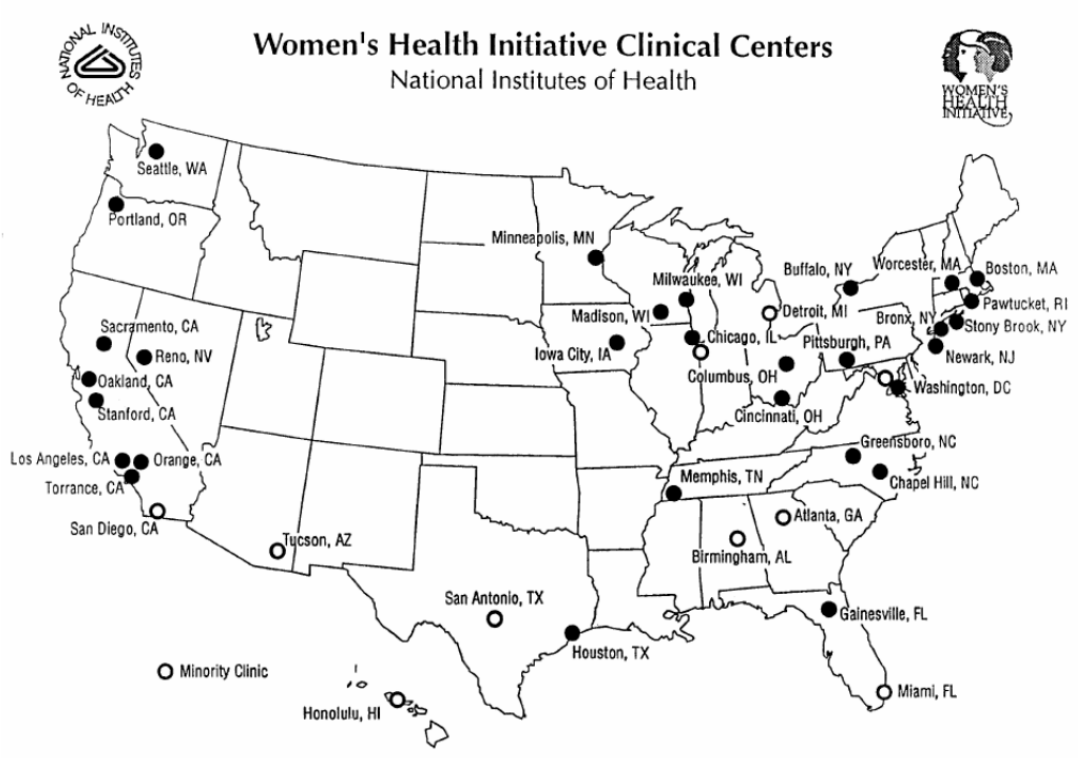


Figure 1. Women's Health Initiative Clinical Centers (www.whi.org)

# Chapter 3

## Interaction of vitamin D and vitamin A with lung cancer risk in the Carotene and Retinol Efficacy Trial (CARET)

## 3.1 Abstract

**Background**: Data on vitamin D and lung cancer prevention among smokers are limited. We investigated whether vitamin D intake was associated with lung cancer and whether effect modification by vitamin A intake and high-dose vitamin A supplementation existed among heavy smokers and workers with occupational exposure to asbestos.

**Methods**: A case-cohort study selected 749 incident lung cancers and 679 subcohort members from the Carotene and Retinol Efficacy Trial (CARET), 1985-2005. Baseline total intake included both dietary (from food frequency questionnaire) and personal supplements (from brand names linked to the labeled potencies). The CARET active intervention (30 mg β-carotene+25,000 IU retinyl palmitate) was modeled as a time-dependent covariate with a 3-year extended effect post-intervention.

**Results**: No association of total vitamin D intake with lung cancer was observed overall. Total vitamin D intake ≥600 versus <200 IU/d was associated with a lower non-small cell lung cancer risk among former smokers (hazard ratio [HR]=0.36, 95% confidence interval [CI]=0.14-0.97). Total vitamin D intake ≥400 versus <400 IU/d was associated with a lower risk of total lung cancer among all participants who received the CARET intervention (HR=0.56, 95% CI=0.32-0.98) and among those who had total vitamin A intake ≥1,500 μg/d Retinol Activity Equivalent (RAE; HR=0.46, 95% CI=0.23-0.91). The inverse associations were attenuated among participants who did not receive the CARET intervention and among those who had total vitamin A intake <1,500 μg/d RAE. The interaction between total vitamin D and vitamin A intake was statistically significant among current smokers (*P*=0.01).

**Conclusion**: Higher vitamin D intake is associated with a lower non-small cell lung cancer risk among former smokers. Vitamin A may assist vitamin D in preventing lung cancer among the study population.

## 3.2 Introduction

Lung cancer has been a major disease burden in the United States for six decades.(1) It is estimated that more than 226,000 new cases of lung cancer occurred in 2012.(1) *In vitro* and *in vivo* evidence has suggested that vitamin D status is a potential modifier of lung cancer risk.(2-4) Human tracheobronchial epithelial cells express high levels of vitamin D metabolic enzymes and vitamin D receptor (VDR), which forms a heterodimer with retinoid X receptor (RXR) to regulate gene transcriptions.(5, 6) Vitamin D enhances innate immunity in the lung by promoting transcriptions of cathelicidin antimicrobial peptide genes and CD14, a co-receptor for detecting bacterial lipopolysaccharide.(6) Vitamin D inhibits lung cancer signaling pathways including mutations in epidermal growth factor receptor and vascular endothelial growth factor and Wnt-β-catenin dysregulation.(4, 7)

Current epidemiological evidence on vitamin D and lung cancer association is inconsistent. In a small Finnish study, serum concentrations of 25-hydroxyvitamin D, the standard biomarker for assessing vitamin D status, were not associated with lung cancer risk overall although an inverse association was seen in women.(8) Also, another Finnish study recruiting solely male smokers observed inverse associations of serum 25-hydroxyvitamin D with lung cancer only in subgroups including those whose blood was drawn during darker months and who had higher vitamin D intake.(9) In U.S. populations, high serum 25-hydroxyvitamin D concentrations were associated with higher lung cancer mortality in men,(10) but with lower lung cancer mortality in never-

smoking men and women.(11) Another U.S. study showed no association between lung cancer incidence and plasma 25-hydroxyvitamin D levels predicted from vitamin D intake, demographic and lifestyle factors in men.(12) Finally, in the Women's Health Initiative (WHI) randomized, placebo-controlled, clinical trial, supplementation with daily calcium carbonate (1 g) and vitamin $D_3$ (400 IU) in otherwise healthy postmenopausal women showed a non-significant risk reduction in lung cancer (hazard ratio [HR]=0.86, 95% confidence interval [CI]=0.67-1.12).(13) However, an analysis combining both the clinical trial and observational components of WHI observed an inverse association of vitamin D intake from food and personal supplements with lung cancer risk among never smokers (HR=0.69, 95% CI=0.49-0.97; ≥400 versus <400 IU/d).(14) The totality of evidence suggests that the association of vitamin D with lung cancer risk is likely to be observed among never smokers. For studies with solely smokers and analyses that did not stratify by smoking status, the evidence remains controversial.

The primary objective of this study was to investigate whether high vitamin D intake from food and dietary supplements was associated with a lower risk of lung cancer among heavy smokers and workers with occupational exposure to asbestos recruited in the Carotene and Retinol Efficacy Trial (CARET). CARET was a multicenter, randomized, double-blind placebo-controlled chemoprevention trial to test whether 30 mg β-carotene plus 25,000 IU retinyl palmitate supplementation daily reduced lung cancer risk.(15) The secondary objective of the current study was to investigate whether high or excess vitamin A intake from diet and supplements modified the association of

vitamin D intake with lung cancer. We explored this potential effect modification because vitamin A converts to 9-*cis*-retinoic acid, the ligand of RXR, to assist with VDR-RXR-regulated gene transcriptions. However, excess 9-*cis*-retinoic acid may compete RXR with VDR and thus attenuate vitamin D activity.(11, 16-18) CARET provided a unique opportunity for this investigation because the "supraphysiologic" dose of vitamin A in the active intervention might have resulted in an increase in circulating and cellular 9-*cis*-retinoic acid concentrations.(19) We therefore hypothesized that an inverse association of vitamin D intake with lung cancer would be stronger among participants who did not receive the CARET active intervention or consumed a lower level of vitamin A from diet and supplements, compared to that among those who received the intervention or consumed a higher level of vitamin A.

## 3.3 Methods

*CARET overview*

The detailed methodology of CARET has been described elsewhere.(15, 20) Briefly, eligible participants were men and women aged 50-69 years who were current or former smokers (within the previous 6 years) with a history of at least 20 pack-years of cigarette smoking (n=14,254), and men aged 45-69 years who were current or former smokers and exposed to asbestos in the workplace beginning at least 15 years prior (n=4,060). Recruitment began in 1985, and two pilot studies with various doses of β-carotene and retinol intervention vitamins were conducted separately for heavy smokers and asbestos-exposed participants. Beginning in July 1988, the full-scale, "efficacy" trial,

in which the active intervention was 30 mg β-carotene plus 25,000 IU retinyl palmitate daily was implemented; pilot participants were also transitioned to the new protocol. The efficacy trial was stopped early in 1996 due to an increase in lung cancer risk in the treatment arm; 94% of participants remained in active follow-up until 2005. Average length of follow-up during administration of the CARET intervention was 4 years; the capsule consumption rate was 77% at four years. CARET had a well-established endpoint assessment protocol. For participants reporting a lung cancer diagnosis through 1997, pathology reports along with tumor tissue samples for independent central pathology review were obtained from the diagnosing institutions. Cases were centrally adjudicated by three physicians; a consensus was required on the primary cancer site, its histology, and the date of diagnosis. Central pathology was discontinued in 1998, and endpoints were adjudicated by endpoint specialists in CARET and a single physician based on pathology reports and other medical records obtained from participants' health care providers. The date and underlying cause of death were obtained by medical records and death certificates. The Institutional Review Board of the Fred Hutchinson Cancer Research Center and each of the five other participating institutions approved all procedures for the study; participants provided written informed consent at recruitment and throughout the trial.

*Case and subcohort selection*

The current study was a case-cohort design. The cases were CARET participants who developed lung cancer during the efficacy trial and post-intervention follow-up. A "subcohort" serving as the comparison group was a random sample of the same size as

the cases from all participants at the outset. As of September 30, 2005, 1,445 lung cancer cases were ascertained among all CARET participants. After excluding those who did not successfully transition from the pilot to the efficacy protocol (n=39), who provided no supplemental vitamin intake data (n=7) or one after a lung cancer diagnosis (n=6), and who attended the Portland clinical center (due to no access to their charts, n=377), 1,016 cases were selected. Eligible for the subcohort selection was a total of 13,457 participants form the CARET cohort: the 1,016 eligible lung cancer cases and 12,441 participants who were free of lung cancer, had charts available for review (i.e., from enrollment centers other than the Portland site), and were enrolled in or successfully transitioned to the efficacy protocol. From the cohort pool, a subcohort of 1,016 participants was randomly selected, including 66 lung cancer cases. We further excluded participants who had a history of gastrointestinal diseases (colitis or diverticulosis, n=176), kidney diseases (nephritis, kidney infection, kidney stones, or kidney failure, n=191), and liver diseases (yellow jaundice, hepatitis, or cirrhosis, n=122) at baseline as they can affect vitamin D metabolism,(21) no data on disease history at baseline (n=34) or a food frequency questionnaire through follow-up (n=97), and implausibly low or high body mass index (<15.0 or >50.0 kg/m$^2$) at baseline (n=13), whose supplemental vitamin use chart was missing (n=1), and whose food frequency questionnaires were completed after lung cancer diagnosis (n=5). Consequently, 749 cases (including 44 cases arising in the subcohort) and 679 lung cancer free subcohort members entered statistical analyses. Estimated energy intake levels from a baseline food frequency questionnaire were in the plausible range (<800 or >5,000 kcal/d for men and <600 or >4,000 kcal/d for women) for all cases and subcohort members.

*Dietary intake assessment*

Dietary intake data over the previous year were collected by a self-administered food frequency questionnaire at baseline clinic visits. The questionnaire was designed to be especially sensitive to major sources of fat and carotenoids. The nutrient database was derived from the University of Minnesota Nutrition Coordinating Center (NCC) database (version 4.02, food and nutrient database version 30) and the 1999 USDA–NCC Carotenoid Database for United States foods.(22)

*Personal supplemental vitamin use*

A personal supplemental vitamin was defined as a dietary supplement used by participants other than the CARET active intervention. Participants were asked to bring their currently used vitamin bottles to clinic centers. Interviewers recorded up to 6 brand names and doses of vitamin A, β-carotene, and vitamin E contained in the supplements. If participants used more than 6 different supplements, priority of recording was given to vitamin A or β-carotene supplements that participants took regularly and then occasionally. The daily dosage was calculated based on the dose on the label and frequency of use per week. For the supplements that participants did not bring in, the brand names and doses were recorded based on participants' self-report. At baseline and each intervention phase visit, participants were advised to keep personal supplemental vitamin A intake under 5,500 IU (1,650 μg) per day and to take no β-carotene supplements. The use of β-carotene supplements decreased from 10% at baseline to 3% during follow-up; the use of vitamin A supplements of any dose and high dose (>5,500

IU) decreased from 19% and 3% at baseline to 12% and 1% during follow-up, respectively.

Since the dosage of vitamin D contained in these supplements was not recorded and the brand names recorded in the participant charts were not computerized, for the current study we retrospectively extracted all the brand names on the baseline charts of the eligible cases and subcohort members who indicated *any* supplement use (n=813 of 1,016 cases and 950 subcohort members free of lung cancer, less one chart that was not available for review). A total of 175 extracted brands were identified as single or multivitamin supplements containing vitamin D. The dosage of vitamin D of each brand was obtained via *Physicians' Desk Reference for Nonprescription Drugs and Dietary Supplements* (23), Dietary Supplement Label Database,(24) and internet searches. For brands unidentifiable from the above sources (n=96 out of 175 extracted brands, 55%), 400 IU, the most common dosage of vitamin D supplements,(23) was assigned. For charts without any information on brand names or vitamin D doses (n=25 out of 812 charts, 3%), 0 IU were assigned. The investigator (TYC) extracting and entering data was blinded to the case-subcohort status. Another investigator (MLN) reviewed a 10% (n=81) random sample of the charts for the quality control; the agreement rate was 98.8%.

Average daily intake of total vitamin D and vitamin A at baseline were calculated by summing together amounts from food and personal supplements. Vitamin A intake was expressed as μg Retinol Activity Equivalent (RAE) because it consists of a wide

range of compounds including retinol and carotenoids. The calculations of RAE for dietary and supplemental intake were:

Dietary intake = μg retinol + (μg β-carotene equivalent/12), where β-carotene equivalent= μg β-carotene + ½ (μg α-carotene + μg β-cryptoxanthin);

Supplemental intake = μg retinol + (μg β-carotene/2).(25)

Supplemental intake of other carotenoids was omitted because the data was unavailable. Based on the conversion, the dosage of CARET active intervention was 22,500 μg RAE (7,500 μg of retinol and 15,000 μg RAE of β-carotene)

*Covariates assessment*

Covariates including age, gender, race/ethnicity, education level, smoking habits, number of years in high-risk (asbestos) trade, and medical history at baseline were collected by standardized, self-administered questionnaires. Current smokers were defined as those who smoked any cigarettes in the past month. Number of cigarettes smoked per day and years as a regular smoker were also queried. Baseline alcohol consumption was assessed by the food frequency questionnaire. Height and weight were measured at baseline visits.

*Statistical analysis*

Baseline characteristics between cases and subcohort members were examined using t-tests for continuous variables or $\chi^2$ tests for categorical variables. Dietary variables were natural-log transformed for t-tests to improve normality. Lung cancer risks

were estimated for categorical (<200, 200 to <400, 400 to <600, 600 to <800, and ≥800 IU/d) and linear (per 100 IU/d increment) total vitamin D intake in separate models. We chose these cutoffs because they are reference intakes for bone health: 400 IU/d, the Estimated Average Requirement for all ages; 600 and 800 IU/d, the Recommended Dietary Allowance for ≤70 years and 71 years or older, respectively.(26) Total vitamin D intake <200 IU/d was chosen as the reference group to provide precise risk estimates due to a relatively small number of participants with total vitamin D intake <100 IU/d. For histological subtype analyses, we combined the top 2 categories (600 to <800 and ≥800 IU/d) due to the reduced number of lung cancer cases. As well, to increase statistical power and be able to compared with prior research,(14) comparisons of total vitamin D intake ≥400 versus <400 IU/d were made for effect modification analyses. HR and 95% CI for lung cancer were estimated by Cox's proportional hazards models with the Self-Prentice method computing robust standard error estimates to account for the case-cohort design.(27) Participants contributed follow-up time from the randomization of CARET to the date of lung cancer diagnosis, date of death from causes other than lung cancer, the last documented follow-up contact, or September 30, 2005, whichever came first. The proportionality assumption was examined by testing whether scaled Schoenfeld residuals for total vitamin D intake were associated with survival time;(28) the assumption was fulfilled. Multivariate models for assessing the association of total vitamin D intake included baseline covariates that were chosen *a priori*: age (continuous), study center, race/ethnicity (White, Black, others), education level (no high school diploma, high school, college degree or higher, unknown), enrolled as asbestos exposure worker (yes, no), number of years in high-risk trade (0, 1–20, ≥21), CARET active intervention (yes or

no), body mass index (continuous), smoking status (current or former), amount of smoking (<40, 40 to 59, ≥60 pack-years), total vitamin A intake (<800, 800 to <1,500, 1,500 to <2,500, ≥2,500 μg/d RAE), and energy intake (continuous). The CARET active intervention was modeled as a time-dependent variable (Figure 1), allowing the exposure to vary 3 years (1999 onward) after the trial.(28) We classified the first 3 years of post-intervention follow-up as the active intervention because the adverse effect of active intervention for lung cancer risk had remained statistically significant until approximately the 4$^{th}$ year after the intervention was stopped.(29) We also evaluated whether dietary calcium intake and history of asbestosis, chronic bronchitis, and emphysema were confounders because research suggests that they are associated with lung cancer risk.(30, 31) They made no changes to risk estimates so were not included in the final model. Linear trends of risk estimates were examined by Wald tests (1 df) of an ordinal variable of total vitamin D intake categories. Cox models were performed for all participants and by *a priori* smoking status subgroups (current and former smokers) and for histological subtypes of lung cancer among cases who had complete histological data (n=592, 79% of all cases). We evaluated whether pre-clinical lung cancer affected vitamin D intake by excluding lung cancer cases diagnosed within the first two years of the efficacy trial. To evaluate effect modification, we stratified the associations of total vitamin D intake by the CARET treatment arm during the trial and total vitamin A intake (<1,500 or ≥1,500 μg/d RAE, approximately the 75$^{th}$ quartile of all participants). Statistical evidence of interaction was examined by Wald tests of the cross-product term of total vitamin D intake categories and CARET active interaction or total vitamin A intake categories (all ordinal variables; 1 df). Interaction between vitamin D intake and smoking status was

also explored by the same approach. All statistical tests were two-sided; statistical significance was defined as $P<0.05$. Statistical analyses were conducted using STATA (12.0, College Station, Texas).

### 3.4 Results

**Table 1** shows baseline characteristics of lung cancer cases and subcohort members. Compared with subcohort members, the cases were older, had a lower education attainment, had more current smokers, smoked more pack-years of cigarette, had a higher number of years working in asbestos trade, and had lower body mass index (all $P<0.05$).

There were no associations of total vitamin D intake with lung cancer risk among all participants or current smokers in either the linear (for every 100 IU/d increment) or categorical models (**Table 2**). However, among former smokers, total vitamin D intake ≥800 versus <200 IU/d was suggestively associated with a lower risk of lung cancer (HR=0.26, 95% CI=0.06-1.05; *P*-trend=0.06). In the analysis of histological subtypes of lung cancer (**Table 3**), total vitamin D intake ≥600 versus <200 IU/d was associated with a lower risk of non-small cell lung cancer among former smokers (HR=0.36, 95% CI=0.14-0.97). The associations of vitamin D intake with total lung cancer and non-small cell lung cancer significantly differed between current and former smokers (*P*-interaction=0.002 and 0.003, respectively). The observed associations did not materially change after excluding lung cancer cases diagnosed within the first two years of the

efficacy trial (data not shown). A decreasing trend in risk across the increasing total vitamin D intake was also observed for adenocarcinoma and squamous cell carcinoma, but not small cell lung cancer among former smokers.

**Table 4** shows analyses stratified by receiving the CARET active intervention and baseline total vitamin A intake. We compared total lung cancer risks for participants with vitamin D intake ≥400 versus <400 IU/d to increase statistical power for these analyses. When stratifying by the CARET active intervention, total vitamin D intake ≥400 versus <400 IU/d was associated with a lower risk of lung cancer among all participants (HR=0.56, 95% CI=0.32-0.98) and among former smokers (HR=0.24, 95% CI=0.08-0.73) who received the CARET active intervention. This inverse association was not observed among all participants and former smokers who did not receive the CARET active intervention, although the interactions were not statistically significant. When stratifying by total vitamin A intake from diet and personal supplements, total vitamin D intake ≥400 versus <400 IU/d was associated with a lower risk of lung cancer among all participants with total vitamin A intake ≥1500 μg/d RAE (HR=0.46, 95% CI=0.23-0.91), but not those with total vitamin A intake <1500 μg/d RAE. The difference in the association of total vitamin D with lung cancer by the total vitamin A intake category was statistically significant among current smokers (*P*-interaction=0.01).

### 3.5 Discussion

In this study population of heavy smokers and/or workers with occupational asbestos exposure, total vitamin D intake ≥600 versus <200 IU/d was associated with a lower risk for non-small cell lung cancer among former smokers. Previous studies have suggested that an inverse association of vitamin D is more likely to be observed for non-small cell lung cancer compared to small cell lung cancer regardless of smoking status.(9, 14) Also, an inverse association is more likely to be observed among distant quitters (e.g., quit ≥20 years) or never smokers compared to recent quitters or current smokers.(11, 14) Therefore, our findings are consistent with prior research. Nevertheless, this study provides novel evidence that vitamin D intake is inversely associated with non-small cell lung cancer among relatively recent quitters. The CARET recruited heavy former smokers quitting within 6 years although allowed for quitting up to 15 years for asbestos exposure workers (60% and 40% of all former smokers, respectively).

Cigarette smoking may affect vitamin D intake and its metabolism. Smokers have lower intake of vitamin D from both food and supplements compared to nonsmokers.(32, 33) In addition, smoking may decrease the expression of CYP2R1, the enzyme synthesizing 25-hydroxyvitamin D,(34) resulting in a lower vitamin D status.(35, 36) Also, smoking-produced carcinogen benzo[a]pyrene enhances CYP24A1 activity, which degrades 1,25-dihydroxyvitamin D.(37) Therefore, among current smokers, higher vitamin D intake or vitamin D status may not necessarily lead to a larger biological effect. This is one explanation for null associations of vitamin D intake and serum 25-

hydroxyvitamin D with lung cancer among current smokers observed in our study and other studies.(9, 11, 14) On the contrary, quitting smoking is associated with increases in both vitamin D intake and vitamin D status to a level that resembles those of never smokers.(38, 39) Among the subcohort members in our study, former smokers consumed approximately 10% more vitamin D compared to current smokers (median total intake= 279 versus 256 IU/d, *P*=0.13; data not shown). If the vitamin D metabolic functions affected by smoking can be restored after smoking cessation, it is plausible that we are more likely to observe an association of vitamin D intake with lung cancer among former smokers compared to current smokers.

The effect modification of the CARET active intervention and total vitamin A intake from food and personal supplements showed a consistent pattern that there was a lower lung cancer risk associated with higher vitamin D intake among participants consuming higher levels of vitamin A, and the association was attenuated among those consuming lower levels of vitamin A. Our observation is consistent with prior research that investigated current smokers. In the Alpha-Tocopherol, Beta Carotene Cancer Prevention Study (ATBC), which recruited only male current smokers, lung cancer risk associated with serum 25-hydrovitamin D (quintiles 4–5 versus 1–3) was lower among participants receiving the active interventions (odds ratio [OR]=0.76, 95% CI=0.46–1.27 for 20 mg β-carotene supplement daily; OR=0.69, 95% CI=0.40–1.17 for 20 mg β-carotene plus 50 mg α-tocopherol supplements daily), compared to that among those receiving placebo (OR=1.34, 95% CI=0.78–2.32; *P*-intervention >0.05).(9) In the Women's Health Initiative (WHI), an inverse association of total vitamin D intake with

lung cancer (HR=0.50, 95% CI=0.26–0.98; ≥400 versus <400 IU/d) was observed among current smokers with total vitamin A intake ≥3,000 μg/d RAE, but not among those with vitamin A intake <1,000 μg/d RAE (HR=1.04, 95% CI=0.64–1.70; *P*-interaction=0.26).(14) These observations and our findings are clearly contrary to our hypothesis. Since retinol can reverse smoking-induced preneoplastic lesions,(40, 41) and provide the essential ligand, i.e., 9-*cis*-retinoic acid, for RXR-VDR-regulated transcription,(42, 43) it may be important for vitamin D's effect on protecting against lung cancer among smokers. We also observed that among participants who received the CARET active intervention, the vitamin D intake-lung cancer association for current smokers (HR=0.54, 95% CI=0.25–1.15) was not as strong compared to that for former smokers (HR=0.24, 95% CI=0.08–0.73). An explanation of this observation was that CARET current smokers underwent an additional adverse effect that high-dose β-carotene together with cigarette smoke enhanced lung carcinogenesis.(44)

Although we cannot isolate the effect of retinol from β-carotene in the CARET active intervention because participants in the intervention arm received both micronutrients, it is possible that retinol is the major contributor to 9-*cis*-retinoic acid. The cleavage of β-carotene to retinol is a negative feedback mechanism associated with vitamin A status, retinol intake, and the dose of β-carotene supplementation. Human studies have shown that the ability of bioconversion of β-carotene to retinol is reduced when vitamin A status is improved from deficient to normal.(45, 46) High-dose vitamin A supplementation (10,000 IU/d) leads to a modest reduction in β-carotene cleavage.(47) In addition, the standard conversion factor of β-carotene supplements to retinol is 2:1;

however, the factor increases to 12:1 and 55:1 when high doses, that are 12 mg and 126 mg of β-carotene oil capsules are consumed, respectively.(48, 49) Taken together, although high-dose β-carotene was given to CARET participants, the conversion of β-carotene to retinol was likely minor because high-dose retinol was given at the same time and the study participants were at normal vitamin A status.(50, 51)

Major strengths of our study include the prospective design and a large number of incident lung cancer cases for stratified analyses by smoking status and histological subtype. All reports of lung cancers were confirmed by medical records and pathology reports and adjudicated. Lung cancer was the primary endpoint of CARET and thus had high completion rate and accuracy. Nevertheless, limitations of this study should be noted. Dietary vitamin D intake measured by food frequency questionnaires may only modestly correlate with the true intake.(52) In addition, supplemental vitamin D intake was subject to measurement error because the ascertainment of vitamin D potencies from bottle labels was incomplete, and we assumed labeled potencies as daily intake doses. Also, we were unable to address potential effects from long-term systematic or random variations in nutrient intake since only the baseline assessment was used. These potential measurement errors might have biased the risk estimates toward the null. In addition, CARET did not assess sun exposure, the other major source of vitamin D exposure. Nevertheless, our regression models included study center as a proxy to latitude, an important determinant for photosynthesis of vitamin D.(53) Lastly, it may not be appropriate to generalize our findings to other populations because CARET participants were heavy smokers and/or had occupational asbestos exposure.

In conclusion, high total vitamin D intake is associated with a lower risk for non-small cell lung cancer among CARET former smokers. Vitamin A intake from diet and supplements may assist vitamin D in preventing lung cancer among heavy smokers and workers with occupational exposure to asbestos.

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Table 1. Baseline characteristics among the lung cancer cases (n=749) and subcohort members (n=679) in the Carotene and Retinol Efficacy Trial (CARET)

| Characteristic | Lung cancer cases[1] | | Subcohort members | | *P*-value[2] |
|---|---|---|---|---|---|
| All participants (n, %)[3] | 749 | (100.0) | 679 | 100.0 | |
| Age, y (mean, SD) | 60.8 | ±5.7 | 57.8 | ±6.1 | <0.001 |
| Female | 206 | (27.5) | 195 | (28.7) | 0.61 |
| Race/ethnicity | | | | | |
| White | 695 | (92.8) | 621 | (91.5) | 0.26 |
| African American | 33 | (4.4) | 28 | (4.1) | |
| Other | 21 | (2.8) | 30 | (4.4) | |
| Education | | | | | |
| No high school diploma | 114 | (15.2) | 71 | (10.5) | 0.03 |
| High school diploma | 181 | (24.2) | 159 | (23.4) | |
| Some college degree or above | 319 | (42.6) | 326 | (48.0) | |
| Unknown | 135 | (18.0) | 123 | (18.1) | |
| CARET randomization assignment | | | | | |
| Active | 404 | (53.9) | 349 | (51.4) | 0.34 |
| Placebo | 345 | (46.1) | 330 | (48.6) | |
| Smoking status | | | | | |
| Current | 527 | (70.4) | 364 | (53.6) | <0.001 |
| Former[4] | 222 | (29.6) | 315 | (46.4) | |
| Smoking pack-years | | | | | |
| <40 | 178 | (23.8) | 276 | (40.7) | <0.001 |
| 40 to 59 | 307 | (41.0) | 246 | (36.2) | |
| ≥60 | 264 | (35.3) | 157 | (23.1) | |
| Enrolled as asbestos exposure worker | 208 | (27.8) | 193 | (28.4) | 0.79 |
| Years in high-risk (asbestos) trade | | | | | |
| 0 | 577 | (77.0) | 518 | (76.3) | 0.04 |
| 1-20 | 58 | (7.7) | 76 | (11.2) | |
| 21+ | 114 | (15.2) | 85 | (12.5) | |
| Alcohol intake | | | | | |
| Non-drinkers (intake <0.39 g) | 254 | (33.9) | 219 | (32.3) | 0.30 |
| <10 g/d | 191 | (25.5) | 198 | (29.2) | |
| ≥10 g/d | 304 | (40.6) | 262 | (38.6) | |
| Body mass index, kg/m$^2$ (mean, SD) | 26.9 | ±4.4 | 27.9 | ±4.9 | <0.001 |
| History of asbestosis | 99 | (13.2) | 83 | (12.2) | 0.57 |
| History of asthma | 55 | (7.3) | 44 | (6.5) | 0.52 |
| History of bronchitis or emphysema | 111 | (14.8) | 68 | (10.0) | 0.006 |
| Any supplemental vitamin use | 305 | (40.7) | 292 | (43.0) | 0.38 |
| Total vitamin A intake, µg RAE (median, IQR)[5] | 835 | (535-1,412) | 892 | (568-1,584) | 0.07 |
| Dietary vitamin A intake, µg RAE (median, IQR) | 724 | (502-1,078) | 731 | (511-1,087) | 0.78 |
| Vitamin A/β-carotene supplement users | 127 | (17.0) | 126 | (18.6) | 0.43 |
| Supplemental vitamin A intake *among the* | 3,000 | (1,500-3,000) | 3,000 | (1,500-3,000) | 0.91 |

| | | | | | |
|---|---|---|---|---|---|
| *users*, μg RAE (median, IQR)[5] | | | | | |
| Total vitamin D intake, IU/d (median, IQR)[5] | 261.2 | (160-512) | 265 | (157-519) | 0.57 |
| Dietary vitamin D intake, IU/d (median, IQR) | 201 | (139-295) | 205 | (130-308) | 0.93 |
| Supplemental vitamin D users | 191 | (25.5) | 177 | (26.1) | 0.81 |
| Supplemental vitamin D intake *among the users*, IU/d intake (median, IQR) | 400 | (400-400) | 400 | (400-400) | 0.12 |

Abbreviations: SD, standard deviation; IQR, interquartile range; RAE, Retinol Activity Equivalent.

[1] Including 44 lung cancer cases arising in the subcohort.

[2] t-tests for continuous variables and $\chi^2$ tests for categorical variables. Dietary variables were natural-log transformed for t-tests to improve normality.

[3] Numbers are number of participants and percentages unless otherwise noted.

[4] Including 7 never smokers, representing <1% of all participants. They were recruited in CARET because of their occupational asbestos exposure.

[5] The level did not include the CARET active intervention (30 mg β-carotene plus 25,000 IU retinyl palmitate daily)

Table 2. Multivariate-adjusted[1] associations of total vitamin D intake with lung cancer risk, CARET, 1989-2005

| | Total vitamin D intake (IU/d) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Per 100 IU | <200 | 200 to <400 | 400 to <600 | 600 to <800 | ≥800 | *P*-trend |
| All participants | | | | | | | |
| No. Cases/p-y in subcohort | 749/8,364 | 281/2,928 | 209/2,386 | 145/1,757 | 84/888 | 30/405 | |
| HR (95% CI) | 0.98 (0.92-1.05) | 1.00 (Ref) | 0.77 (0.55–1.07) | 0.74 (0.49-1.13) | 0.83 (0.49-1.40) | 0.67 (0.32-1.39) | 0.26 |
| Current smokers | | | | | | | |
| No. Cases/p-y in subcohort | 527/4,429 | 191/1,674 | 145/1,278 | 106/901 | 59/395 | 26/181 | |
| HR (95% CI) | 1.01 (0.93-1.10) | 1.00 (Ref) | 0.75 (0.49-1.16) | 0.72 (0.41-1.25) | 1.07 (0.55-2.09) | 0.94 (0.37-2.36) | 0.91 |
| Former smokers[2] | | | | | | | |
| No. Cases/p-y in subcohort | 222/3,935 | 90/1,255 | 64/1,108 | 39/856 | 25/492 | 4/224 | |
| HR (95% CI) | 0.91 (0.81-1.03) | 1.00 (Ref) | 0.87 (0.49-1.53) | 0.66 (0.32-1.34) | 0.51 (0.22-1.23) | 0.26 (0.06-1.05) | 0.06 |

Abbreviations: HR, hazard ratio; CI, confidence interval; p-y, person-years; ref, reference.

[1] Adjusted for age, study center, race/ethnicity, education, enrolled as asbestos exposure worker, number of years in high-risk trade, smoking status (for all participants only), smoking pack-years, body mass index, energy intake, total vitamin A intake, and CARET active intervention (time-dependent covariate with a 3-year extended effect post-intervention).

[2] *P*-value for interaction between total vitamin D intake categories and smoking =0.002 (both ordinal variables; Wald test, 1 df).

Table 3. Associations of total vitamin D intake with lung cancer risk by histological subtype of tumor[1]

| | Total vitamin D intake (IU/d) | | | | | |
|---|---|---|---|---|---|---|
| | Per 100 IU | <200 | 200 to <400 | 400 to <600 | ≥600 | *P*-trend |
| *Non-small cell lung cancer*[2] | | | | | | |
| All participants | | | | | | |
| No. Cases/p-y in subcohort | 476/8,238 | 181/2,902 | 126/2,329 | 97/1,722 | 72/1,285 | |
| HR (95% CI) | 0.95 (0.88-1.03) | 1.00 (Ref) | 0.69 (0.47–1.01) | 0.78 (0.49-1.22) | 0.68 (0.39-1.17) | 0.16 |
| Current smokers | | | | | | |
| No. Cases/p-y in subcohort | 324/4,329 | 121/1,658 | 79/1,238 | 69/865 | 55/568 | |
| HR (95% CI) | 0.98 (0.89-1.08) | 1.00 (Ref) | 0.61 (0.37-1.00) | 0.73 (0.39-1.33) | 0.91 (0.45-1.85) | 0.58 |
| Former smokers[3] | | | | | | |
| No. Cases/p-y in subcohort | 152/3,909 | 60/1,244 | 47/1,091 | 28/857 | 17/717 | |
| HR (95% CI) | 0.89 (0.78-1.02) | 1.00 (Ref) | 1.06 (0.56-2.00) | 0.81 (0.38-1.74) | 0.36 (0.14-0.97) | 0.09 |
| *Adenocarcinoma* | | | | | | |
| All participants | | | | | | |
| No. Cases/p-y in subcohort | 198/8,093 | 81/2,834 | 52/2,298 | 38/1,701 | 27/1,260 | |
| HR (95% CI) | 1.01 (0.91-1.11) | 1.00 (Ref) | 0.80 (0.50–1.29) | 0.96 (0.54-1.70) | 0.96 (0.48-1.94) | 0.88 |
| Current smokers | | | | | | |
| No. Cases/p-y in subcohort | 132/4224 | 50/1,623 | 33/1,206 | 26/851 | 23/544 | |
| HR (95% CI) | 1.05 (0.92-1.19) | 1.00 (Ref) | 0.81 (0.44-1.50) | 0.91 (0.42-1.96) | 1.45 (0.60-3.53) | 0.60 |
| Former smokers[3] | | | | | | |
| No. Cases/p-y in subcohort | 66/3,869 | 31/1,211 | 19/1,091 | 12/850 | 4/717 | |
| HR (95% CI) | 0.95 (0.78-1.15) | 1.00 (Ref) | 1.14 (0.50-2.64) | 0.97 (0.35-2.68) | 0.43 (0.10-1.93) | 0.49 |
| *Squamous cell carcinoma* | | | | | | |
| All participants | | | | | | |
| No. Cases/p-y in subcohort | 143/8,116 | 49/2,847 | 41/2,301 | 28/1,695 | 25/1,273 | |
| HR (95% CI) | 0.94 (0.83-1.06) | 1.00 (Ref) | 0.68 (0.38–1.20) | 0.70 (0.34-1.42) | 0.64 (0.28-1.49) | 0.29 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Current smokers | | | | | | |
| No. Cases/p-y in subcohort | 104/4,237 | 37/1,618 | 28/1,214 | 20/848 | 19/557 | |
| HR (95% CI) | 0.97 (0.84-1.12) | 1.00 (Ref) | 0.61 (0.30-1.25) | 0.64 (0.26-1.60) | 0.85 (0.30-2.38) | 0.61 |
| Former smokers[3] | | | | | | |
| No. Cases/p-y in subcohort | 39/3,879 | 12/1,228 | 13/1,087 | 8/847 | 6/717 | |
| HR (95% CI) | 0.85 (0.68-1.06) | 1.00 (Ref) | 0.98 (0.33-2.93) | 0.76 (0.20-2.86) | 0.33 (0.07-1.64) | 0.19 |
| *Small-cell lung cancer* | | | | | | |
| All participants | | | | | | |
| No. Cases/p-y in subcohort | 116/8,048 | 38/2,807 | 36/2,298 | 23/1,678 | 19/1,265 | |
| HR (95% CI) | 1.08 (0.96-1.22) | 1.00 (Ref) | 0.98 (0.54–1.78) | 1.12 (0.54-2.32) | 1.32 (0.55-3.15) | 0.63 |
| Current smokers | | | | | | |
| No. Cases/p-y in subcohort | 88/4,186 | 28/1,605 | 30/1,195 | 17/838 | 13/548 | |
| HR (95% CI) | 1.10 (0.95-1.28) | 1.00 (Ref) | 1.16 (0.58-2.32) | 1.20 (0.48-2.99) | 1.53 (0.53-4.36) | 0.46 |
| Former smokers[3] | | | | | | |
| No. Cases/p-y in subcohort | 28/3,862 | 10/1,202 | 6/1,103 | 6/841 | 6/716 | |
| HR (95% CI) | 1.06 (0.84-1.33) | 1.00 (Ref) | 0.35 (0.08-1.47) | 0.63 (0.15-2.71) | 0.84 (0.16-4.43) | 0.99 |

Abbreviations: HR, hazard ratio; CI, confidence interval; p-y, person-years; ref, reference.

[1] Adjusted for age, study center, race/ethnicity, education, enrolled as asbestos exposure worker, number of years in high-risk trade, smoking status, smoking pack-years, body mass index, energy intake, total vitamin A intake, and CARET active intervention (time-dependent covariate with a 3-year extended effect post-intervention).

[2] Non-small cell lung cancer included adenocarcinoma, squamous cell carcinoma, and non-small cell lung cancer not otherwise specified or subtypes other than small-cell lung cancer.

[3] *P*-interaction between total vitamin D intake and smoking status: 0.003 for non-small cell lung cancer, 0.001 for adenocarcinoma, 0.21 for squamous cell carcinoma, and 0.45 for small-cell lung cancer. The *P*-values were obtained by Wald tests of the cross-product term of total vitamin D intake categories and smoking status (both ordinal variables; 1 df).

Table 4. Associations of total vitamin D intake with lung cancer risk, stratified by receiving the CARET active intervention (30 mg β-carotene plus 25,000 IU retinyl palmitate daily or 22,500 µg RAE) during the trial and total vitamin A intake levels for all participants, current smokers, and former smokers

| | Total vitamin D intake (IU/d) | | | | |
|---|---|---|---|---|---|
| | <400 | | ≥400 | | |
| Main effects and stratifications[1] | No. cases/p-y in subcohort | HR (95% CI) | No. cases/p-y in subcohort | HR (95% CI) | *P*-interaction[3] |
| **All participants, main effect** | 490/5,314 | 1.00 (Ref) | 259/3,050 | 0.91 (0.65-1.27) | |
| *CARET active intervention*[2] | | | | | |
| Yes | 129/1,603 | 1.00 (Ref) | 60/988 | 0.56 (0.32-0.98) | 0.24 |
| No | 361/3711 | 1.00 (Ref) | 199/2,062 | 1.08 (0.75-1.54) | |
| *Total vitamin A intake* | | | | | |
| ≥1,500 µg/d RAE | 39/404 | 1.00 (Ref) | 138/1,719 | 0.46 (0.23-0.91) | 0.08 |
| <1,500 µg/d RAE | 451/4,900 | 1.00 (Ref) | 121/1,331 | 1.06 (0.73-1.55) | |
| **Current smokers, main effect** | 336/2,952 | 1.00 (Ref) | 191/1,477 | 0.97 (0.62-1.53) | |
| *CARET active intervention*[2] | | | | | |
| Yes | 86/879 | 1.00 (Ref) | 45/481 | 0.54 (0.25-1.15) | 0.61 |
| No | 250/2,073 | 1.00 (Ref) | 146/996 | 1.10 (0.68-1.78) | |
| *Total vitamin A intake* | | | | | |
| ≥1,500 µg/d RAE | 29/166 | 1.00 (Ref) | 104/847 | 0.38 (0.14-1.07) | 0.01 |
| <1,500 µg/d RAE | 307/2,786 | 1.00 (Ref) | 87/630 | 1.33 (0.82-2.15) | |
| **Former smokers, main effect** | 154/2,362 | 1.00 (Ref) | 68/1,573 | 0.64 (0.37-1.13) | |
| *CARET active intervention*[2] | | | | | |
| Yes | 43/724 | 1.00 (Ref) | 15/508 | 0.24 (0.08-0.73) | 0.18 |
| No | 111/1,638 | 1.00 (Ref) | 53/1,065 | 0.82 (0.44-1.53) | |
| *Total vitamin A intake* | | | | | |
| ≥1,500 µg/d RAE | 10/248 | 1.00 (Ref) | 34/872 | 0.81 (0.27-2.50) | 0.79 |
| <1,500 µg/d RAE | 144/2,114 | 1.00 (Ref) | 34/701 | 0.59 (0.30-1.16) | |

Abbreviations: HR, hazard ratio; CI, confidence interval; p-y, person-years; ref, reference; RAE, Retinol Activity Equivalent.

[1] Adjusted for age, study center, race/ethnicity, education, enrolled as asbestos exposure worker, number of years in high-risk trade, smoking status (for all participants only), smoking pack-years, body mass index, energy intake, total vitamin A intake (except for models stratified by total vitamin A intake), and CARET active intervention (time-dependent covariate with a 3-year extended effect post-intervention; except for models stratified by the CARET active intervention).

[2] Modeled as time-dependent variable with a 3-year extended effect post-intervention.

[3] Wald tests of the cross-product term of total vitamin D intake categories and the CARET active intervention or total vitamin A intake categories (all ordinal variables; 1 df).

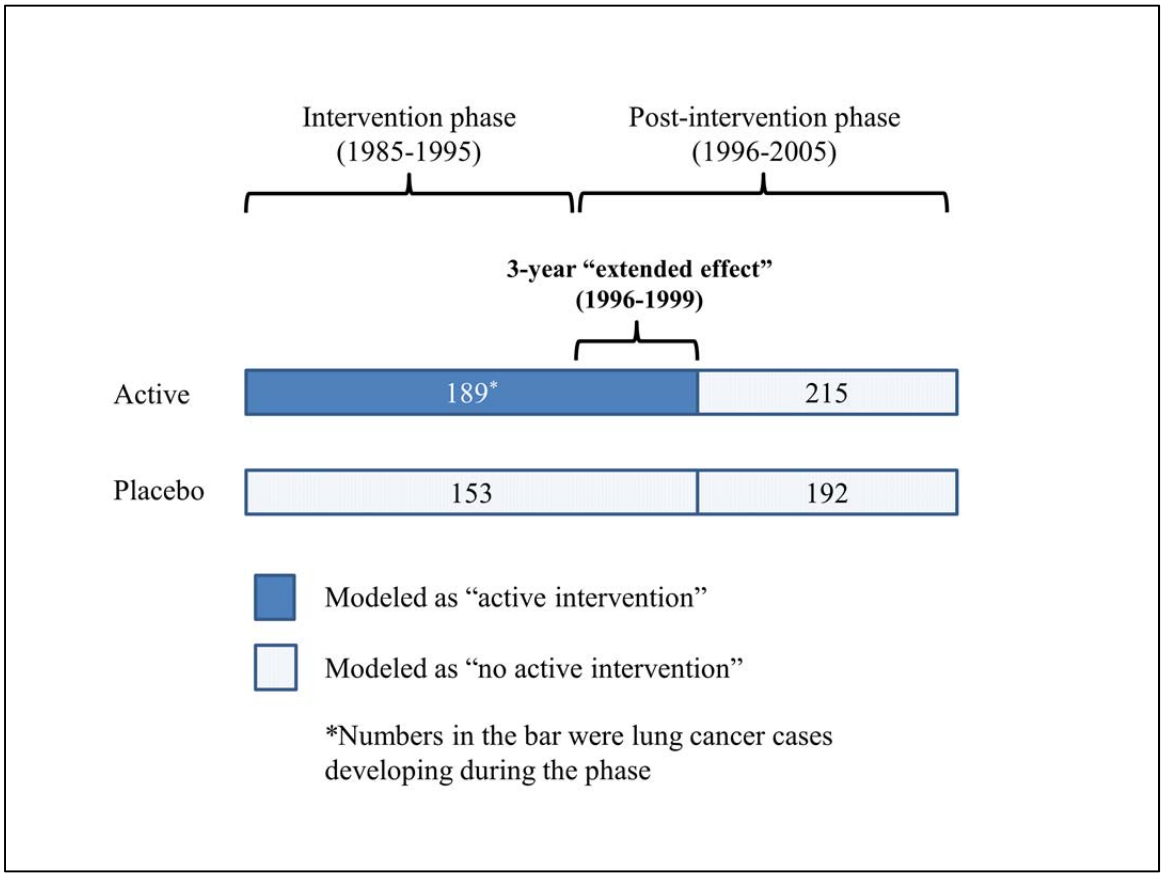


Figure 1. Illustration of modeling the active intervention (30 mg β-carotene plus 25,000 IU retinyl palmitate daily) and placebo during (1985-1995) and after (1996-2005) the Beta-Carotene and Retinol Efficacy Trial (CARET). "CARET active intervention" was modeled as time-dependent covariate with a 3-year extended effect in the post-intervention phase.

# Chapter 4

## Evaluation of determinants of serum 25-hydroxivitamin D concentrations among postmenopausal women

## 4.1 Abstract

**Background:** Postmenopausal women have a lower vitamin D status compared to other age- and gender-groups. Whether conventional influencing factors of vitamin D status remain important in postmenopausal women is unknown. We evaluated both conventional and novel determinants of serum 25-hydroxyvitamin D [25(OH)D] concentrations in a national-wide sample of postmenopausal women.

**Methods:** Data from the Women's Health Initiative Observational Study were analyzed (n=3,347). Questionnaire (diet, lifestyle behaviors, secondhand smoke), inventory (dietary supplements and medication use), and anthropometric data at baseline and sun exposure at year 4 were collected. Linear regression of baseline fasting serum 25(OH)D concentrations (nmol/L) on potential determinants was performed with forward-stepwise selection.

**Results**: Significant determinants were total vitamin D intake (food+ supplements; regression coefficient, β=2.08) and fat intake (β=–0.03), years of supplemental vitamin D use (β=0.15), smoking status (β=–2.64, current versus never), regional solar irradiance (β=6.26, 475–500 versus 300–325 Langleys), time spent outdoors in summer (β=5.15, >2 hours versus <30 minutes/day), recreational physical activity (MET-h/wk, β=0.13), waist circumference (cm, β=–0.26), and race/ethnicity (β=–11.94, black versus white). The model $R^2$ was 0.292. Secondhand smoke exposure was not a determinant. The association between total vitamin D intake and serum 25(OH)D concentrations was stronger among participants who spent <30 minutes (β=2.23) compared to those who spent >2 hours outdoors in summer daytime (β=1.50; *P*-interaction=0.026).

**Conclusion**: Sun exposure and diet remain important determinants of vitamin D status in postmenopausal women. Vitamin D intake should be emphasized for those with less chance to receive sun exposure. Smoking may independently influence vitamin D status.

## 4.2 Introduction

Low vitamin D status has been linked to a wide range of skeletal and extra-skeletal diseases,(1, 2) while approximately one-third of U.S. adults are at risk of vitamin D insufficiency.(3) The human body absorbs dietary vitamin D into chylomicrons in the small intestine when fat intake stimulates bile acids and pancreatic lipase. In addition, skin synthesizes vitamin D from 7-dehydrocholesterol after receiving a sufficient dose of solar ultraviolet B radiation (UVB, 290–315 nm). A fraction of circulating vitamin D is stored by adipose tissue and skeletal muscle. Another fraction of vitamin D is converted to 25-hydroxyvitamin D [25(OH)D] by 25-hydroxylase in the liver.(4, 5) Serum 25(OH)D is currently the standard biomarker of vitamin D status.(1) Dietary vitamin D intake and cutaneous photosynthesis clearly modulate serum 25(OH)D concentrations.(6, 7) However, factors potentially influencing vitamin D metabolism, such as obesity, may also affect serum 25(OH)D concentrations.(8)

Identifying determinants of serum 25(OH)D concentrations has received broad attention. Published reports have identified dietary, demographic, and lifestyle variables as determinants.(9-21) However, few data were from female populations, particularly those who are post-menopausal.(11, 12, 21) To evaluate determinant of serum 25(OH)D concentrations in postmenopausal women is important because among all gender- and age- groups postmenopausal women (≥51 years) has the lowest mean serum 25(OH)D concentrations.(1) Paradoxically, postmenopausal women have a higher level of vitamin D intake from food and supplements compared to younger female populations.(22) Thus,

it is important to know that to what extent vitamin D intake and other dietary factors, such as fat intake, contribute to serum 25(OH)D concentrations after the menopause. Also, since cutaneous photosynthesis decreases with age,(23) it is unknown whether sun exposure remains as an important determinant of serum 25(OH)D concentrations in postmenopausal women. In addition, chronic diseases such as diabetes, hyperlipidemia, and hypertension are common in older, postmenopausal women.(24) The potential influence of these conditions and their pharmacologic treatments on vitamin D status has been postulated,(25, 26) but population-level data are very limited.

The objective of this study is to evaluate both conventional and novel determinants of serum 25(OH)D concentrations in Women's Health Initiative Observational Study (WHI-OS), one of the largest studies of postmenopausal women in the U.S. In particular, we investigate the contribution of dietary intake to serum 25(OH)D concentrations by the degree of sun exposure. In addition, we investigate whether secondhand smoke exposure is associated with serum 25(OH)D concentrations. Biologically, secondhand smoke may affect serum 25(OH)D concentrations among nonsmokers;(27) however, no study has investigated this factor. Findings from this study can provide better understanding on the determinants of serum 25(OH)D concentrations and contribute to developing strategies for preventing vitamin D insufficiency in postmenopausal women.

## 4.3 Methods

### Sample selection

The WHI-OS is a prospective cohort study that enrolled 93,676 women between 1993 and 1998 at 40 U.S. clinical institutions. The study was approved by the human subjects review committees at each of the participating institutions and written informed consent was obtained from each study participant. Women were eligible for the WHI-OS if they were postmenopausal, not participating in any clinical trial, aged 50 to 79 years at the time of enrollment, unlikely to relocate within three years, and unlikely to die from a pre-existing medical condition within three years. Characteristics of WHI-OS participants have been described elsewhere.(28)

The current study included 4,458 participants from 3 ancillary studies (2 nested case-control studies a cohort study) that measured baseline serum 25(OH)D in the WHI-OS (**Supplemental Table 1**).(29-31) We excluded participants with conditions affecting vitamin D metabolism (ulcerative colitis, Crohn's disease, part of intestines removed, high blood calcium, liver diseases, and dialysis for kidney failure; n=852)(32), implausible body mass index (≤15.0 or ≥50.0 kg/m$^2$; n=107), and extreme energy intake (<600 or >5,000 Kcal/d; n=203) estimated from a baseline food frequency questionnaire (FFQ). Consequently, 3,347 participants entered statistical analyses.

### Data collection

At WHI-OS baseline, concurrent with blood draw and anthropometry measurements, data on demographics, diet, medical history, hormone use, physical activity, smoking, and secondhand smoke exposure were assessed by self-administered questionnaires at clinics.(33) Medication and dietary supplements inventory were also conducted during baseline clinical visits. Sunlight exposure variables were assessed by a mailed follow-up questionnaire at year 4.

*Dietary intake.* A food frequency questionnaire (FFQ) developed specifically for the WHI was used to assess usual dietary intake over the previous 3 months. In a subset of WHI participants, correlation coefficients between the FFQ and 8 days of dietary intake (four 24-hr recalls and a 4-day food record) for vitamin D were 0.70.(34) Information on usual use of vitamin and mineral supplements was collected by a simplified inventory system.(35) Participants were asked to bring their supplement bottles to the baseline clinic visit, and trained staff entered doses of vitamins and minerals based on the bottle labels. Only supplements used once per week or more were transcribed. The frequency (pills per week) and duration (months taken last year and total years taken) of use were also queried. Total vitamin D intake was the summation of vitamin D intake from food and supplements. Intake of alcohol, cholesterol and total fat was assessed by the FFQ. Whether participants were currently on low-fat or low-cholesterol diet was queried by a questionnaire regarding lifestyle behaviors.

*Latitude and regional solar irradiance*. Based on the location of the 40 WHI clinic centers that participants attended, regional latitude categories: northern (>40 °N), middle

(>37 °N to 40 °N), and southern (≤37 °N) were assigned. Also, mean annual regional solar irradiance estimates in measures of Langleys (g-cal/$cm^2$; 1 Langley=41.84 KJ/$m^2$) (36) and Watts (J/$m^2$-s) (7) corresponding to each of the WHI clinic centers were assigned for each participant.(37) Langley measures total solar radiation (all wavelengths) as energy distribution over a unit area on the ground; Watt measures UVB flux (i.e., the rate of transfer of energy through a unit area) reaching the ground. The dose-response relationship between solar radiation measured in Langley and serum 25(OH)D concentrations has been established.(38)

*Sun exposure variables*. WHI-OS collected selected baseline variables and additional information through mailed questionnaires each year after baseline up to year 8. Up to two additional mailings and telephone contacts were conducted for non-responders; the response rate was 94%.(39) The Year 4 questionnaire queried skin reaction, i.e., tan or burn, after unprotected sun exposure for 45–60 minutes, average time per day spent outdoors during summer and non-summer daylight hours in the lifetime (childhood, teens, thirties, and this year), and sun protection measures (hat wearing and usual sunscreen use when being outside for more than 10 minutes).

*Smoking and secondhand smoke*. Participants were asked if they had smoked at least 100 cigarettes in their lifetime. Never smokers were defined as those who had not smoked more than 100 cigarettes in their lifetime.(40) Former smokers were defined as those who did not smoke currently but had smoked in the past. Number of cigarettes smoked per day and years as a regular smoker were also queried. Participants were also asked if they ever

or currently lived and worked with a smoker after the age of 18. Numbers of years living and working with a smoker were queried.

*Physical activity.* Recreational physical activity was measured as metabolic equivalent task (MET)-hours per week.(41) In addition, duration of walking was considered as a potential determinant of serum 25(OH)D concentrations because the time walking outdoors can expose to sunlight significantly compared to other activities that might be conducted indoors. Time and MET-hr/week doing yardwork were also calculated and treated as a separate variable.

*Medical history and medication use.* Medical history of osteoporosis, fracture at age 50 or older, hypertension, and type 2 diabetes were self-reported as ever or never having the diseases. Self-reported ever requiring pills for high cholesterol was defined as ever having hyperlipidemia. For medication use, participants brought their current medication to baseline visits. Research staff transcribed the product or genetic name of the medication, prescribed and actual doses, and frequency and duration of use. National Drug Code (NDC) for the medication is assigned based on the Master Drug Database (MDDB®) supplied by the Medi-Span Division of Wolters Kluwer Health. Based on their NDC, we selected the following drug classes that may influence the vitamin D metabolism as potential determinants: steroids/corticosteroids, anti-hypertensive medication, anti-convulsants, hematopoietic agents, anti-hyperlipidemic agents, anti-diabetic medication, and osteoporosis-related medication (bisphosphates and calcitonin).(1, 11, 32)

*Serum 25-hydroxyvitamin D assay*. 12-hour fasting blood samples were collected at baseline visits and stored at –80°C until assay. Serum 25(OH)D concentrations were measured in 3 different laboratories, with 2 using a chemiluminescent immunoassay and 1 using a radioimmunoassay (all DiaSorin, Stillwater, MN). Measurements of 25(OH)D between these two assays are highly correlated (r=0.83–0.91).(42, 43) The coefficient of variation (CV) from blinded duplicates ranged from 13.6%–13.9% (Supplementary Table 1).

**Statistical analysis**

Descriptive analyses were conducted to investigate the distributions of potential determinants by serum 25(OH)D concentration category, with using analysis of variance (ANOVA) and $\chi^2$ tests for continuous and categorical variables, respectively. Further, since month of blood draw and the 3 ancillary studies' case-control and exposure status might be associated with the serum 25(OH)D concentrations, we examined 25(OH)D concentrations by categories of potential determinants with and without adjustment for these 2 factors. The adjustment was performed by residual method.(44) The relationships between determinants of serum 25(OH)D concentrations and smoking status among all participants and secondhand smoke exposure were examined using t-tests. Analyses regarding secondhand smoke exposure were restricted to never smokers to avoid the influence of active smoking.

To obtain a smaller set of independent variables that are mostly important, forward-stepwise selection with *P*-values <0.1 as inclusion and >0.05 as exclusion criteria was performed in a linear regression. Based on the results from descriptive analyses and biological knowledge, we excluded the following variables from the stepwise selection to avoid reverse causality: history of osteoporosis and fractures, sun protection measures (sun screening use and wearing hat outdoors), and use of prescribed vitamin D supplements as medication. For example, because vitamin D deficiency is a risk factor of osteoporosis, women with history of osteoporosis are likely to have lower serum 25(OH)D concentrations. However, in our data, the osteoporotic group had higher serum 25(OH)D concentrations compared to the non-osteoporotic group (60.8 versus 56.4 nmol/L; Supplementary Table 2). This discrepancy was likely due to a higher total vitamin D intake in the osteoporotic group (444 versus 372 IU/d; data not shown) in part because high levels of vitamin D intake might have been advised.(45) Similarly, sunscreen users had higher serum 25(OH)D concentrations compared to nonusers (59.8 versus 54.7 nmol/L; Supplementary Table 2) likely because the users engaged more recreational physical activity (15.8 versus 11.3 MET-h/wk; data not shown) compared to the nonusers. We excluded participants who used prescribed vitamin D supplements as medication (n=2) from regression analyses because their underlying serum 25(OH)D concentrations were likely very low. In addition, we excluded the following variables from stepwise regression because they were not associated with serum 25(OH)D concentrations in descriptive analyses: years as regular smoked, number of cigarettes smoked per day, time spent outdoors in non-summer time in the age of 30–40 years, and living or working with smokers after age 18 (ever/never status and number of years;

*P*>0.05; Supplementary Table 2). Factors that were established or potentially important determinants (age, race/ethnicity, smoking status)(16, 46, 47) and resulted from study design and protocol (month of blood draw and study case-control and exposure status) were forced to enter the stepwise regression model. Waist circumference and body mass index, but not waist-to-hip ratio and body weight, were selected by stepwise regression; we only entered waist circumference in the final model because it had a higher partial $R^2$ compared to body mass index. After the stepwise procedure, regression coefficients (β) for the selected variables and model $R^2$ were re-estimated by a robust linear regression to reduce potential influence of outliers and high leverage data points. Robust regression assigns lower weights to deviant cases (e.g., weight=0 for data point with Cook's distance >1).(48) In addition, since the relationship between total vitamin D intake and serum 25(OH)D concentrations is likely curvilinear,(1) total vitamin D intake modeled in the logarithmic scale was investigated. Because the model $R^2$ did not materially changed, results from total vitamin D intake in the linear scale were presented for the ease of interpretation. A separate forward-stepwise selection model was performed among never smokers to investigate secondhand smoke exposure variables, which have a larger effect among never smokers. As a sensitivity analysis, β coefficients were estimated among participants who were not fracture cases in the 2 nested case-control studies (Supplementary Table 1) to avoid potential influence of lower serum 25(OH)D concentrations due to the case selections.

To investigate the potential influence of sun exposure on the association of total vitamin D intake with serum 25(OH)D concentrations, β coefficients of total vitamin D

intake were estimated for each category of sun exposure related variables—time spent outdoors this summer, regional solar irradiance, and season of blood draw. The difference in β across the categories was tested by including a cross-product term of total vitamin D intake and a sun exposure variable; the *P* value of Wald test for the product term was regarded as evidence of statistical interactions. All statistical analyses were conducted using SAS (version 9.3, Cary, NC).

### 4.4 Results

**Table 1** shows the distributions of selected baseline characteristics by 25(OH)D concentration category. Serum 25(OH)D concentrations were associated with demographic (age, race, and education), anthropometric (body mass index and waist circumference), behavioral (recreational physical activity levels and cigarette smoking), and dietary factors (total vitamin D intake, during of supplemental vitamin D use, low-fat or low-cholesterol diet, total fat intake, and alcohol consumption). The proportion of secondhand smoke exposure at home was higher among never smokers with either lower (<25 nmol/L) or higher (≥100 nmol/L) serum 25(OH)D concentrations, compared to that among those with serum 25(OH)D concentrations in the middle range. Having history of hypertension, hyperlipidemia, and diabetes and use of the respective medication were associated with lower serum 25(OH)D concentrations, but current use of hormone therapy was associated with higher serum 25(OH)D concentrations. Serum 25(OH)D concentrations were also associated with factors related to skin vitamin D photosynthesis: regional solar irradiance of clinical centers, skin reaction to sun, time spent outdoors

during summer daytime this year, wearing hat outdoors this year, and sunscreen use. **Supplemental Table 2** shows mean serum 25(OH)D concentrations by potential determinants. Generally, the values adjusted for month of blood draw and study status were similar compared to the unadjusted values.

**Table 2** shows the relationships between smoking variables, including active smoking and secondhand smoke exposure, and potentially important determinants of serum 25(OH)D concentrations. Current smokers had significant lower levels of total vitamin D intake and recreational physical activity compared to never smokers. Among never smokers, those who was currently living with a smoker had lower total vitamin D intake and larger waist circumferences compared to those who was not living with a smoker.

**Table 3** presents the final model of serum 25(OH)D concentrations with predictors selected by the forward stepwise procedure. Black or African-Americans had on average 12 nmol/L lower serum 25(OH)D concentrations compared to Caucasians. Current versus never smoking was associated with 2.64 nmol/L lower serum 25(OH)D concentrations. Every increment of 100 IU/d of total vitamin D intake was associated with 2.08 nmol/L higher serum 25(OH)D concentrations. The dose-response relationship is shown in Figure 1. Other dietary factors associated with serum 25(OH)D concentrations were duration of supplemental vitamin D use ($\beta$=0.15 per year of use) and total fat consumption ($\beta$=–0.03 per g/d intake). A unit (centimeter) higher of waist circumference was associated with 0.26 nmol/L lower serum 25(OH)D concentrations.

Conversely, recreational physical activity levels were positively associated with serum (OH)D concentrations (β=0.13 per MET-h/wk). Sun exposure related variables—time spent outdoors in summer daytime this year (β=5.15, >2 hours versus <30 minutes) and regional solar irradiance (β=6.26, 475–500 versus 300–350 Langleys) were also significantly associated with serum 25(OH)D concentrations. All variables in the final model explained 29.2% of variability of 25(OH)D concentrations between participants. In the sensitivity analysis among participants who were not fracture cases, the β coefficients did not change materially (**Supplemental Table 3**). Secondhand smoke exposure variables were not selected in the final model either among all participants or among never smokers (data not shown).

**Table 4** shows the total vitamin D intake-25(OH)D association stratified by sun exposure related variables. Every 100 IU of total vitamin D intake was associated with higher concentrations of serum 25(OH)D among participants spending less time outdoors this summer (β=2.23 versus 1.50), residing in a region with less solar irradiance (β=2.15 versus 1.76), and having their blood draw testing for vitamin D in winter/springs (β=2.36 versus 1.83), compared to their counterparts. The difference in β coefficient by time spent outdoors this summer daytime was statistically significant (*P*-value for interaction=0.026).

## 4.5 Discussion

Among this nation-wide sample of postmenopausal women, we confirmed that vitamin D intake and factors related to skin vitamin D photosynthesis were associated with serum 25(OH)D concentrations. We also identified cigarette smoking and total fat intake as important determinants. However, medical history, medication use, and secondhand smoke exposure were not significant determinants of serum 25(OH)D concentrations. The WHI-OS collected detailed information on a wide range of variables including secondhand smoke and sun exposure that are not always available in large population studies. This study improves the model $R^2$ (0.29) compared to a previous model ($R^2$=0.21) developed in the WHI Calcium/Vitamin D trial (11) in part because we are able to additionally include sun exposure variables, which were not collected in the trial.

To our knowledge, our study is the first to investigate the association between secondhand smoke exposure and serum 25(OH)D concentrations. In the descriptive analysis we observed a potential U-shaped relation between secondhand smoke exposure at home and serum 25(OH)D concentrations among never smokers. However, no significant associations were observed in the multivariate analysis. The influence of secondhand smoke on serum 25(OH)D concentrations may not be as strong as active smoking. Future studies should use tools with a higher sensitivity such as serum or hair cotinine compared to self-report to measure secondhand smoke exposure.(49)

Smoking influences vitamin D status through several mechanisms. First, from our data and other cross-sectional observations,(46, 50, 51) current smokers were more likely to consume less total vitamin D and engage lower levels of recreational physical activity, which is associated with sun exposure, compared to never smokers. Second, smokers tend to have lower body weight and waist circumference,(52) which are associated with higher serum 25(OH)D concentrations. Our analysis was able to control these variables, suggesting that smoking may have independently influence on serum 25(OH)D concentrations. Research has shown that smoking may decrease the expression of CYP2R1, the enzyme synthesizing 25(OH)D.(27) Also, smoking-produced carcinogen benzo[a]pyrene enhances the activity of CYP24A1,(53) which degrades 25(OH)D. Both actions can decrease serum 25(OH)D concentrations.

Whether diabetes, hypertension, and hyperlipidemia and their medication use are associated with lower vitamin D status remains uncertain. Cross-sectional studies have shown that diabetes is associated with lower serum 25(OH)D concentrations.(16, 54, 55) However, these studies did not consider sun exposure variables. In our multivariate analysis including the adjustment for sun exposure variables, both self-reported disease history and medication use of diabetes were not associated with serum 25(OH)D concentrations. Therefore, the influence of these disease factors on serum 25(OH)D concentrations may not be as strong as non-disease factors. In addition, a large portion of variation in serum 25(OH)D concentrations between diabetic and non-diabetic participants may be explained by non-disease factors. For example, recreational physical activity levels (9.2 versus 13.4 MET-hr/wk; $P<0.001$) and total vitamin D intake (304

versus 381 IU/d; $P<0.001$) were significantly lower among participants with history of diabetes compared to those without diabetes history in our study population. We also observed similar patterns for hypertension and hyperlipidemia. However, our findings are limited by the cross-sectional design, while prospective studies with long-term follow up have showed that lower vitamin D status is associated with a higher diabetes risk.(56, 57)

Measuring sun exposure and its contribution to vitamin D status is a difficult task. A questionnaire of personal sun exposure can include time of day, latitudes of residence, season, terrestrial features (e.g., tree cover and over water), and personal sun protection behaviors (wearing hats, clothing, and sunscreen use).(58) However, the correlation between personal report of sun exposure and UV radiation assessed by personal dosimetry is relatively low.(58) Thus, although many factors can be inquired through questionnaires, measurement errors remain large and recall bias may be substantial.(59) In addition, modulating factors including blood cholesterol and baseline serum 25(OH)D concentrations are not readily assessable by questionnaires.(60) WHI's sun exposure questionnaire was not validated by personal dosimetry; however, the duration of sun exposure and skin reaction to the sun assessed by the questionnaire were able to predict the risk of cutaneous melanoma,(61) a condition highly related to sun exposure, in the WHI-OS. Other studies have also supported that the duration of sun exposure specifically in summer is an important determinants of serum 25(OH)D concentrations.(62, 63)

Our estimation of total vitamin D intake associated with serum 25(OH)D concentrations (2.08 nmol/L per 100 IU) is very similar to that from a randomized trial of

vitamin D supplementation in postmenopausal women (approximately 2 nmol/L per 100 IU until 2,400 IU).(6) The estimates are lower than the "pure effect" of vitamin D intake without influence from sun exposure (5.8 per 100 IU in Antarctica men during the winter season). In addition, the relationship between vitamin D intake and serum 25(OH)D is likely curvilinear. In our study population, the β coefficient of log total vitamin D intake was 6.36 (SE=0.37, P<0.001; data not shown). The corresponding β coefficient is 10.9 in adults aged >71 years living in northern latitudes in Europe and Antarctica during their respective winter seasons.(1) These observations and our stratified analyses by sun exposure variables suggest that the association of total vitamin D intake with serum 25(OH)D concentrations is stronger, i.e., a higher β coefficient, as the influence of skin vitamin D photosynthesis decreases. This has important implication that people with less sun exposure due to sedentary lifestyle or living in high latitude regions may largely benefit from vitamin D intake from food and supplements in maintaining sufficient vitamin D status. It is noteworthy that higher vitamin D intake among postmenopausal women heavily relies on dietary supplements. An analysis of all WHI participants showed that among those with total vitamin D intake >400 IU/d, over 90% used vitamin D supplements.(64)

We have acknowledged several limitations of this study. First, WHI-OS did not comprehensively collect all factors that influence serum 25(OH)D concentrations, such as genetic variants in *CYP2R1* and *CYP24A1*.(65, 66) Second, the variables related to sun exposure were measured on average 4 years after the baseline blood draw. Women might have changed their sun exposure behavior during this period. Third, our observations

were based on cross-sectional design; causality cannot be inferred. Lastly, observations from the three ancillary studies might not be generalizable to a large population of all WHI-OS participants. Although the samples of two studies investigating fracture risks were drawn based on all WHI-OS participants,(30, 31) the study investigating eye health recruited WHI-OS participants from 3 clinical centers in relatively higher latitudes (Madison, WI [43 °N], Iowa City, IA [42 °N], and Portland, OR [46 °N]).(29) Therefore, the generalizability of our study findings might have been affected.

In conclusion, several modifiable factors including smoking are identified to be associated with serum 25(OH)D concentrations among postmenopausal women. Vitamin D intake from food and supplements is an important determinant of serum 25(OH)D concentrations, particularly for postmenopausal women who spent 2 hours or less outdoors in summer daytime.

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Table 1. Distribution of baseline characteristics by serum 25-hydroxyvitamin D [25(OH)D] category: the Women's Health Initiative Observational Study (n=3,347)[1]

| Characteristic | Serum 25(OH)D concentrations (nmol/L) <25 (n=235) | 25 to <50 (n=1,170) | 50 to <75 (n=1,322) | 75 to <100 (n=467) | ≥100 (n=151) | *P*-value[3] |
|---|---|---|---|---|---|---|
| ***Demographics*** | | | | | | |
| Age (y) | 62.9 (7.4)[2] | 64.8 (7.5) | 65.1 (7.8) | 64.9 (7.7) | 63.6 (7.7) | <0.001 |
| Black or African-American (%) | 40.4 | 22.1 | 11.2 | 6.6 | 7.3 | <0.001 |
| College or higher degree (%) | 31.2 | 34.5 | 38.5 | 39.1 | 43.6 | 0.027 |
| ***Anthropometry*** | | | | | | |
| Body mass index (kg/m$^2$) | 30.1 (6.4) | 28.9 (5.8) | 27.0 (5.1) | 25.8 (4.7) | 25.1 (4.9) | <0.001 |
| Waist circumference (cm) | 91.2 (15.7) | 88.4 (13.8) | 83.9 (12.2) | 80.9 (11.4) | 78.7 (11.6) | <0.001 |
| ***Healthy behaviors*** | | | | | | |
| Recreational physical activity (MET-h/wk)[4] | 8.6 (11.0) | 11.0 (12.6) | 13.8 (13.8) | 16.0 (15.1) | 19.6 (17.5) | <0.001 |
| Current smoker (%) | 11.3 | 7.0 | 4.4 | 4.6 | 6.8 | 0.004 |
| ***Diet*** | | | | | | |
| Total vitamin D intake (IU/d) | 196.0 (194.5) | 305.5 (244.5) | 415.5 (304.4) | 479.9 (305.5) | 514.7 (350.3) | <0.001 |
| Duration of supplemental vitamin D use (y) | 1.1 (4.4) | 2.8 (6.9) | 4.9 (8.4) | 5.8 (9.6) | 7.4 (11.8) | <0.001 |
| Low-fat or low cholesterol diet (%) | 36.8 | 43.4 | 50.0 | 49.3 | 46.3 | <0.001 |
| Dietary total fat (g/d) | 59.3 (34.2) | 56.5 (32.8) | 53.3 (29.2) | 54.0 (31.2) | 49.2 (26.3) | 0.003 |
| Alcohol intake (%) | 19.8 | 28.2 | 34.9 | 36.6 | 41.1 | <0.001 |
| ***Secondhand smoke exposure*** | | | | | | |
| Currently living with a smoker (%)[5] | 8.5 | 6.1 | 5.0 | 4.1 | 7.1 | 0.38 |
| Currently working with a smoker (%)[5] | 3.1 | 3.0 | 2.0 | 1.9 | 1.2 | 0.64 |
| ***Disease history /medication use*** | | | | | | |
| History of osteoporosis (%) | 6.0 | 5.2 | 6.1 | 8.6 | 11.3 | 0.012 |
| History of hypertension (%) | 49.4 | 40.1 | 32.9 | 31.7 | 27.2 | <0.001 |
| Anti-hypertensive medication use (%) | 19.6 | 11.8 | 10.7 | 9.0 | 10.6 | 0.001 |
| History of hyperlipidemia (%) | 14.9 | 16.3 | 14.0 | 13.2 | 9.5 | 0.15 |
| Anti-hyperlipidemic agents use (%) | 9.8 | 8.8 | 8.3 | 7.7 | 6.6 | 0.78 |
| History of type 2 diabetes (%) | 13.2 | 9.2 | 6.4 | 4.7 | 4.6 | <0.001 |
| Anti-diabetic medication use (%) | 11.5 | 6.5 | 3.7 | 3.2 | 2.7 | <0.001 |
| Current hormone therapy use (%) | 14.5 | 17.5 | 19.5 | 24.4 | 21.2 | 0.010 |

| | | | | | | |
|---|---|---|---|---|---|---|
| ***Sun exposure*** | | | | | | |
| Regional solar irradiance of clinical centers ≥475 Langleys (%) | 10.2 | 14.4 | 17.9 | 20.8 | 21.9 | <0.001 |
| Skin reaction to sun as burns, then no or minimal tans (%)[6] | 26.0 | 28.3 | 30.1 | 31.0 | 22.1 | <0.001 |
| Spent outdoors in summer this year >2 hours (%) | 16.2 | 17.1 | 21.6 | 25.0 | 24.1 | <0.001 |
| Wear hat outdoors this year (%) | 45.5 | 44.9 | 49.0 | 48.7 | 55.8 | 0.16 |
| Usually use sunscreen outside (%) | 26.9 | 38.6 | 47.7 | 50.6 | 51.1 | <0.001 |

[1] A sample of participants from 3 ancillary studies providing baseline serum 25(OH)D measurements.
[2] Numbers are mean (standard deviation) for continuous variables or percentages for categorical variables within each of the serum 25(OH)D concentration category.
[3] Analysis of variance (ANOVA) for continuous variables and $\chi^2$ test for all categories of a categorical variable. See Supplementary Table 2 for detailed information on categories.
[4] Recreational physical activities included walking, mild, moderate, and strenuous exercise.
[5] Among never smokers.
[6] Skin phototypes (I & II) that achieve maximal vitamin D photosynthesis more rapidly compared to other phototypes. See reference (67).

Table 2. Relationship between smoking status and secondhand smoke exposure and potential determinants of serum 25-hydroxyvitamin D concentrations

| | | Total vitamin D intake (IU/d) | Recreational physical activity (MET-h/wk) | Time spent outdoor in summer this year >2 hours | Waist circumference (cm) |
|---|---|---|---|---|---|
| | N[1] | mean | mean | % | mean |
| **Smoking status** | | | | | |
| Current | 196 | 316.9 | 9.4 | 25 | 85 |
| Former | 1,227 | 374.0 | 14.1 | 20 | 86 |
| Never | 1,881 | 382.6 | 12.8 | 20 | 84 |
| P-value (current vs. never) | | 0.002 | <0.001 | 0.47 | 0.39 |
| **Currently living with a smoker**[1] | | | | | |
| Yes | 105 | 309.4 | 11.4 | 23% | 88 |
| No | 1,776 | 386.9 | 12.9 | 20% | 84 |
| P-value | | 0.001 | 0.28 | 0.69 | 0.007 |
| **Currently working with a smokers**[2] | | | | | |
| Yes | 44 | 389.6 | 13.1 | 21 | 87 |
| No | 1,837 | 382.4 | 12.8 | 20 | 85 |
| P-value | | 0.87 | 0.88 | 0.45 | 0.27 |

[1] Number of participants did not add up due to missingness.
[2] Among never smokers.

Table 3. Final model of determinants of serum 25-hydroxyvitamin D

| Independent variable[1] | β (SE)[2] | *P* value |
|---|---|---|
| **Age** | –0.08 (0.04) | 0.05 |
| **Race/ethnicity** | | |
| White (not of Hispanic origin) | Reference | |
| Am. Indian/Alaskan Native | –8.49 (2.38) | <0.001 |
| Asian/Pacific Islander | –3.22 (1.51) | 0.033 |
| Black or African-Am. | –11.94 (1.05) | <0.001 |
| Hispanic/Latino | –7.67 (1.30) | <0.001 |
| Other/unknown | –3.18 (5.39) | 0.55 |
| **Smoking** | | |
| Never smokers | Reference | |
| Former smokers | 0.84 (0.64) | 0.19 |
| Current smokers | –2.64 (1.32) | 0.045 |
| **Total vitamin D intake (per 100 IU/d)** | 2.08 (0.12) | <0.001 |
| **Years of supplemental vitamin D use** | 0.15 (0.04) | <0.001 |
| **Dietary fat intake (g/d)** | –0.03 (0.01) | 0.043 |
| **Waist circumference (cm)** | –0.26 (0.02) | <0.001 |
| **Recreational physical activity (MET-h/wk)** | 0.13 (0.02) | <0.001 |
| **Time spent outdoors in summer daytime this year** | | |
| <30 minutes | Reference | |
| 30 minutes – 2 hours | 3.07 (0.76) | <0.001 |
| >2 hours | 5.15 (1.01) | <0.001 |
| **Solar irradiance (Langleys)** | | |
| 300–325 | Reference | |
| 350 | –0.18 (0.78) | 0.82 |
| 375–380 | 0.71 (1.34) | 0.60 |
| 400–430 | 3.16 (1.15) | 0.006 |
| 475–500 | 6.26 (1.09) | <0.001 |
| Model $R^2$ =0.292 | | |

SE: standard error

[1] Obtained during the forward-selection process (n=2,766). Month of blood draw, study case-control and exposure status, age, race/ethnicity, and smoking status were forced in the model.

[2] Adjusted for month of blood draw and study case-control and exposure status. Re-estimated using robust regression after the forward-selection process (n=3,270). The intercept was 67.65.

Table 4. Regression coefficient (β) for total vitamin D intake from food and supplements on serum 25-hydroxyvitamin (nmol/L) stratified by variables related to skin vitamin D photosynthesis

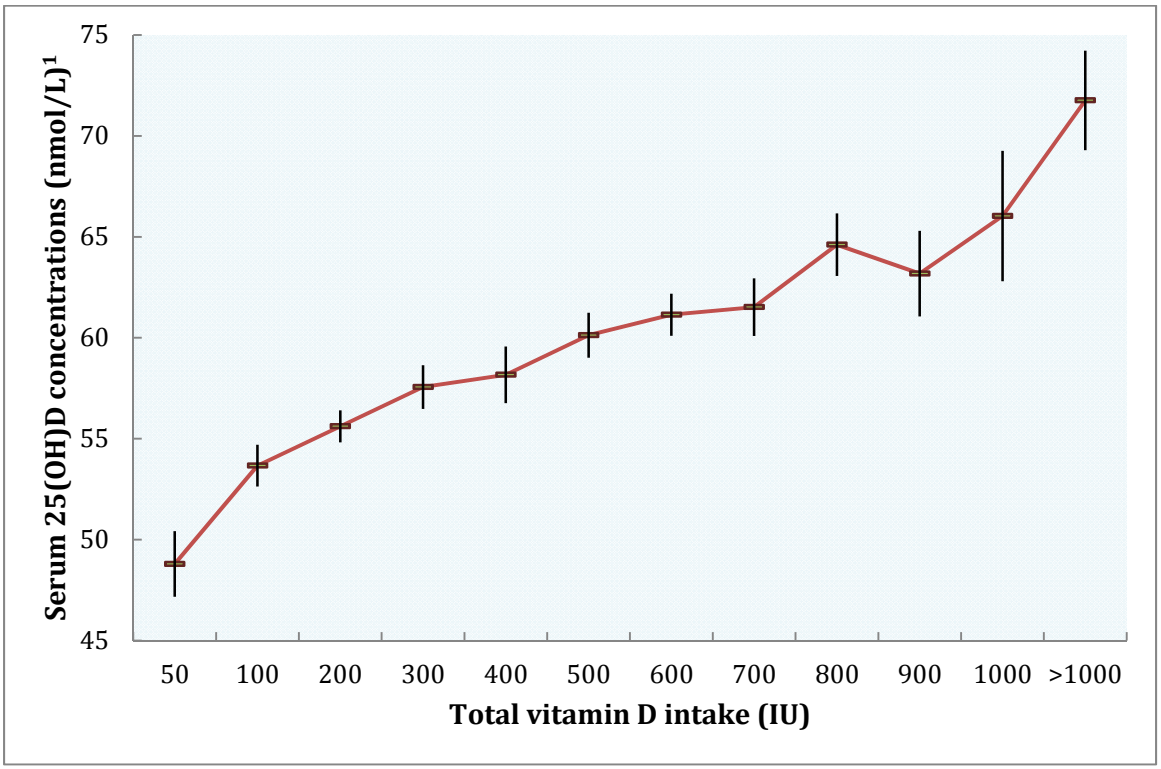
| Sun exposure variables | N[2] | β (SE) of total vitamin D intake per 100 IU[3] | *P* value |
|---|---|---|---|
| **Time spent outdoors this summer** | | | |
| <30 minutes | 891 | 2.23 (0.22) | <0.001 |
| 30 minutes – 2 hours | 1,432 | 2.23 (0.20) | <0.001 |
| >2 hours | 591 | 1.50 (0.28) | <0.001 |
| | | *P*-interaction | 0.026 |
| **Regional solar irradiance (Langleys)** | | | |
| 300–380 | 2,378 | 2.15 (0.14) | <0.001 |
| 400–500 | 909 | 1.76 (0.24) | <0.001 |
| | | *P*-interaction | 0.08 |
| **Season of blood draw[1]** | | | |
| Winter/spring | 1,471 | 2.36 (0.18) | <0.001 |
| Summer/fall | 1,816 | 1.83 (0.17) | <0.001 |
| | | *P*-interaction | 0.15 |

N: number of participants; SE: standard error

[1] Winter/spring: December–May; summer/fall: June–November.

[2] Number of participants did not add up due to missingness.

[3] Adjusted for the variables in the final model shown in Table 3 except for the stratified variable.




Note: Vertical bars indicate standard errors.
[1] Adjusted for month of blood draw, study case-control and exposure status, age, race/ethnicity, smoking status, waist circumference (cm), solar irradiance of study center (Langley), recreational physical activity (MET-h/wk), time spent outdoors this summer daytimes using residual method with robust regression.

Figure 1. Mean serum 25-hydroxyvitamin D [25(OH)D] concentrations in every 100 IU of total vitamin D intake from foods and supplements: the Women's Health Initiative Observational Study (n=3,270)

Supplemental Table 1. Studies nested in the Women's Health Initiative Observation Study providing baseline serum 25-hydroxyvitamin D measurements

| Study | Participants | Serum 25(OH)D assay | No. participants with 25(OH)D available | Serum 25(OH)D concentrations, Mean (SD), nmol/L | Intra-assay coefficient of variation (%)[1] |
|---|---|---|---|---|---|
| Carotenoids in Age-Related Eye Disease Study (CAREDS) [Ref.: (29)] | 1,313 women with intake of lutein plus zeaxanthin >75$^{th}$ or <25$^{th}$ percentiles in 3 WHI centers | Chemiluminescence, LIAISON DiaSorin | 1,475 | 57.6 (23.7) | 13.6 |
| Estradiol, cytokines, and bone turnover: effects on hip fracture[2] [Ref.: (30)] | 400 hip fracture cases and 400 matched controls | Radioimmunoassay (RIA), DiaSorin | 799 | 57.8 (19.3) | 13.7 |
| Biochemical antecedents of fracture in minority women[2] [Ref.: (31)] | 1,132 total fracture cases and 1,132 matched controls | Radioimmunoassay (RIA), DiaSorin | 2,260 | 53.6 (25.0) | 13.9 |

[1] Duplicated samples (the number was approximately 5% of the tested samples) were randomly inserted in analytical batches. The study investigators and lab personals were blinded for the quality control process.

[2] There were 76 women included in both fracture studies. Their serum 25(OH)D concentrations were averaged. After accounting for the overlapping, a total of 4,458 individuals entered statistical analyses.

Supplemental Table 2. Mean serum 25-hydroxyvitamin D concentrations by potential predictors (n=3,347)

| | | Serum 25(OH)D concentrations, mean (SE), nmol/L | |
|---|---|---|---|
| Characteristic | No. of participants[2] | Unadjusted | Adjusted for month of blood draw and study case-control/exposure status[3] |
| Age at baseline screening (y) | | | |
| 50–54 | 394 | 56.2 (1.26) | 56.4 (1.24) |
| 55–59 | 501 | 57.0 (1.04) | 57.1 (1.03) |
| 60–64 | 721 | 54.7 (0.91) | 54.8 (0.90) |
| 65–69 | 696 | 57.4 (0.94) | 57.5 (0.93) |
| 70–74 | 655 | 57.5 (0.86) | 57.3 (0.85) |
| 75–79 | 380 | 56.7 (1.12) | 56.3 (1.11) |
| *P*-value (ANOVA) | | 0.23 | 0.30 |
| | | | |
| Race/ethnicity | | | |
| American Indian or Alaskan Native | 59 | 52.8 (3.28) | 53.9 (3.29) |
| Asian or Pacific Islander | 193 | 63.0 (1.85) | 64.3 (1.85) |
| Black or African-American | 544 | 45.3 (0.96) | 46.8 (0.97) |
| Hispanic/Latino | 293 | 53.0 (1.16) | 54.5 (1.16) |
| White (not of Hispanic origin) | 2,248 | 59.3 (0.49) | 58.6 (0.49) |
| Other/unknown | 10 | 55.2 (5.29) | 56.2 (5.55) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Latitude of clinic center | | | |
| Northern (>40 ºN) | 2,110 | 56.1 (0.52) | 55.6 (0.51) |
| Middle (>37–40 ºN) | 385 | 56.4 (1.20) | 57.3 (1.19) |
| Southern (≤37 ºN) | 852 | 57.8 (0.80) | 58.7 (0.80) |
| *P*-value | | 0.22 | 0.004 |
| | | | |
| Regional solar irradiation of clinic center (Langleys, g-cal/cm$^2$) | | | |
| 300–325 | 873 | 58.2 (0.80) | 57.6 (0.79) |
| 350 | 1,301 | 54.8 (0.65) | 54.4 (0.64) |
| 375–380 | 236 | 51.6 (1.42) | 52.8 (1.40) |
| 400–430 | 377 | 55.6 (1.27) | 56.3 (1.27) |
| 475–500 | 560 | 60.7 (0.97) | 61.7 (0.96) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Regional solar irradiation of clinic center [Watts, (J/s)/m$^2$] | | | |
| 0.4–0.5 | 633 | 52.4 (0.92) | 53.1 (0.90) |
| 0.7 | 1,166 | 57.3 (0.69) | 56.6 (0.68) |
| 1.0 | 665 | 56.3 (0.92) | 55.7 (0.92) |
| 1.4 | 468 | 57.5 (1.13) | 58.1 (1.13) |
| 1.5–1.9 | 415 | 60.2 (1.09) | 61.2 (1.08) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Education | | | |
| 0–8 y | 107 | 52.1 (1.98) | 53.7 (2.00) |
| Some high school | 142 | 54.2 (2.09) | 54.8 (2.06) |
| High school diploma/GED | 619 | 55.7 (1.00) | 56.0 (1.01) |
| School after high school | 1230 | 55.9 (0.65) | 55.9 (0.64) |

| | | | |
|---|---|---|---|
| College degree or higher | 1227 | 58.3 (0.67) | 57.9 (0.67) |
| *P*-value | | 0.009 | 0.10 |
| | | | |
| Weight (kg)[1] | | | |
| Quintile 1: 34.5–59.0 | 674 | 62.5 (0.95) | 62.4 (0.95) |
| Quintile 2: 59.1–66.2 | 668 | 62.1 (0.95) | 61.9 (0.95) |
| Quintile 3: 66.3–73.2 | 673 | 57.7 (0.86) | 57.4 (0.86) |
| Quintile 4: 73.3–83.3 | 666 | 52.0 (0.85) | 52.1 (0.84) |
| Quintile 5: 83.5–143 | 666 | 48.5 (0.82) | 49.0 (0.80) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Waist circumference (cm) | | | |
| Quintile 1: 45.0–74.0 | 724 | 64.0 (0.95) | 63.5 (0.94) |
| Quintile 2: 74.1–80.5 | 619 | 60.8 (0.96) | 60.7 (0.96) |
| Quintile 3: 80.6–87.0 | 690 | 56.8 (0.85) | 56.7 (0.85) |
| Quintile 4: 87.2–96.0 | 662 | 53.4 (0.86) | 53.6 (0.85) |
| Quintile 5: 96.1–194.2 | 647 | 47.3 (0.79) | 47.7 (0.77) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Waist-to-hip ratio[1] | | | |
| Quintile 1: 0.48–0.75 | 668 | 61.4 (0.98) | 60.9 (0.98) |
| Quintile 2: 0.76–0.78 | 670 | 58.3 (0.87) | 58.0 (0.86) |
| Quintile 3: 0.79–0.82 | 667 | 57.8 (0.93) | 57.8 (0.93) |
| Quintile 4: 0.83–0.87 | 667 | 53.4 (0.87) | 53.7 (0.86) |
| Quintile 5: 0.88–2.55 | 667 | 51.9 (0.87) | 52.4 (0.86) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Body mass index (kg/m$^2$)[1] | | | |
| Underweight (< 18.5) | 46 | 68.7 (4.03) | 68.3 (4.02) |
| Normal (18.5 - 24.9) | 1,146 | 62.8 (0.72) | 62.5 (0.72) |
| Overweight (25.0 - 29.9) | 1,214 | 56.1 (0.64) | 56.1 (0.64) |
| Obesity I (30.0 - 34.9) | 594 | 51.2 (0.90) | 51.3 (0.89) |
| Obesity II (35.0 - 39.9) | 231 | 45.8 (1.23) | 46.5 (1.22) |
| Extreme Obesity III (≥ 40) | 116 | 44.3 (1.94) | 45.3 (1.88) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Total vitamin D intake (IU/d) | | | |
| <100 | 604 | 47.0 (0.93) | 47.7 (0.92) |
| 100–199 | 676 | 51.2 (0.86) | 51.4 (0.85) |
| 200–399 | 600 | 55.3 (0.92) | 55.1 (0.91) |
| 400–599 | 786 | 60.6 (0.79) | 60.8 (0.79) |
| 600–799 | 445 | 65.1 (1.09) | 64.2 (1.09) |
| >=800 | 236 | 70.0 (1.53) | 69.2 (1.52) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Duration of supplemental vitamin D use (y) | | | |
| 0 (non-users) | 1,746 | 51.2 (0.55) | 51.5 (0.54) |
| ≤1.5 | 264 | 56.6 (1.36) | 56.5 (1.36) |
| 1.6-6 | 748 | 61.9 (0.84) | 61.8 (0.84) |
| ≥7 | 598 | 65.7 (0.92) | 65.1 (0.93) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Total time spent walking per wk[4] | | | |

| | | | |
|---|---|---|---|
| 0 h | 650 | 53.2 (0.91) | 53.5 (0.90) |
| <1 h | 1,081 | 54.5 (0.72) | 54.6 (0.71) |
| 1 to <2 h | 529 | 57.4 (0.94) | 57.1 (0.92) |
| ≥2 h | 1,087 | 60.2 (0.74) | 60.0 (0.74) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Recreational physical activity (MET-h/wk)[5] | | | |
| Quintile 1: 0–1.5 | 692 | 51.0 (0.85) | 51.4 (0.84) |
| Quintile 2: 1.6–6.3 | 636 | 53.4 (0.95) | 53.8 (0.95) |
| Quintile 3: 6.4–12.5 | 674 | 56.4 (0.87) | 56.2 (0.86) |
| Quintile 4: 12.6–22.0 | 634 | 58.2 (0.86) | 58.0 (0.84) |
| Quintile 5: 22.1–121.3 | 658 | 64.1 (0.98) | 63.7 (0.99) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Smoking status | | | |
| Never smokers | 1,882 | 57.0 (0.55) | 56.9 (0.54) |
| Past smokers | 1,227 | 56.8 (0.65) | 56.8 (0.645) |
| Current smokers | 197 | 51.6 (1.78) | 52.2 (1.75) |
| Missing | 41 | - | - |
| *P*-value | | 0.009 | 0.026 |
| | | | |
| Years as a regular smoker[1] | | | |
| 0 (never smokers) | 1,882 | 57.0 (0.55) | 56.9 (0.54) |
| <5 | 243 | 57.0 (1.47) | 57.1 (1.46) |
| 5-9 | 119 | 57.4 (2.13) | 57.1 (2.14) |
| 10-19 | 311 | 56.1 (1.36) | 56.1 (1.36) |
| 20-29 | 303 | 55.6 (1.29) | 55.6 (1.27) |
| 30-39 | 214 | 54.4 (1.44) | 54.8 (1.41) |
| 40-49 | 152 | 56.1 (2.04) | 56.4 (2.01) |
| ≥50 | 53 | 53.8 (3.53) | 54.2 (3.55) |
| *P*-value | | 0.78 | 0.89 |
| | | | |
| Cigarettes/day smoke or smoked[1] | | | |
| 0 (never smokers) | 1,882 | 57.0 (0.55) | 56.9 (0.54) |
| <1 | 85 | 57.4 (2.50) | 57.2 (2.48) |
| 1-4 | 289 | 56.3 (1.46) | 56.6 (1.44) |
| 5-14 | 470 | 54.9 (1.01) | 54.8 (1.00) |
| 15-24 | 364 | 57.4 (1.25) | 57.5 (1.25) |
| 25-34 | 97 | 55.2 (2.31) | 55.0 (2.33) |
| 35-44 | 56 | 52.2 (2.92) | 53.7 (2.84) |
| ≥45 | 24 | 55.3 (4.79) | 55.1 (4.49) |
| *P*-value | | 0.56 | 0.67 |
| | | | |
| Prescribed vitamin D supplement use (as medication)[1] | | | |
| No | 3,345 | 56.5 (0.41) | 56.5 (0.40) |
| Yes | 2 | 120.5 (14.9) | 119.5 (14.6) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Hip fracture age ≥55[1] | | | |
| No | 2,639 | 56.8 (0.45) | 56.8 (0.45) |
| Yes | 11 | 52.5 (6.28) | 52.4 (5.91) |
| *P*-value | | 0.54 | 0.54 |

| | | | |
|---|---|---|---|
| Fracture at Age ≥55[1] | | | |
| No | 2,156 | 56.6 (0.50) | 56.6 (0.50) |
| Yes | 532 | 57.9 (1.04) | 58.0 (1.03) |
| *P*-value | | 0.26 | 0.19 |
| | | | |
| History of osteoporosis[1] | | | |
| No | 3,090 | 56.3 (0.42) | 56.4 (0.42) |
| Yes | 211 | 61.2 (1.72) | 60.8 (1.71) |
| *P*-value | | 0.004 | 0.007 |
| | | | |
| Osteoporosis-related medication use | | | |
| No | 3,327 | 56.5 (0.41) | 56.5 (0.40) |
| Yes | 20 | 59.4 (4.27) | 59.7 (4.44) |
| *P*-value | | 0.59 | 0.55 |
| | | | |
| History of hypertension | | | |
| No | 2,117 | 58.4 (0.51) | 58.2 (0.51) |
| Yes | 1,199 | 53.4 (0.68) | 53.7 (0.67) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Anti-hypertensive medication use | | | |
| No | 2,963 | 57.0 (0.43) | 57.0 (0.43) |
| Yes | 384 | 53.9 (1.27) | 52.8 (1.28) |
| *P*-value | | 0.001 | 0.001 |
| | | | |
| History of hyperlipidemia | | | |
| No | 2,784 | 57.0 (0.45) | 57.0 (0.45) |
| Yes | 474 | 54.6 (1.03) | 54.7 (1.02) |
| *P*-value | | 0.039 | 0.050 |
| | | | |
| Anti-hyperlipidemic agents use | | | |
| No | 3,065 | 56.7 (0.43) | 56.7 (0.42) |
| Yes | 282 | 54.9 (1.34) | 54.8 (1.32) |
| *P*-value | | 0.20 | 0.18 |
| | | | |
| History of type 2 diabetes | | | |
| No | 3,093 | 57.1 (0.42) | 57.1 (0.42) |
| Yes | 252 | 49.7 (1.53) | 50.4 (1.52) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Anti-diabetic medication use | | | |
| No | 3,176 | 57.1 (0.42) | 57.0 (0.41) |
| Yes | 171 | 47.8 (1.90) | 48.6 (1.87) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Anti-convulsant use | | | |
| No | 3,303 | 56.6 (0.41) | 56.6 (0.41) |
| Yes | 44 | 56.7 (3.67) | 57.0 (3.62) |
| *P*-value | | 0.97 | 0.90 |
| | | | |
| Hematopoietic agents use | | | |
| No | 3,315 | 56.6 (0.41) | 56.6 (0.41) |

| | | | |
|---|---|---|---|
| Yes | 32 | 50.3 (4.09) | 50.4 (3.95) |
| *P*-value | | 0.13 | 0.13 |
| | | | |
| Steroids/corticosteroids use | | | |
| No | 3,289 | 56.6 (0.41) | 56.6 (0.41) |
| Yes | 58 | 52.9 (3.31) | 56.5 (3.28) |
| *P*-value | | 0.23 | 0.32 |
| | | | |
| Hormone therapy use | | | |
| None | 2,063 | 55.3 (0.52) | 55.6 (0.51) |
| Past | 641 | 57.9 (0.95) | 58.3 (0.94) |
| Current, estrogen alone | 328 | 58.9 (1.29) | 57.4 (1.26) |
| Current, estrogen + progesterone | 315 | 60.0 (1.32) | 58.3 (1.31) |
| *P*-value | | <0.001 | 0.032 |
| | | | |
| Low-fat or low cholesterol diet | | | |
| No | 1,753 | 55.6 (0.58) | 55.7 (0.57) |
| Yes | 1,523 | 57.9 (0.58) | 57.7 (0.57) |
| *P*-value | | 0.005 | 0.017 |
| | | | |
| Dietary cholesterol (mg/d) | | | |
| Quintile 1: 149–105.9 | 670 | 57.3 (0.96) | 57.7 (0.95) |
| Quintile 2: 106.0–148.9 | 669 | 57.2 (0.88) | 57.4 (0.86) |
| Quintile 3: 149.0–200.0 | 670 | 56.0 (0.91) | 55.9 (0.90) |
| Quintile 4: 200.1–279.2 | 669 | 57.6 (0.91) | 57.3 (0.90) |
| Quintile 5: 279.4–1,477.2 | 669 | 54.8 (0.91) | 54.6 (0.90) |
| *P*-value | | 0.16 | 0.082 |
| | | | |
| Dietary total fat (g/d) | | | |
| Quintile 1: 7.6–30.3 | 670 | 57.3 (0.94) | 57.8 (0.94) |
| Quintile 2: 30.4–41.9 | 669 | 57.8 (0.90) | 57.9 (0.90) |
| Quintile 3: 42.0–54.3 | 670 | 56.5 (0.88) | 56.3 (0.88) |
| Quintile 4: 54.4–74.0 | 669 | 58.0 (0.95) | 57.7 (0.93) |
| Quintile 5: 74.1–248.0 | 669 | 53.2 (0.88) | 54.1 (0.87) |
| *P*-value | | 0.001 | <0.001 |
| | | | |
| Alcohol intake | | | |
| Non drinkers | 511 | 55.4 (1.07) | 55.7 (1.07) |
| Past drinkers | 696 | 54.3 (0.90) | 54.6 (0.89) |
| Current drinkers, <1 drink/month | 401 | 53.7 (1.13) | 53.5 (1.13) |
| Current drinkers, <1 drink/week | 649 | 56.2 (0.87) | 56.1 (0.86) |
| Current drinkers, 1 to <7 drinks/week | 761 | 59.6 (0.86) | 59.3 (0.86) |
| Current drinkers, 7+ drinks/week | 304 | 61.8 (1.43) | 61.6 (1.40) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Skin reaction to sun | | | |
| No change in skin color | 356 | 51.5 (1.28) | 52.1 (1.30) |
| Tans but does not burn | 1,130 | 56.9 (0.69) | 57.1 (0.69) |
| Burns, then tans | 592 | 60.5 (0.98) | 60.1 (0.99) |
| Burns, then tans a minimal amount | 585 | 58.6 (0.93) | 58.3 (0.92) |
| Burns but does not tan | 262 | 54.1 (1.34) | 53.8 (1.32) |
| *P*-value | | <0.001 | <0.001 |
| | | | |

| | | | |
|---|---|---|---|
| Time outdoors/summer/thirties | | | |
| <30 minutes | 406 | 54.8 (1.03) | 55.0 (1.02) |
| 30 minutes – 2 hours | 1,551 | 56.7 (0.60) | 56.5 (0.59) |
| >2 hours | 996 | 58.6 (0.78) | 58.6 (0.77) |
| *P*-value | | 0.016 | 0.019 |
| | | | |
| Time outdoors/summer/this year | | | |
| <30 minutes | 904 | 53.6 (0.74) | 53.7 (0.73) |
| 30 minutes – 2 hours | 1,454 | 57.7 (0.62) | 57.6 (0.61) |
| >2 hours | 602 | 60.9 (0.99) | 60.7 (0.98) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Time outdoors/other seasons/thirties[1] | | | |
| <30 minutes | 674 | 56.0 (0.88) | 56.0 (0.87) |
| 30 minutes – 2 hours | 1,680 | 57.8 (0.59) | 57.6 (0.59) |
| >2 hours | 584 | 56.6 (0.92) | 56.7 (0.91) |
| *P*-value | | 0.19 | 0.28 |
| | | | |
| Time outdoors/other seasons/this year | | | |
| <30 minutes | 1,132 | 53.9 (0.66) | 53.9 (0.65) |
| 30 minutes – 2 hours | 1,475 | 58.8 (0.63) | 58.7 (0.63) |
| >2 hours | 355 | 60.3 (1.25) | 60.2 (1.23) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Wear hat outdoors/thirties[1] | | | |
| No | 2,303 | 57.2 (0.49) | 56.9 (0.48) |
| Yes | 617 | 56.6 (0.97) | 57.1 (0.98) |
| Don't know | 61 | 57.7 (2.84) | 58.3 (2.79) |
| *P*-value | | 0.85 | 0.89 |
| | | | |
| Wear hat outdoors/this year[1] | | | |
| No | 1,554 | 55.9 (0.58) | 55.8 (0.58) |
| Yes | 1422 | 58.4 (0.64) | 58.4 (0.63) |
| Don't know | 10 | 66.3 (7.66) | 66.0 (8.65) |
| *P*-value | | 0.009 | 0.005 |
| | | | |
| Usually use sunscreen outside | | | |
| No | 1,655 | 54.6 (0.58) | 54.7 (0.57) |
| Yes | 1,286 | 60.2 (0.64) | 59.8 (0.64) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Usual sunscreen SPF[1] | | | |
| 0 (non-users) | 1,655 | 54.6 (0.58) | 54.7 (0.57) |
| 2-9 | 40 | 64.2 (3.14) | 64.5 (3.19) |
| 10-14 | 78 | 53.6 (2.28) | 53.3 (2.30) |
| 15-24 | 670 | 60.5 (0.91) | 60.1 (0.90) |
| ≥25 | 427 | 61.3 (1.10) | 60.9 (1.10) |
| Don't know | 68 | 55.7 (2.44) | 56.3 (2.47) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Time doing yard work per week | | | |
| 0 h | 1,599 | 54.3 (0.58) | 54.5 (0.58) |
| <1 h | 765 | 56.5 (0.83) | 56.4 (0.82) |

| | | | |
|---|---|---|---|
| 1 to <2 h | 334 | 61.5 (1.35) | 61.4 (1.34) |
| ≥2 h | 600 | 60.2 (0.98) | 59.7 (0.98) |
| *P*-value | | <0.001 | <0.001 |
| | | | |
| Ever lived with a smoker after age 18[1,6] | | | |
| No | 734 | 57.7 (0.87) | 57.4 (0.87) |
| Yes | 1,132 | 56.7 (0.70) | 56.7 (0.70) |
| *P*-value | | 0.38 | 0.54 |
| | | | |
| Years as adult lived with a smoker[1,6] | | | |
| 0 (never living with a smoker) | 734 | 57.7 (0.88) | 57.4 (0.87) |
| <1 | 45 | 55.7 (3.41) | 55.3 (3.44) |
| 1-4 | 178 | 58.3 (1.59) | 58.2 (1.56) |
| 5-9 | 162 | 53.4 (1.81) | 53.8 (1.80) |
| 10-19 | 235 | 54.4 (1.43) | 54.6 (1.41) |
| 20-29 | 222 | 58.9 (1.65) | 58.7 (1.63) |
| 30-39 | 156 | 59.3 (2.27) | 59.2 (2.27) |
| ≥40 | 130 | 56.0 (2.11) | 55.9 (2.13) |
| *P*-value | | 0.15 | 0.24 |
| | | | |
| Currently living with a smoker[6] | | | |
| No | 1,777 | 57.2 (0.56) | 57.0 (0.56) |
| Yes | 150 | 53.2 (2.46) | 53.7 (2.49) |
| *P*-value | | 0.09 | 0.16 |
| | | | |
| Ever worked with a smoker[1,6] | | | |
| No | 667 | 57.7 (0.90) | 57.7 (0.89) |
| Yes | 1,201 | 56.6 (0.69) | 56.4 (0.69) |
| *P*-value | | 0.32 | 0.25 |
| | | | |
| Years worked where people smoked[1,6] | | | |
| 0 (never working with a smoker) | 667 | 57.7 (0.90) | 57.7 (0.89) |
| <1 | 87 | 59.5 (2.50) | 59.8 (2.49) |
| 1-4 | 268 | 59.5 (1.58) | 58.8 (1.56) |
| 5-9 | 246 | 56.2 (1.50) | 56.1 (1.49) |
| 10-19 | 290 | 54.9 (1.30) | 54.8 (1.29) |
| 20-29 | 190 | 55.3 (1.75) | 55.2 (1.74) |
| 30-39 | 75 | 53.0 (2.62) | 53.3 (2.57) |
| ≥40 | 34 | 57.9 (3.74) | 57.7 (3.65) |
| *P*-value | | 0.17 | 0.22 |
| | | | |
| Currently work with a smoker[6] | | | |
| No | 1,838 | 57.1 (0.55) | 57.0 (0.55) |
| Yes | 44 | 51.3 (4.04) | 52.1 (4.17) |
| *P*-value | | 0.11 | 0.18 |

[1] Not entered to forward-stepwise selection in regression analysis (see text for details)
[2] Number of participants did not add up due to missingness.
[3] Adjust using residual method. Case/control status in fracture studies; exposure status = high versus low dietary lutein plus zeaxanthin intake in CAREDS (See Supplementary Table 1).
[4] Walked outside the home for more than 10 minutes without stopping (frequency x duration using midpoint of categories).
[5] Recreational physical activities included walking, mild, moderate, and strenuous exercise.
[6] Among never smokers.

Supplemental Table 3. Regression coefficients for serum 25-hydroxyvitamin D concentrations among participants who were not fracture cases (n=2,175)

| Independent variable[1] | β (SE)[1] | *P* value |
|---|---|---|
| **Age** | –0.10 (0.05) | 0.08 |
| **Race/ethnicity** | | |
| White (not of Hispanic origin) | Reference | |
| Am. Indian/Alaskan Native | –6.46 (3.33) | 0.05 |
| Asian/Pacific Islander | –5.31 (2010) | 0.003 |
| Black or African-Am. | –12.65 (1.42) | <0.001 |
| Hispanic/Latino | –8.60 (1.87) | <0.001 |
| Other/unknown | –2.91 (6.21) | 0.64 |
| **Smoking** | | |
| Never smokers | Reference | |
| Former smokers | 0.42 (0.80) | 0.60 |
| Current smokers | –2.09 (1.79) | 0.24 |
| **Total vitamin D intake (per 100 IU/d)** | 2.06 (0.15) | <0.001 |
| **Years of supplemental vitamin D use** | 0.13 (0.05) | 0.01 |
| **Dietary fat intake (g/d)** | –0.01 (0.01) | 0.57 |
| **Waist circumference (cm)** | –0.28 (0.03) | <0.001 |
| **Recreational physical activity (MET-h/wk)** | 0.12 (0.03) | <0.001 |
| **Time spent outdoors in summer daytime this year** | | |
| <30 minutes | Reference | |
| 30 minutes – 2 hours | 3.80 (0.95) | <0.001 |
| >2 hours | 6.59 (1.26) | <0.001 |
| **Solar irradiance (Langleys)** | | |
| 300–325 | Reference | |
| 350 | –0.37 (0.93) | 0.69 |
| 375–380 | –0.26 (1.79) | 0.88 |
| 400–430 | 1.96 (1.57) | 0.21 |
| 475–500 | 5.74 (1.53) | <0.001 |

SE: standard error

[1] Estimated using robust regression adjusting for month of blood draw and study status. The intercept was 70.50.

## Conclusion

In this dissertation, we aimed to investigate whether vitamin D intake was associated with lung cancer risk and whether vitamin A intake modified the vitamin D-lung cancer association. In addition, since vitamin D intake is not the only source of vitamin D in the human body, we investigated the correlation between vitamin D intake and serum 25-hydroxyvitamin D concentrations, which represent the internal dose of vitamin D or vitamin D status. We also examined several novel determinants of serum 25-hydroxyvitamin D concentrations, such as smoking and secondhand smoke. To achieve these specific aims, we analyzed data from two large prospective studies—the Women's Health Initiative (WHI) and the Carotene and Retinol Efficacy Trial (CARET). The results are summarized as follows.

First, in the WHI, no association was observed in all participants. There was a clear dose-response relationship that high vitamin D intake was associated with a lower risk of lung cancer among never smokers. Compared to <100 IU/day, 400 to <800 IU/day of total vitamin D intake was associated with a 44% lower total lung cancer risk, and 800 IU/day or above was associated with a 63% lower total lung cancer risk among never smokers. The beneficial association of total vitamin D intake was also observed for non-small cell lung cancer, particularly adenocarcinoma, in never smokers. The effect modification of total vitamin A intake on the total vitamin D intake-lung cancer association was not observed among never smokers or all participants. However, analyses on the other vitamin D exposure in the WHI: 1g calcium+400 IU vitamin D

supplementation daily suggested that total vitamin A intake at 1,000 μg/day Retinal Activity Equivalent (RAE) or above might attenuate a beneficial association of the supplementation with lung cancer regardless of smoking status.

Second, in the CARET study, no association was observed in all participants. Total vitamin D intake ≥600 versus <200 IU/d was associated with a 64% lower risk of non-small cell lung cancer among former smokers. The effect modification analysis showed that higher total vitamin A intake (≥1,500 μg/day RAE) and high-dose vitamin A supplementation (22,500 μg/day RAE) might assist vitamin D in in preventing lung cancer among current/former heavy smokers and workers with occupational exposure to asbestos.

Third, total vitamin D intake remains an important determinant of vitamin D status in postmenopausal women, particularly those with less chance to receive sun exposure due to high residential latitudes and less time spent outdoors. Smoking is associated with lower serum 25-hydroxyvitamin D concentrations. We did not find the evidence on the association of secondhand smoke exposure with serum 25-hydroxyvitamin D concentrations.

The main effects of total vitamin D intake on lung cancer risk in the two study populations are not completely consistent. We observed an inverse association in CARET former smokers, but not in WHI former smokers. It is unknown whether specific characteristics of the study populations have led to the inconsistency because these two

study populations are heterogeneous. However, findings for current smokers are consistent, as both studies showed no association of total vitamin D intake with lung cancer. The null finding may result from the additional variation created by cigarette smoking because smoking adversely influences both vitamin D metabolism and lung tissues. Future study for current smokers should investigate whether high-dose, e.g., 2,000 IU/day, vitamin D supplement use, is associated with a lower lung cancer risk.

Our analyses of lung cancer histology provided important biological insights. A beneficial association of total vitamin D intake was more likely to be observed for adenocarcinoma compared to other lung cancer histologies. Adenocarcinoma is less tobacco-related compared to other histological type of lung cancer. Therefore, our finding suggests that vitamin D may be more effective in preventing lung carcinogenesis that are not tobacco-related compared to tobacco-related carcinogenesis.

Our findings also have important public health implications. One-third of U.S. adults are at risk of vitamin D insufficiency, defined as serum 25-hydroxyvitamin D concentration <50 nmol/L. This high prevalence of vitamin D insufficiency reflects the fact that the mean total vitamin D intake level in the population is below the Estimated Average Requirement, 400 IU/day. If the inverse association of total vitamin D intake with lung cancer in our study implies a causal relationship, increasing total vitamin D intake to 400 IU/day or above would decrease lung cancer risk in the population. Nevertheless, the intake level should be no more than 4,000 IU/day, the Tolerable Upper Intake Level, to avoid potential adverse effects.

Our observations on effect modification of vitamin A should be cautiously interpreted. Based on our data, whether vitamin A assists or attenuates the protective association of vitamin D intake with lung cancer risk may depend on smoking status. Nevertheless, this conclusion has important limitations. First, in the WHI study the attenuating effect of high vitamin A intake on the Calcium/Vitamin D intervention was not found on total vitamin D intake. Second, the statistical evidence of the effect modification in both studies was only suggestive. Studies with a larger size of lung cancer cases are required to verify the findings. Also, future investigations should use biomarkers of excess vitamin A intake, such as serum retinyl esters, to reflect the effective dose. The feasibility and utility of serum 9-*cis*-retinoid acid as a biomarker should also be assessed.

In sum, this work has provided important information on vitamin D in relation to lung cancer prevention. The study findings shed light on the potential use of vitamin D and vitamin A for the chemoprevention of lung cancer among high-risk population.

Appendix. Data extraction on brand names of dietary supplements and their vitamin D doses in the CARET

| Brand name or generic name (in alphabetical order) | Vitamin D doses (IU) | Note[1] | Source[2] |
|---|---|---|---|
| 2 Bee | 0 | | |
| 2 do E | 0 | Vitamin E=100 | |
| 2.2.2 vitamin science | 400 | MV | assigned |
| 2000 mg/d | 0 | | |
| AARP Activitamins | 400 | MV | assigned |
| AARP multivitamin | 400 | MV | assigned |
| AARP Stress formula | 0 | | |
| Acidophilus | 0 | | |
| ALBERTSON'S daily multivitamin | 400 | MV (Vitamin A=5,000; Vitamin E=15; no β-carotene) | assigned (generic) |
| All four +5 | 0 | | |
| American Health Vitamin C | 0 | | |
| Amway vit C | 0 | | |
| B complex 50 | 0 | | |
| B1 complex | 0 | | |
| B-100 | 0 | | |
| B12 Shots | 0 | | |
| B-150 + E | 0 | | |
| B-50 | 0 | | |
| Bartell brand | 400 | MV (Vitamin A=2,500; vit E=30; β-carotene =2,500) | assigned (generic) |
| Bartell Centabs | 400 | MV | assigned (generic) |
| Bee Pollen | 0 | | |
| Bee Pollen 500 mg | 0 | | |
| Beta-carotene | 0 | | |
| Bio Niacin 500 mg | 0 | | |
| Bioflavonoids | 0 | | |
| Braelly multivitamin | 400 | MV | assigned |
| BRONSON MATURE | 400 | MV | DSLD |
| Brooks multivitamin | 400 | MV | assigned |
| Ca, Mg with A & D | 400 | | DSLD (mode) |
| Ca++ 500 mg QD | 0 | | |
| Cafol | 0 | | |
| Calcium | 0 | | |
| Calcium & Potassium | 0 | | |
| Calcium 1000mg | 0 | | |
| Calcium 1200 mg | 0 | | |
| Calcium 1750mg | 0 | | |
| Calcium 2000mg | 0 | | |
| Calcium 250 | 0 | | |
| Calcium 500 mg | 0 | | |

| | | | |
|---|---|---|---|
| Calcium 600 mg | 0 | | |
| Calcium 600 mg with vit D | 200 | | DSLD (mode) |
| Calcium magnesium | 0 | | |
| calcium magnesium zinc | 0 | | DSLD (mode) |
| Calcium plus | 400 | | DSLD (mode) |
| Calcium w/ vit D | 400 | | DSLD (mode) |
| Calthag w/ vitamin A | 0 | Vitamin A=4,000 | |
| CALTRATE 600 | 0 | | PRD 1993 |
| Can Vita | 400 | MV | assigned |
| Celt multi | 400 | MV | assigned |
| Centab multi | 400 | MV | website |
| Central Vita | 400 | MV | website |
| Central Vite | 400 | MV | website |
| Centrum | 400 | MV | PDR 1993 |
| CENTRUM ADVANCED FORMULA | 400 | MV | DSLD |
| Centrum Silver | 400 | MV | PDR 1993 |
| CENTURY | 400 | MV | DSLD (21th Century) |
| Chewable children's little animals | 400 | MV | DSLD (mode) |
| Citracal 1500 + d | 200 | | website |
| Cod liver oil | 135 | | DSLD (mode) |
| Cooperative multiple vitamin | 400 | MV | assigned |
| Costco multi | 400 | MV | DSLD |
| CVS multivitamin | 400 | MV | website |
| CVS zinc | 0 | | |
| Dac Flex | 400 | MV | assigned |
| Daily calcium | 0 | | |
| Daily multi | 400 | MV (Vitamin A=5,000) | assigned |
| DAILY PAK FOR MEN | 400 | MV | assigned |
| Daily vitamin C | 0 | | |
| Daily vitamin formula | 400 | MV | assigned |
| Dynamite | 100 | MV | website |
| E cono-E formula | 0 | | |
| EdiGuard B-complex | 0 | | |
| EdiGuard high potency vit & minerals | 400 | MV | assigned |
| EdiGuard vitamin C | 0 | | |
| Energer multi | 400 | MV | assigned |
| EPA Marine lipid concertrate | 0 | | DSLD |
| Ezyme | 0 | | |
| FAMILY IMPROVEMENT | 400 | MV (Vitamin A=10,000; Vitamin E=30) | assigned |
| FEDCO | 400 | MV | assigned |
| FEDCO β-carotene | 0 | | |
| FEDCO Niacin 500 mg | 0 | | |
| FEDCO Stress Formula | 0 | | |
| FEDCO Vitamin C | 0 | | |

| | | | |
|---|---|---|---|
| Feosol 200 mg | 0 | | |
| FeSO4 60 ng | 0 | | |
| Fish liver oil-1600 mg | 540 | | website |
| Fish oil Omega 3 - 3,000 mg | 0 | | website |
| Folic acid | 0 | | |
| Formula | 400 | MV | assigned |
| Formula 101 multi-vitamin | 400 | MV | assigned |
| Formula 199 | 0 | | |
| Formula 50 Vit B complex | 0 | | |
| Fosfree | 300 | MV | website |
| Fred Meyer multi | 400 | MV | assigned (generic) |
| Fruit of the Land | 0 | | |
| G.H. multi | 400 | MV | assigned |
| Garlic | 0 | | |
| GENOVESE | 400 | | assigned (generic) |
| Gerital | 400 | MV | PRD |
| Gerital Complete | 400 | MV | PDR |
| Geritol Extend | 200 | MV | PDR |
| Gero vita | 0 | | |
| GER-TABS | 400 | MV | assigned |
| Giant High Potency Vitamins | 400 | Mv | assigned (generic) |
| Giant multivitamin | 400 | MV | assigned (generic) |
| Giant One a Day vitamins | 400 | MV | assigned (generic) |
| GNC Mega EPA -1000 | 0 | | website |
| GNC Stress B-complex | 0 | | website |
| Golden Seal Root | 0 | | |
| GOLDLINE | 400 | MV | assigned |
| GOLDLINE E | 0 | | |
| GOOD NEIGHBOR (vit E) | 0 | | |
| GOOD NEIGHBOR Calcium | 0 | | |
| Great Earth | 400 | (β-carotene 10,000 IU) | website |
| Great Earth vit C | 0 | | |
| Great Earth vitamin E 400 | 0 | | |
| Great Steert | 0 | (Vitamin A 10,000 IU) | |
| Grocery store brand | 400 | | assigned |
| Group Health multi vit | 400 | MV | assigned |
| Hall B-12 | 0 | | |
| HEALTH BALANCE | 400 | MV (Vitamin A 5000; Vitamin E 30) | assigned |
| HEALTH BALANCE calcium | 0 | | |
| HEALTH BALANCE stress formula w/ iron | 0 | | |
| HEALTH BALANCE Vitamin C 1000 mg | 0 | | |
| Health Plus | 0 | | |
| Health Plus sentabs | 400 | MV (Vitamin A=2,500; Vitamin E=30; β-carotene | assigned |

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|---|---|---|---|
| | | =2,500) | |
| Hematinic 3-way multi | 0 | MV | Dailymed |
| Herbal Cellulex | 0 | | |
| Herbal Deictic | 0 | tea | |
| Herbal vitamin | 0 | | |
| HERBALIFE | 67 | MV | catalog.md |
| Hi B-complex | 0 | | catalog.md |
| Iron tablet | 0 | | |
| Jenasol | 0 | Male enhancement (Vitamin A=10,000 IU) | |
| Jenny Craig | 400 | MV | assigned |
| Kausar natural vitamin E | 0 | | |
| Kelp Leather | 0 | | |
| KM herbal concoction vitamins | 0 | | |
| Lecithin | 0 | | |
| Life line B12 250 | 0 | | |
| Life line B-complex | 0 | | |
| Linseed oil | 0 | | |
| Living Source | 0 | | |
| Lloyd's mega daily | 400 | MV | assigned |
| L-Lysine | 0 | | |
| Long Cod Liver Oil - 1,250 IU A/135 IU D | 135 | | participant |
| LONG'S B-12 | 0 | | |
| LONG'S B-6 | 0 | | |
| LONG'S daily vitamin | 400 | MV | assigned |
| LONG'S multi | 400 | MV | assigned |
| LONG'S vit E | 0 | | |
| LONG'S Vital | 400 | MV | assigned |
| Lucky HIGH POTENCY A - Z | 400 | MV | assigned |
| Lucky- Vit C | 0 | | |
| MAGNA ONE | 400 | MV | assigned |
| Magnesium | 0 | | |
| Mel-vita calcium vitamin d vitamin c | 400 | | assigned |
| Mel-vita multivitamin | 133 | MV | catalog.md |
| Mineral supplement | 0 | | |
| MOR-B-PLEX | 0 | MV | |
| Mrs. Gooch vitamin C 5000 | 0 | | |
| Mrs. Gooch vitamin E | 0 | | |
| Multi & Minerals | 400 | | assigned |
| Multi II | 400 | MV | website |
| Multi Vite | 30 | MV | catalog.md |
| Multi-Guard | 200 | MV | website |
| Multi-System multiple vitamin | 400 | MV | assigned |
| Multivitamin | 400 | MV | assigned |
| Multivitamin w/ Cal + Iron | 400 | MV | assigned |
| Multivitamin w/ Iron | 400 | MV | assigned |

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|---|---|---|---|
| Multi-Vitamin with Fluoride | 400 | MV | assigned |
| Myadec | 400 | MV | PDR |
| Natural selenium | 0 | | |
| Natural Total | 0 | | |
| Natural vit E | 0 | | |
| NATURAL VIT stress B | 0 | | |
| Nature Made | 400 | MV | website |
| Nature Made - Daily Combo | 400 | MV | DSLD |
| Nature Made - Essential Balance | 400 | MV | DSLD |
| Nature Made - fish oil | 0 | | DSLD |
| Nature Made A+D | 400 | | DSLD |
| Nature Made calcium/magnesium | 0 | | website |
| Nature Made Therapeutic M | 400 | MV (Vitamin A =5,000; Vitamin E=30; β-carotene=2,500) | assigned |
| Nature Made Vit C | 0 | | |
| Nature Made vitamin E | 0 | | |
| Nature multi | 400 | MV | assigned |
| NATURE'S BLEND thera-vits | 400 | MV | assigned |
| Niacin | 0 | | |
| Niacin 1500 mg | 0 | | |
| Niacin 2000 mg | 0 | | |
| Niacin 3000 mg | 0 | | |
| Niacin 4000 /d | 0 | | |
| Niacin 500 | 0 | | |
| Nutri-action multiple | 400 | MV | assigned |
| Nutrilite multivitamin | 400 | MV | website |
| Nutri-Plus | 400 | MV | assigned |
| Nutrisystem multivitamin | 400 | MV (Vitamin A=5,000; Vitamin E=30; no β-carotene) | assigned |
| Nutrition Headquarters | 0 | | |
| Ocuvite | 0 | MV | catelog.md |
| Omaga 3 | 0 | | |
| One a day | 400 | MV | PDR 1993 |
| One a day - Womens formula | 400 | MV | PDR 1993 |
| One Daily | 400 | MV | assigned |
| Opti - Zinc | 0 | MV (Vitamin E=30 ; β-carotene=5,000) | catelog.md |
| OSCO | 0 | MV | website |
| OSCO A to Z | 400 | MV | website |
| OSCO One a Day | 400 | MV | assigned |
| OSCO vitamin C 500 mg | 0 | | |
| OSCO Vitamin E | 0 | | |
| OSCO zinc BEC | 0 | | |
| Oyster Shell Ca + Vit D | 800 | | website |
| Oyster Shell Ca 500 | 0 | | |

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|---|---|---|---|
| Oyster Shell Ca+ | 0 | | |
| Pace | 400 | MV | assigned |
| Pantothenic acid | 0 | | |
| Pantothenic acid 1000 | 0 | | |
| Papaya enzyme | 0 | | |
| Parade 1-A-day | 400 | MV (Vitamin A=5,000; Vitamin E=30) | assigned |
| PATHMARK MULTI VIT | 400 | | assigned |
| PAY n SAVE | 400 | MV | assigned |
| PAY n SAVE THERAPEUTIC M | 400 | MV | assigned |
| PAYLESS | 400 | MV | assigned |
| PAYLESS A to Z | 400 | MV | assigned |
| PAYLESS daily | 400 | MV | assigned |
| PAYLESS vit C 2000 mg | 0 | | |
| PAYLESS vit E | 0 | | |
| Pay'n Save | 400 | MV | assigned |
| People B50 | 0 | | |
| PERFECT CHOICE multi/mineral | 400 | MV | assigned |
| Phoenix vitamin & mineral | 400 | | assigned |
| Phycotene | 400 | MV (Vitamin A 25,600; Vitamin E= 55; β-carotene=25,600) | assigned |
| PLENAMINS vitamin QOP | 400 | MV | website |
| PNS Central Vite | 400 | MV | assigned |
| Potassium | 0 | | |
| Potassium 260 mg | 0 | | |
| Potassium 550 | 0 | | |
| Potent 75 | 400 | MV | DSLD |
| Power All | 0 | | |
| Pre-natal multi-vit | 400 | MV | assigned |
| Price Clees E | 0 | | |
| Prime Natural Health | 400 | MV | assigned |
| PURITAN PRIDE | 400 | MV (Vitamin A 5,000; Vitamin E 30) | DSLD |
| RAINBOW nutritional system | 400 | MV | DSLD |
| Ralph's brand multiple vits plus iron | 400 | MV | assigned (generic) |
| Rugby | 0 | | |
| Ruts 500 | 0 | | |
| Safeway brand | 400 | MV | website |
| Safeway multi | 400 | MV | website |
| Safeway One Tablet | 800 | MV | website |
| Safeway stress vitamin +Zinc | 0 | | |
| Schiff B complex | 0 | | |
| Schiff multi | 400 | MV | DSLD |
| SELENIUM | 0 | | |
| Sentinel | 400 | MV | website |

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|---|---|---|---|
| Sentinel Amino acids | 0 | | |
| Sentinel C | 0 | | |
| Sentral Vite | 400 | MV | catelog.md (Central Vite) |
| Shackley's milk | 0 | | |
| SHAKLEE B complex | 0 | | |
| SHAKLEE Ca, Mg, vit D | 200 | | DSLD |
| SHAKLEE Calcium | 0 | | |
| SHAKLEE multi | 400 | MV | DSLD |
| SHAKLEE Vit C | 0 | | |
| Shaklee's Vita-Lea | 400 | MV | website |
| Skinny up | 400 | MV | assigned |
| Smokers Pac | 0 | | |
| Spirulina | 400 | Vitamin A=7000 | DSLD |
| Spring Valley | 400 | MV | website |
| Squibb multi-vitamin | 400 | MV | assigned |
| Stress B-complex | 0 | | |
| STRESS FORMULA | 0 | | |
| Stressquard | 0 | | |
| Stresstabs | 0 | | |
| Stresstabs + iron | 0 | | |
| Stresstabs 600 | 0 | | |
| Stuart | 400 | MV | PDR |
| STUART NATAL | 400 | MV | website |
| Summertime one day diet | 0 | | |
| Super - multiple | 500 | MV | DSLD |
| SUPER -B | 0 | | |
| Super High V with 25000 vitA-palmitate and b/c | 400 | MV | catalog.md |
| Super Hy-vites | 400 | MV | catalog.md |
| Supreme B-100 | 0 | | |
| TARGET high potency | 400 | MV | assigned |
| Thera viles | 0 | | |
| Theragenerix | 400 | MV | assigned |
| Theragran | 400 | MV | PDR |
| Theragran Multiple | 400 | MV | PDR |
| Theragran Stress | 0 | | PDR |
| Theragran with mineral | 400 | MV | PDR |
| THERAGRAN-M | 400 | MV | PDR |
| Theramin vitamin c | 0 | | |
| THERAPEUTIC M | 400 | MV | website |
| THERAPEUTIC multivitamin | 400 | MV | assigned |
| Thera-plus multi | 400 | MV | assigned |
| Thex Forte B complex | 0 | | |
| Thompson multi | 400 | MV (Vitamin A=10,000) | DSLD |
| Thompson Vitamin D | 400 | MV | DSLD |
| Thrifty alpha beta | 400 | MV | assigned |
| Thrifty C | 0 | | |

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|---|---|---|---|
| THRIFTY drug store | 400 | MV | assigned |
| Thrifty kelp b6 cider viingegar | 0 | | |
| Thrifty naturalized multivitamins w iron & zinc for women | 400 | MV | assigned |
| TRADER DARWIN | 400 | MV | DSLD |
| Trader Joe B100 mg | 0 | | |
| Tums | 0 | Calcium Carbonate | |
| Ultramega | 200 | MV | catalog.md |
| Unicap | 400 | MV | PDR |
| Unicap M | 400 | MV | PDR |
| Unicap T multivit | 400 | MV | PDR |
| Universal Life | 400 | MV | assigned |
| unknown | 0 | | |
| UPJOHN One-a-day | 400 | MV | PDR |
| Uttrum | 400 | MV | assigned |
| Vibrant Health C | 0 | | |
| Vicon -Zinc | 0 | | |
| Vit Imp Program | 0 | | |
| Vita Fresh | 400 | MV, Vitamin E=400 | assigned |
| Vita Fresh multiple | 400 | MV (Vitamin A 500; Vitamin E 30) | assigned |
| Vita gram | 0 | | |
| Vita-Fresh vitamin C | 0 | | |
| Vital life | 0 | MV | catalog.md |
| Vita-Lea | 400 | MV | DSLD |
| VITALERT | 400 | MV | DSLD |
| Vitamin A | 0 | | |
| Vitamin A & D | 400 | | assigned |
| Vitamin A & D 400 IU | 400 | Vitamin A=5,000 | assigned |
| Vitamin A (10,000 IU) & D | 400 | Vitamin A=10,000 | assigned |
| Vitamin A (5,000 IU) & D | 400 | | assigned |
| Vitamin A (5,000 IU) & D (400) | 400 | | assigned |
| Vitamin A fresh vitamin E 400 | 0 | | |
| Vitamin B | 0 | | |
| Vitamin B & vit C | 0 | | |
| Vitamin B complex w/ iron | 0 | | |
| Vitamin B1 (Thiamine) | 0 | | |
| Vitamin B1 400 mg | 0 | | |
| Vitamin B12 | 0 | | |
| Vitamin B12 100 mg | 0 | | |
| Vitamin B12 1000 mg | 0 | | |
| Vitamin B12 1667 mg | 0 | | |
| Vitamin B6 | 0 | | |
| Vitamin B6 100 mg | 0 | | |
| Vitamin C | 0 | | |
| Vitamin C 100 mg | 0 | | |
| Vitamin C 1000 mg | 0 | | |
| Vitamin C 1500 mg | 0 | | |
| Vitamin C 200 mg | 0 | | |

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|---|---|---|---|
| Vitamin C 2000 mg | 0 | | |
| Vitamin C 250 mg | 0 | | |
| Vitamin C 250 QD | 0 | | |
| Vitamin C 2500 mg | 0 | | |
| Vitamin C 400 mg | 0 | | |
| Vitamin C 500 | 0 | | |
| Vitamin C 500 mg | 0 | | |
| Vitamin C 5000 mg | 0 | | |
| Vitamin C QD | 0 | | |
| Vitamin Classics | 400 | MV | assigned |
| Vitamin D | 400 | | assigned |
| Vitamin D 1000 IU | 1000 | | participant |
| Vitamin D 800 IU/d | 800 | | participant |
| Vitamin E | 0 | | |
| VITRUM | 400 | MV | catalog.md |
| Von b-100 | 0 | | |
| Vons | 400 | MV | assigned (generic) |
| Vons C | 0 | | |
| VONS CENTRAL VITE | 400 | MV | assigned (generic) |
| Vons Theradex M | 400 | MV | assigned (generic) |
| Waye's -Mega B | 0 | | |
| Western Family B50 | 0 | | |
| Western Family daily vitamin | 400 | MV (Vitamin A=2,500; Vitamin E=30; β-carotene=2,500) | assigned |
| Western Family Thera -M | 400 | MV | assigned |
| Western Family vit C | 0 | | |
| Your Life | 1000 | MV | DSLD |
| Your Life -B12 | 0 | | |
| Your Life E | 0 | | |
| Your Life Potassium | 0 | | |
| Z-BEC | 0 | MV | PDR |
| Zinc | 0 | | |
| Zinc 15 mg | 0 | | |
| Zinc 30 mg | 0 | | |
| Zinc 50 mg | 0 | | |

[1] Multivitamin (MV) and doses of vitamin A, vitamin E, and β-carotene; unit=IU. The doses were recorded by CARET staff.

[2] DSLD: Dietary Supplement Label Database; PDR: Physicians' Desk Reference for Nonprescription Drugs and Dietary Supplements; website: the manufacture's website.