REVIEW

Vitamin D and multiple health outcomes in the Harvard cohorts

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The hypothesis that vitamin D is inversely associated with multiple health outcomes has been studied in the Harvard cohorts, including the Nurses' Health Study I ($n = 121\,700$ female nurses aged 37–64 at baseline in 1984), Nurses' Health Study II ($n = 116\,671$ female nurses aged 27–44 years at baseline in 1991), Health Professionals Follow-up Study ($n = 51\,529$ male health professionals aged 40–75 years at baseline in 1986), and Physicians' Health Study ($n = 22\,071$ male physicians aged 40–84 years at baseline in 1982). These studies assessed vitamin D through circulating 25-hydroxyvitamin D, dietary and supplemental intake, predicted 25-hydroxyvitamin D, and vitamin D receptor polymorphisms. This review summarizes studies of vitamin D and various endpoints considered in these cohorts, including risk of cardiovascular disease, hypertension, elevated plasma C-peptide, various cancers, bone fractures, and multiple sclerosis. Based on the multiple observed benefits of vitamin D, this article postulates recommendations for vitamin D intake in the US population for reduced incidence of multiple health outcomes.

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1 Introduction

Vitamin D deficiency (<20 ng/mL or 50 nmol/L) and insufficiency (21–29 ng/mL or 52–72 nmol/L) are pandemic. One billion individuals worldwide are vitamin D deficient or insufficient [1, 2], including 40–100% of community (non-nursing home) dwelling US adults [3–5]. Sources of vitamin D include direct skin exposure to sunlight, some foods, and dietary supplements. The predominant source occurs through solar ultraviolet B (UV-B; 290–315 nm wavelength) radiation exposure on the skin, where 7-dehydrocholesterol is converted to previtamin D_3 and then vitamin D_3 in the magnitude of 10 000–25 000 IU of vitamin D_2 for an individual in a bathing suit after 1 minimal erythemal dose (the safest amount of radiation sufficient to produce redness in the skin after application) [6]. Vitamin D_3 intoxication is precluded by the destruction of previtamin D_3 [7]. Vitamin D is rare in food sources and when present is in quantities far below the quantity accrued through solar UV-B exposure [2]. Particularly in northern latitudes and during the winter months, food sources alone are considered inadequate for maintaining sufficient, let alone optimal circulating vitamin D levels [2].

Vitamin D deficiency has been hypothesized to increase the risk of a number of chronic diseases, including cancer,

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Abbreviations: 1,25(OH)2D3, 1, 25-dihydroxyvitamin D; **25(OH)D,** 25-hydroxyvitamin D; **CI,** confidence interval; **HPFS,** Health Professionals Follow-up Study; **MI,** myocardial infarction; **NHS I,** Nurses' Health Study I; **NHS II,** Nurses' Health Study II; **OR,** odds ratio; **PHS,** Physicians' Health Study; **RR,** relative risk; **UV-B,** ultraviolet B; **VDR,** vitamin D receptor

cardiovascular disease, and some autoimmune diseases [8–15]. Some of the initial evidence supporting the hypothesized associations has been based on ecologic studies on sun exposure. For example, in the early 1980s, Garland and Garland hypothesized that inadequate vitamin D status resulting from lower solar UV-B radiation exposure accounted for the association between higher latitudes and increased mortality from colon cancer [16], breast cancer [8], and ovarian cancer [17]. In the past several decades, experimental studies have revealed that many tissues express a signal transduction mechanism involving the nuclear vitamin D receptor (VDR) and possess the ability to convert 25-hydroxyvitamin D (25(OH)D) to the active form 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$) [18]. Through binding the VDR, $1,25(OH)_2D_3$ has been shown to activate or inhibit numerous genes that offer plausible biologic explanations for many of the hypothesized benefits of vitamin D. There are also nongenomic VDRs located in the plasma membrane that modulate a complex signaling system through rapidly opening calcium channels [19], and there may be crosstalk between the genomic and nongenomic pathways [20].

The establishment of a risk factor as causal may require randomized controlled trials. However, for many of the conditions hypothesized to be associated with vitamin D, definitive randomized data are not available. The paucity of data does not reflect low interest, but rather the practical and technical difficulties of undertaking such an endeavor. Endpoints such as cancer would require long-term study, perhaps of decades, and tens of thousands of individuals. Further, the optimal doses of vitamin D and targeted levels of 25(OH)D have been unclear. Observational studies, either case-control or prospective cohort, can offer important evidence regarding the vitamin D hypothesis. Short of ''definitive'' randomized trials and complementary with other types of studies, these studies can offer the best evidence for a role of vitamin D for the various proposed endpoints.

Four Harvard cohort studies, the Nurses' Health Study I (NHS I), Nurses' Health Study II (NHS II), the Health Professionals Follow-Up Study (HPFS) and the Physicians' Health Study (PHS) have examined a number of vitamin D-related hypotheses over the past several decades. This brief review summarizes some of these studies and highlights strengths and limitations of the cohort studies of vitamin D in general. We first provide an overview of the cohorts, summarize the ways vitamin D status has been assessed, and then describe some results for various endpoints including cardiovascular disease, hypertension, plasma C-peptide, bone fractures, multiple sclerosis, and cancers of the colon, rectum, breast, prostate, and pancreas (Table 1).

2 Overview of the cohorts

All four Harvard cohorts are comprised of health professionals living in the United States. The NHS I is a longitudinal study of 121 700 female registered nurses aged 37–64 years at baseline in 1976. Information on various lifestyle factors and medical history is collected through self-administered questionnaires. A dietary component based on a food frequency questionnaire began in 1980, and blood samples were collected in 1989–1990. Information is updated every 2 years, and diet is updated every 4 years. The women are followed for various health outcomes, most of which are confirmed through medical records and pathology reports. The NHS II is an ongoing prospective cohort study of 116 671 female registered nurses aged 27–44 years at baseline in 1989. Participants are followed similarly as NHS I through biennial questionnaires that gather information on health-related behaviors and medical events. During the years 1997–1999, participants contributed blood samples. The HPFS is a cohort of 51 529 male health professionals aged 40–75 years when the cohort commenced in 1986 with questionnaires and was extended to blood-based biomarkers in 1993. As for the NHS cohorts, the men are followed every 2 years in general and every 4 years for dietary information. The follow-up rate is similarly high as in the NHS. The PHS is a placebo-controlled trial of aspirin and β-carotene among 22 071 healthy US male physicians, aged 40–84 years, in 1982. Questionnaire data for this cohort are limited, but blood samples were collected at baseline in 1982. The participants in these cohorts are predominantly Caucasians (over 95%), reflecting the make-up of these health professionals in the United States at the time of recruitment. The follow-up rates for these cohorts are high, typically exceeding 90%. The prevalence of vitamin D deficiency and insufficiency in the Harvard cohorts is similar to that in the US population, with 76% of women [9] and 77% of men [10] found to be vitamin D deficient or insufficient.

3 Assessment of vitamin D status in the cohorts

Studies based on circulating 25(OH)D level are arguably the ''gold standard'' among observational studies for examining vitamin D status. Circulating 25(OH)D accounts not only for skin exposure to UV-B radiation, but also for the multiple factors that determine vitamin D status, such as total vitamin D intake, sun exposure and skin pigmentation. In addition, 25(OH)D has a relatively long half-life ($t_{1/2}$) in the circulation, and thus can provide a reasonably good indicator of long-term vitamin D status. In the HPFS, the correlation of two 25(OH)D measures approximately 3 years apart was 0.7 [21]. A limitation of studies of 25(OH)D is that typically only one measurement has been made, and levels fluctuate seasonally throughout the year due to variation in sun exposure, and over time vitamin D status may change. The NHS and PHS have collected a second blood sample in recent years, so future analyses based on two measurements are possible.

Table 1. Vitamin D and chronic health outcomes investigated in the Harvard cohorts

First author, year	Cohort[a], follow-up	No. of cases, controls	Serum or predicted 25(OH)D (ng/mL) or vitamin D intake (IU/d) in quantile (Q) medians, ranges or cut-points	Relative risk or odds ratio (95% confidence interval)	*p* for trend	Adjusted for
Cardiovascular disease (nonfatal myocardial infarction or fatal coronary heart disease)						
Giovannucci 2008	HPFS 1993–2004	454 900[b]	Serum 25(OH)D ≥30 <15	 1.0 2.09 (1.24–3.54)	 $p = 0.02$	Matching variables plus BMI, physical activity, race, region, history of diabetes mellitus, history of hypertension, high- and low-density lipoprotein cholesterol and triglyceride levels, family history of MI before age 60, multivitamin use, fasting status, and intakes of alcohol and marine ω-3 fatty acids
Hypertension						
Forman 2005	HPFS 1986–2002	8834	Dietary intake Q1 median 99 Q5 median 748	 1.0 1.03 (0.93–1.15)	 $p = 0.48$	Age, BMI, physical activity, smoking history, family history of hypertension, baseline systolic and diastolic blood pressure, and intakes of alcohol, caffeine, total protein, fiber, folate, vitamins B_6 and B_{12}, calcium, magnesium, sodium, potassium
	NHS I 1984–2002	27 084	Dietary intake Q1 median 79 Q5 median 646	 1.0 0.98 (0.93–1.04)	 $p = 0.84$	Age, BMI, physical activity, smoking history, family history of hypertension, and intakes of alcohol, caffeine, total protein, fiber, folate, vitamins B_6, B_{12}, calcium, magnesium, sodium, potassium
	NHS II 1991–1999	7371	Dietary intake Q1 median 128 Q5 median 742	 1.0 1.13 (0.99–1.29)	 $p = 0.11$	Age, BMI, physical activity, smoking history, family history of hypertension, baseline systolic and diastolic blood pressure, and intakes of alcohol, caffeine, total protein, fiber, folate, vitamins B_6 and B_{12}, calcium, magnesium, sodium, potassium
Forman 2007	HPFS 1986–2002	133	Serum 25(OH)D ≥30 <15	 1.0 3.53 (1.02–12.3)	 Not provided	Age, BMI, physical activity, race, smoking status, family history of hypertension, and intakes of alcohol, vitamin D, folate, calcium, magnesium, sodium, potassium
		9029	Predicted 25(OH)D Decile 1 7.3–22.8 Decile 10 32.5–37.6	 1.0 1.86 (1.70–2.04)	 $p < 0.001$	Age, race, BMI, physical activity
	NHS I 1984–2002	274	Serum 25(OH)D ≥30 ng/mL <15 ng/mL	 1.0 1.70 (0.92–3.16)	 Not provided	Age, race, BMI, physical activity, menopausal status
		26 525	Predicted 25(OH)D Decile 1 7.3–22.8 Decile 10 32.5–37.6	 1.0 2.27 (2.15–2.39)	 $p < 0.001$	Age, BMI, physical activity, race, smoking status, family history of hypertension, menopausal status, and intakes of alcohol, vitamin D, folate, calcium, magnesium, sodium, potassium

Table 1. Continued

First author, year	Cohort[a)], follow-up	No. of cases, controls	Serum or predicted 25(OH)D (ng/mL) or vitamin D intake (IU/d) in quantile (Q) medians, ranges or cut-points	Relative risk or odds ratio (95% confidence interval)	*p* for trend	Adjusted for
Forman 2008	NHS II 1989–2005	742 742[c)]	Serum 25(OH)D Q4 median 37.9 Q1 median 16.7	1.0 1.66 (1.11–2.48)	$p = 0.01$	Matching factors plus BMI, physical activity, family history of hypertension, oral contraceptive use, and plasma levels of PTH, calcium, phosophorous, creatinine, uric acid
Plasma C-peptide						
Wu 2009	HPFS Cross sectional		Serum 25(OH)D Q1 median 15.2 Q4 median 35.2	21% lower C-peptide level Q4 *versus* Q1	$p = 0.08$	Age, BMI, physical activity, smoking, race, region, month and time of blood draw, lab batch, fasting status, and intakes of alcohol, caffeine, total energy, glycemic load, dairy foods, cereal fiber, calcium, retinol
	NHS II Cross sectional		Serum 25(OH)D Q1 median 17.6 Q4 median 44.8	7.7% lower Q4 *versus* Q1	$p = 0.30$	Above plus postmenopausal status, hormone use
Colorectal adenoma						
Kampman 1994	HPFS 1986–1990	589	Total intake Q1 median 125 Q5 median 851	1.0 1.29 (0.87–1.93)	$p = 0.32$	Age, BMI, indication for endoscopy, previous endoscopy, family history, and intakes of alcohol, saturated fat, total energy, fiber, folate
	NHS I 1980–1988	509	Total intake Q1 median 61–87 Q5 median 653–683	1.0 0.68 (0.41–1.13)	$p = 0.09$	Age, BMI, indications for endoscopy, history of endoscopy, family history, and intakes of alcohol, saturated fat, fiber, total energy, folate
Oh 2007	NHS I 1980–2002	2747	Total intake Q1 median 135 Q5 median 601	1.0 0.79 (0.63–0.99)	$p = 0.07$	Age, BMI, physical activity, smoking, previous endoscopy, family history, aspirin use, menopausal status and hormone use, and intakes of alcohol, red meat, total energy, total fiber, folate, phosphorus, calcium, retinol
Colorectal cancer						
Kearney 1996	HPFS 1986–1992	203	Total intake Q1 < 161 Q5 ≥ 613	1.0 0.66 (0.42–1.05)	$p = 0.02$	Age, BMI, physical activity, smoking status, previous polyp and screening, family history, aspirin use, intakes of alcohol, red meat, total calories, saturated fat, fiber
Martinez 1996	NHS I 1980–1992	501	Total intake < 120 > 550	1.0 0.42 (0.19–0.91)	$p = 0.04$	Age, BMI, physical activity, smoking status, family history, aspirin use, intake of alcohol and red meat

Table 1. Continued

First author, year	Cohort[a], follow-up	No. of cases, controls	Serum or predicted 25(OH)D (ng/mL) or vitamin D intake (IU/d) in quantile (Q) medians, ranges or cut-points	Relative risk or odds ratio (95% confidence interval)	*p* for trend	Adjusted for
Wu 2007	HPFS 1994–2002	179	Serum 25(OH)D Q1 median 18.4 Q5 median 39.4	 1.0 0.83 (0.45–1.52)	 $p = 0.24$	Physical activity, smoking status, family history, aspirin use, and intakes of alcohol, meat, folate, calcium, retinol
	NHS I 1990–2000	223	Serum 25(OH)D Q1 median 14.1 Q5 median 45.5	 1.0 0.51 (0.26–1.00)	 $p = 0.02$	Physical activity, smoking status, family history, menopausal status, postmenopausal hormone use, aspirin use, and intakes of alcohol, meat, folate, calcium, retinol
Prostate cancer						
Giovannucci 1998	HPFS 1986–1994	1414	Total intake <150 ≥ 800	 1.0 1.21 (0.92–1.58)	 $p = 0.87$	Age, BMI at age 21, intakes of total energy, total fat, fructose, lycopene, phosphorus
Platz 2004	HPFS 1993–1998	460 460[d]	Serum 25(OH)D quartile median ranges Q1 9.3–20.1 Q4 24.6–32.9	 1.0 1.19 (0.79–1.79)	 $p = 0.59$	Matching variables plus vigorous physical activity, smoking status, height, diabetes mellitus, vasectomy, family history, vitamin E and selenium supplement use, and intakes of total energy, red meat, fish, fructose, lycopene, α-linolenic acid
Gann 1996	HPFS 1982–1992	232 414[e]	Serum 25(OH)D Q1 6.3–21.4 Q4 not provided	 1.0 0.92 (0.56–1.50)	 $p = 0.82$	Matching variables plus BMI, physical activity, alcohol intake
Pancreatic cancer						
Skinner 2006	HPFS 1986–2000	187	Total intake <150 ≥ 600	 1.0 0.49 (0.29–0.82)	 $p = 0.01$	Age, time period, BMI, height, region, smoking status, history of diabetes, multivitamin use, total energy intake
	NHS I 1984–2000	178	Total intake <150 ≥ 600	 1.0 0.76 (0.42–1.38)	 $p = 0.47$	Above plus parity
Giovannucci 2006	HPFS 1986–2000	170	Predicted 25(OH)D *per* 10 ng/mL increment	0.49 (0.28–0.86)	Not provided	Age, height, smoking history, and intakes of total calories, alcohol, red meat, fruits, vegetables, calcium, retinol
Breast cancer						
Shin 2002	NHS I 1980–1996	827	Total intake ≤ 150 >500	 1.0 0.72 (0.55–0.94)	 $p = 0.01$	(All premenopausal) Age, time period, physical activity, BMI at age 18, weight change since age 18, height, age at menarche and first birth, parity, family history, history of benign breast disease, glycemic index, and intakes of alcohol, total energy, total fat, total vitamin E, β-carotene

Table 1. Continued

First author, year	Cohort[a)], follow-up	No. of cases, controls	Serum or predicted 25(OH)D (ng/mL) or vitamin D intake (IU/d) in quantile (Q) medians, ranges or cut-points	Relative risk or odds ratio (95% confidence interval)	*p* for trend	Adjusted for
		2345	Total intake			(All postmenopusal) Same as above
			≤150	1.0		
			>500	0.94 (0.80–1.10)	$p = 0.27$	
Bertone-Johnson 2005	NHS II 1989–1996	701 701[f)]	Serum 25(OH)D Q1 cutpoint ≤20–28	1.0		Matching factors plus BMI at age 18, age at menarche, first birth and menopause, parity, postmenopausal hormone use, family history, history of benign breast disease, plasma α-carotene, alcohol intake
			Q5 cutpoint ≥37–48	0.65 (0.40–1.06)	$p = 0.06$	
Multiple sclerosis						
Munger 2004	NHS I 1980–1998	76	Total intake			Age in 5-year groups
			Q1 median 58	1.0		
			Q5 median 641	0.55 (0.25–1.22)	$p = 0.05$	
			Supplemental intake			Same as above
			<400	1.0		
			≥400	0.39 (0.18–0.85)	$p = 0.007$	
	NHS II 1991–1999	97	Total intake			Age in 5-year groups
			Q1 median 128	1.0		
			Q5 median 742	0.77 (0.40–1.50)	$p = 0.22$	
			Supplemental intake			Same as above
			<400	1.0		
			≥400	0.75 (0.44–1.26)	$p = 0.20$	
Hip fractures						
Feskanich 2003	NHS I 1980–1998	603	Total intake			Age, BMI, physical activity, smoking status, postmenopausal hormone use, and intakes of alcohol, caffeine, protein, calcium, retinol, vitamin K
			<140	1.0		
			≥400	0.63 (0.42–0.94)	$p = 0.03$	

a) Harvard cohorts include the Health Professionals Follow-up Study (HPFS, 51529 male health professionals 40–75 years of age at baseline in 1986); Nurses' Health Study I (NHS I, 121700 female registered nurses 37–64 years of age at baseline in 1976); Nurses' Health Study II (NHS II, 116671 female registered nurses 27–44 years of age at baseline in 1989); and Physicians' Health Study (PHS, 22071 male physicians 40–84 years of age in 1982).
b) Population-based controls matched by age, month and year of blood collection, smoking status.
c) Population-based controls matched by age, race, hour and month of blood draw, day of menstrual cycle if premenopausal.
d) Population-based controls matched by year of birth, PSA prior to blood draw, and time, season and year of blood draw.
e) Population-based controls matched by age within 1 year, smoking status.
f) Population-based controls matched by age, time and month of blood collection, fasting status at blood draw, menopausal status, postmenopausal hormone use.

A second way to examine vitamin D status is to examine intakes from the food frequency questionnaire. A limitation of this method is that vitamin D intakes are relatively low in general because of the scarcity of vitamin D in natural foods and limited fortification of this vitamin in foods. Thus, vitamin D intake contributes a relatively small proportion of an individual's overall vitamin D status. Nonetheless, vitamin D intake is an important contributor to 25(OH)D levels, especially in winter months in regions at high latitudes, when it is probably the sole contributor. Further, an advantage of the NHS and HPFS is that dietary intake has been assessed every 4 years, which improves the assessment of long-term status.

In the HPFS, we have also utilized known predictors of 25(OH)D level based on data on the individual level to formulate a predicted 25(OH)D index. Specifically, based on the men's reported vitamin D intake, region of residence and outdoor leisure activity level (surrogates of UV-B exposure), skin pigmentation, and body mass index, a quantitative estimate of the expected vitamin D level was made. The weighing of the individual factors is based on a sample of the population who had 25(OH)D measured, and then the index is computed for each cohort member using multiple linear regression models. The predicted 25(OH)D approach has both advantages and disadvantages compared to the use of a single measurement of circulating 25(OH)D in epidemiologic studies. The measurement of 25(OH)D is more direct, intuitive, and encompasses some of the sources of variability of 25(OH)D not taken into account by the predicted 25(OH)D score. The most important of these is actual sun exposure behaviors, such as type of clothing and use of sunscreen. However, the predicted 25(OH)D measure may provide a reasonable assessment of long-term vitamin D status because some factors accounted by the predicted 25(OH)D score are immutable (for example, skin pigmentation) or were updated every 2–4 years (region of residence, body mass index, physical activity, and diet). In contrast, a single measure of circulating 25(OH)D level could be heavily influenced by relatively recent exposures and depend on season, and thus may be less representative of long-term exposure. Given these caveats about individual measurements, we have therefore utilized a multi-tiered approach to evaluate the following endpoints.

4 Coronary heart disease and myocardial infarction (MI)

$1,25(OH)_2D_3$ exerts physiologic effects on vascular smooth muscle cells, vascular endothelium and cardiomyocytes. Low levels of 25(OH)D have been associated with MI, congestive heart failure, and calcific aortic stenosis [22].

Plasma 25(OH)D and incident MI was studied in a nested case-control study in the HPFS [10]. Eighteen thousand two hundred and twenty-five men without a diagnosis of cardiovascular disease at baseline and who provided blood samples between 1993 and 1999 were included. After 10 years of follow-up, 454 men developed nonfatal MI or fatal coronary heart disease, as documented by medical record review. Nine hundred controls were matched by age, date of blood collection and smoking status. Men who were either deficient or insufficient in vitamin D had an increased risk of MI compared with men with 25(OH)D $\geq$ 30 ng/mL. Men with 25(OH)D $\leq$ 15 ng/mL had more than a twofold increased risk of MI compared with men with 25(OH)D $\geq$ 30 ng/mL (relative risk (RR) 2.42, 95% confidence interval (CI): 1.53–3.84; p for trend $<$ 0.001). After full adjustment for known risk factors of coronary artery disease, this relationship was mildly attenuated but remained significant (RR 2.09; 95% CI: 1.24–3.54; p for trend = 0.02). Men with mildly insufficient 25(OH)D (22.6–29.9 ng/mL) also had an increased risk of MI (RR 1.60; 95% CI: 1.10–2.32; p for trend = 0.02). While known cardiovascular disease risk factors (family history of MI, body mass index, alcohol consumption, physical activity, history of diabetes mellitus and hypertension, ethnicity, geographic region, marine ω-3 fatty acid intake, low- and high-density lipoprotein cholesterol levels, and triglyceride levels) were independent risk factors among men in this cohort, vitamin D deficiency remained a strong, independent risk factor. Each 1 ng/mL increment in 25(OH)D corresponded with a 2.1% decreased risk of MI.

5 Hypertension

Antihypertensive features of vitamin D include suppression of the renin–angiotensin–aldosterone system [23, 24], regulation of calcium metabolism through prevention of secondary hyperparathyroidism, renoprotection, and direct effects on vascular cells including endothelial cells, vascular smooth muscle cells and macrophages that all express the VDR and 1-α-hydroxylase [2, 12, 13]. Clinical studies largely although inconsistently demonstrate that additional vitamin D promotes arterial blood pressure lowering, particularly among vitamin D deficient patients with elevated blood pressure [25].

5.1 Measured plasma 25(OH)D

Prospective analyses of 25(OH)D and vitamin D intake and incident hypertension were conducted in the HPFS and NHS I [11]. In the HPFS, among a group of men who had 25(OH)D measured, there were 133 incident cases of hypertension after 8 years of follow-up. These cases were based on self-report of a physician's diagnosis of hypertension. A validation study based on a sample of the self-reports documented that these health professionals were highly accurate in the reporting of high blood pressure. The RR of hypertension for men with vitamin D deficiency (25(OH)D $\leq$ 15 ng/mL) compared with men with

25(OH)D ≥ 30 ng/mL was 3.53 (95% CI: 1.02–12.3). The RR was no longer significant in the fully adjusted model: RR 3.03 (95% CI: 0.94–9.67), although the RR did not change appreciably. In the NHS I, there were 274 incident cases of hypertension after 8 years of follow-up. Women with 25(OH)D ≤ 15 ng/mL compared with ≥ 30 ng/mL did not have an increased risk of incident hypertension in the multivariate model: RR 1.42; (95% CI: 0.79–2.56). Although there was an inverse association between 25(OH)D and hypertension in men but not older women, there was limited power to ascertain whether this is a true biologic difference by sex.

An inverse association between 25(OH)D and incident hypertension of similar magnitude as in the HPFS was reported in a nested case-control study in the NHS II, a younger cohort of female registered nurses [9]. Women in the lowest quartile of 25(OH)D (median 16.7 ng/mL, range 6.2–21.0 ng/mL) compared with the highest quartile of 25(OH)D (median 37.9 ng/mL, range 32.3–89.5 ng/mL) had an increased odds of incident hypertension: odds ratio (OR) of 2.21 (95% CI: 1.57–3.12; p for trend < 0.001). This association was attenuated but remained significant after multivariable adjustment for risk factors for hypertension: OR 1.66 (95% CI: 1.11–2.48; p for trend = 0.01).

5.2 Predicted plasma 25(OH)D

Predicted 25(OH)D levels were computed for individuals in the HPFS and NHS using a prediction model published previously [26]. After 16 years of follow-up in the HPFS, 38,388 cases of hypertension were identified [11]. Men in the first decile of predicted 25(OH)D (mean 23.6 ng/mL, range 13.7–24.8 ng/mL) compared with men in the tenth decile (mean 33.0 ng/mL, range 32.1–36.1 ng/mL) had a 2.31-fold increased risk of hypertension (95% CI: 2.03–2.63; p for trend < 0.001) in the multivariate model. Women followed for 18 years in the NHS I had a similar RR of incident hypertension 2.27 (95% CI: 2.15–2.39; p for trend < 0.001) in the multivariate model for the first decile (mean 21.4 ng/mL, range 7.3–22.8 ng/mL) compared with the tenth decile (mean 33.7 ng/mL, range 32.5–37.6 ng/mL) of 25(OH)D.

5.3 Vitamin D intake

Vitamin D from dietary and supplemental sources was examined in the HPFS, NHS I, and NHS II cohorts [27]. Total vitamin D intake was not associated with reduced incidence of hypertension in the three cohorts each followed for ≥ 8 years. In the HPFS, the RR of incident hypertension was 1.03 (95% CI: 0.83–1.15) for men with the highest compared with lowest vitamin D intake, quartile median 748 IU/day and 99 IU/day, respectively. The RR of incident hypertension among older women in NHS I was 0.98 (95% CI: 0.93–1.04) for the highest (quartile median 646 IU/day) compared with lowest vitamin D intake (quartile median 79 IU/day). In the NHS II of younger women, there was a small, non-significant increased risk of hypertension for those in the highest quartile of vitamin D intake (median 742 IU/day) *versus* the lowest (median 128 IU/day): RR 1.13 (95% CI: 0.99–1.29). Cumulatively, these cohorts suggest increased dietary vitamin D intake does not reduce risk of hypertension. However, individuals in all three cohorts had vitamin D intake that was low and likely below the necessary intake to observe reduced incidence of hypertension. In contrast, increased plasma 25(OH)D was associated with reduced incidence of hypertension in the HPFS, NHS I and NHS II. Alternatively, it is possible that the 25(OH)D levels were confounded by other factors, although BMI and physical activity levels were adjusted for in multivariable analysis.

6 Plasma C-peptide

Vitamin D has been shown to counter the mechanisms through which glucose intolerance and diabetes mellitus type II develop. Through both direct and indirect effects on insulin secretion, insulin action, and cytokines, vitamin D has been shown to improve insulin sensitivity and pancreatic β-cell function and reduce systemic inflammation [28, 29].

Plasma C-peptide level is proportional to the amount of insulin secreted and thus is a measure of insulin resistance and insulin secretion. Circulating C-peptide has a longer half-life than insulin and thus may be a better integrated measure of insulin. Plasma C-peptide was examined in two cross-sectional analyses of healthy men in the HPFS and healthy women in the NHS I [30]. C-peptide level was 23% lower among men with the highest *versus* lowest quartile of plasma 25(OH)D in the multivariate model (p for trend = 0.03). The strength of the association was attenuated and not statistically significant with further adjustment for body mass index and dairy intake: C-peptide was 19% lower for men in the highest compared with lowest quartile of 25(OH)D (p for trend = 0.08). C-peptide level did not vary appreciably among women by 25(OH)D status in the fully adjusted model: plasma C-peptide in the highest quartile was 2.09 μg/L compared with 2.25 μg/L in the lowest quartile (p for trend = 0.30).

7 Cancer

Local production of $1,25(OH)_2D_3$ and extracellular Ca^{2+} act jointly as key regulators in cell proliferation, differentiation, and physiologic function [31]. The association between vitamin D and cancers of the colon, breast, prostate, pancreas, and ovaries has been examined in epidemiologic studies [32]. The findings related to cancer from the Harvard cohorts have been previously reviewed in detail [14]. The most recent findings are briefly summarized here.

7.1 Colorectal cancer and adenoma

In the NHS I and HPFS, colorectal cancer and adenoma have been followed and updated over decades using multiple medical history questionnaires and confirmed pathology reports. In the most recent analysis of the NHS I, 2747 cases of adenoma (1064 large, 1531 small, 2085 distal colon, and 779 rectal) were diagnosed among 48 115 female nurses who had an endoscopy before 2002. Women with the highest quartile of total vitamin D intake (median 601 IU/day) *versus* lowest quartile (135 IU/day) had a 33% reduced risk of distal colon adenoma (RR 0.67; 95% CI: 0.52–0.87, p for trend = 0.004) and 21% reduced risk of distal colorectal adenoma (RR 0.79; 95% CI: 0.63–0.99, p for trend = 0.07) [33]. In the HPFS there was an inverse linear trend for total vitamin D intake and colorectal adenoma through the fourth but not fifth quintile (RR 1.29; 95% CI 0.87–1.93) compared with the lowest quintile [34].

With respect to colorectal cancer, there were 203 incident cases of colon cancer diagnosed between 1986 and 1992 in the HPFS. There was a strong, although not statistically significant association with vitamin D supplements (RR 0.48; 95% CI: 0.22–1.02) and weak inverse association for dietary vitamin D intake (RR 0.88; 95% CI: 0.54–1.42) [35]. In the NHS I, there were 501 incident cases of colorectal cancer documented between 1980 and 1992. Premenopausal women with the highest quartile of total and dietary vitamin D intake had a lower risk of colorectal cancer in the multivariate model (RR 0.84; 95% CI: 0.63–1.13 and RR 0.88; 95% CI: 0.66–1.16, respectively) [36].

Plasma 25(OH)D and risk of colorectal cancer and adenoma were also investigated in the NHS and HPFS. Risk of colorectal cancer was related to 25(OH)D status in women with the highest *versus* lowest quartile of 25(OH)D in a linear inverse relationship (RR 0.51; 95% CI: 0.26–1.00; p for trend = 0.02) [37]. Among men, there was an inverse association between 25(OH)D and risk of colon cancer (RR 0.46; 95% CI: 0.24–0.89; p for trend = 0.005) but not rectal cancer based on only 40 cases (RR 3.32; 95% CI: 0.87–12.69; p for trend = 0.08) [37]. Adenoma risk in women based on 326 cases between 1989 and 1996 was not associated with 25(OH)D [38] although there was a modest but non-significant reduced risk in an updated analysis with higher plasma 25(OH)D levels (unpublished data).

Risk of colorectal cancer was also assessed using a predicted 25(OH)D score based on a multiple linear regression of independent variables of vitamin D status: geographical residence, leisure-time physical activity as a proxy for UV-B exposure, skin pigmentation, dietary and supplemental intake, and body mass intake. Among 691 cases diagnosed between 1986 and 2000 in the HPFS, a 10 ng/mL incremental increase in 25(OH)D was associated with a reduced risk of colorectal cancer (RR 0.63; 95% CI: 0.48–0.83) [26]. The same 25(OH)D score was used to analyze 1017 prospective cases of colorectal cancer mortality in the NHS I and HPFS from 1986 to 2004. Overall survival was highest among individuals with higher predicted 25(OH)D (adjusted hazard ratio 0.50; 95% CI, 0.26–0.95; p for trend = 0.02) [39]. Cancer-specific survival was also improved though the results did not attain statistical significance.

7.2 Breast cancer

Risk of breast cancer by vitamin D intake, 25(OH)D and $1,25(OH)_2D_3$ status were assessed in the NHS I. In a nested case-control study, 701 incident breast cancer cases and 724 controls matched by age, menopausal status, postmenopausal hormone use, and fasting status, date, and time of blood draw were identified [40]. Cases had a significantly lower mean 25(OH)D (31.5 ng/mL) compared with controls (33.1 ng/mL) ($p = 0.01$). In the multivariate model, women with the highest 25(OH)D status had a 35% reduced risk of incidental breast cancer (RR 0.65; 95% CI: 0.40–1.06, p for trend = 0.06). This association was stronger and significant for women at least 60 years of age (RR 0.57; 95% CI: 0.31–1.04, p for trend = 0.03). There was no association between $1,25(OH)_2D_3$ level and incident breast cancer risk.

Vitamin D intake and risk of breast cancer was assessed in the NHS I between 1980 and 1996 [41]. Among premenopausal women, there was an inverse linear dose-response relationship between total vitamin D intake and RR of breast cancer. Women who consumed a mean of >500 IU/day between 1980 and 1996 had a 28% decreased risk of breast cancer (RR 0.72; 95% CI: 0.55–0.94, p for trend = 0.01). There was no significant association between total or dietary vitamin D intake for postmenopausal women. It is unclear why plasma 25(OH)D was more strongly associated with risk in post-menopausal women, whereas vitamin D intake was more predictive of risk in pre-menopausal women. It is possible that these differential associations by menopausal status could have arisen by chance.

7.3 Prostate cancer

Vitamin D intake, 25(OH)D, and risk of prostate cancer were assessed in the HPFS and PHS. In the PHS, 232 cases were diagnosed up to 1992 from 14 916 men who provided plasma samples between 1982 and 1983 [42]. There was a non-significant association between 25(OH)D and prostate cancer incidence for individuals in the highest *versus* lowest quartile of 25(OH)D (RR 0.92; 95% CI: 0.56–1.50; p for trend = 0.82). Aggressive prostate cancer (defined as high grade or advanced stage) was more inversely but non-significantly associated with high *versus* low 25(OH)D (RR 0.82; 95% CI: 0.42–1.61). In the HPFS, 460 incident cases of prostate cancer were diagnosed in men who provided a blood specimen between 1993 and 1995 and were followed through 1998 [43]. There was no association between 25(OH)D status and prostate cancer risk (OR 1.19; 95%

CI:0.79–1.79 for the fourth quartile *versus* first quartile, p for trend = 0.59). Most prostate cancer cases were early cases diagnosed by prostate specific antigen. There was insufficient power to specifically examine advanced stage prostate cancer. In an earlier study in the HPFS, total vitamin D intake was not associated with prostate cancer risk (multivariate RR 1.21; 95% CI: 0.92–1.58 for vitamin D ≥ 800 IU/day *versus* < 150 IU/day; p for trend = 0.87) [44].

7.4 Pancreatic cancer

Vitamin D intake and risk of pancreatic cancer was studied in the HPFS and NHS I [45]. Through follow-up from 1986 (for HPFS) or 1984 (for NHS I) to 2000, 365 incident cases of pancreatic cancer were identified in the combined cohorts. Individuals with vitamin D intake ≥ 600 IU/day had a 41% reduced risk of pancreatic cancer compared with individuals with vitamin D intake < 150 IU/day in the pooled multivariate analysis (RR 0.59; 95% CI: 0.40–0.88, p for trend = 0.01). The association was stronger and significant in men (RR 0.49; 95% CI: 0.29–0.82; p for trend = 0.01) compared with women (RR 0.76; 95% CI: 0.42–1.38; p for trend = 0.47), although greater power would be necessary to determine a true biologic difference.

Predicted vitamin D status and risk of pancreatic cancer was also investigated in the HPFS [26]. Risk of pancreatic cancer was inversely related to predicted 25(OH)D for an incremental increase of 25 nmol/L (RR 0.49; 95% CI: 0.28–0.86).

8 Multiple sclerosis

Vitamin D is thought to exert an immunomodulatory role in the central nervous system [46]. Epidemiologic studies have shown an association between increased latitude and low serum vitamin D with increased prevalence of multiple sclerosis [47, 48].

Vitamin D intake and incidence of multiple sclerosis was examined in the NHS I (1980–1998) and NHS II (1991–1999) cohorts [49]. Vitamin D was measured at baseline and updated every 4 years to assess cumulative average intake. Vitamin D from supplements but not food sources was associated with reduced risk of MS for women in the NHS I. Women in the NHS I who consumed at least 400 IU/day had a significant 65% reduced risk of multiple sclerosis (RR 0.39, 95% CI: 0.18–0.85; p for trend = 0.007) compared to women with less than 400 IU/day of supplemental intake. There was no reduction in MS for younger women in the NHS II with increased intake of vitamin D from food (RR = 0.78, 95% CI: 0.39–1.54; p for trend = 0.56) or supplemental sources (RR = 0.75, 95% CI: 0.44–1.26; p for trend = 0.20). Of note, the median intake of vitamin D from supplements and food sources was higher in women in the NHS II compared with the NHS I. The age-adjusted pooled RR comparing women with vitamin D intake ≥ 400 IU/day *versus* < 400 IU/day of supplemental intake was 0.57 (95% CI: 0.34–0.94; p for trend = 0.04). This association was unchanged after further adjustment for cigarette smoking and latitude at birth: RR 0.58 (95% CI: 0.35–0.96; p for trend = 0.06). This study did not specifically stratify for supplemental intake greater than 800 or 1000 IU/day, as in the 1990s and earlier; vitamin D was most commonly consumed in doses of 200–400 IU daily. A further limitation is that this study did not examine serum 25(OH)D levels.

9 Bone fractures

The role of vitamin D in bone metabolism occurs through calcium and phosphorus regulation as well as the nuclear VDR. Calcium regulation and the $1,25(OH)_2$D-parathyroid hormone axis is well established. Of comparable importance is phosphate regulation through the induction of osteoblast fibroblast growth factor 23 (FGF23), which inhibits renal reabsorption of phosphorus and represses $1,25(OH)_2D_3$ synthesis. $1,25(OH)_2D_3$ also regulates several co-modulators and chromatin remodeling enzymes [50]. Further, the VDR selectively binds certain ω-3/ω-6 polyunsaturated fatty acids to facilitate transcriptionally active VDR-retinoid X receptor complexes and $1,25(OH)_2D_3$-independent signaling pathways in bone, intestine, and other VDR-containing tissues [51].

Dietary and supplemental vitamin D intake and incident hip fractures were assessed in a prospective analysis of 72 337 postmenopausal women in the NHS I. During 18 years of follow-up, 7466 (10%) women reported a diagnosis of osteoporosis, and 603 incident hip fractures were identified [52]. Vitamin D was assessed at baseline in 1980 and updated every 2 years during follow-up to assess cumulative average intake. Increased dietary and total vitamin D intake were associated with decreased fracture risk. Women with ≥ 400 IU/day of total vitamin D intake had a 37% lower risk of hip fracture compared with women who consumed < 140 IU/day (RR 0.63; 95% CI: 0.42–0.94). The inverse association was stronger among women with ≥ 250 IU/day of dietary vitamin D intake compared with < 100 IU/day; the RR of fracture risk was 0.57 (95% CI: 0.41–0.78). These results suggest dietary sources of vitamin D are as important as total vitamin intake, possibly because the high level of retinol in many multivitamin supplements may offset some of the benefits of vitamin D. Strengths of this study include the use of hip fractures as the outcome rather than intermediate markers of bone mass density. Results may be limited to Caucasian women and those residing in latitudes similar to those in the USA.

10 Concluding remarks and recommendations

The studies of vitamin D from the Harvard cohorts have provided evidence for a beneficial role of vitamin D against a

number of major chronic diseases. These include cardiovascular disease, hypertension, bone fractures, some cancers, multiple sclerosis, and possibly insulin resistance. Each of these relationships has already received some support from the literature. A meta-analysis of randomized clinical trials of vitamin D found a lower risk of bone fractures in individuals receiving adequately high doses of vitamin D, typically >400 IU/day [53]. A meta-analysis of prospective studies of 25(OH)D and risk of colorectal cancer indicates similar results as we found [54]. A similar risk reduction of cardiovascular disease has also been reported recently in several other cohort studies [55, 56]. A study based on the Department of Defense Repository found a strong inverse association between 25(OH)D and multiple sclerosis [15]. Interestingly, the association was particularly strong in younger individuals (for example, <20 years old), which could not be studied in the cohorts of older individuals.

Short of randomized clinical trials, data from long-term, prospective cohort studies are generally considered to represent the strongest data to assess an exposure-disease relationship. Because vitamin D status is not randomly allocated, it is important to evaluate the potential for confounding. One of the advantages of the Harvard cohorts and similarly designed studies is the extensive information on numerous covariates including diet, physical activity, and body habitus, all of which have been reported to influence vitamin D levels. In general, controlling for these and other potential confounding factors did not change the findings or conclusions for 25(OH)D. Exceptions include the association between 25(OH)D and blood pressure, which was attenuated after controlling for potential confounders, and the 25(OH)D and C-peptide association, which became non-significant in the fully adjusted multivariate model. The attenuation of the 25(OH)D and C-peptide association may have been due to over-adjustment from dairy intake, which partially contributes to 25(OH)D status. Nonetheless, confounding by factors that were not considered or measured adequately could have occurred. While further data, including from randomized trials, are forthcoming, recommendations regarding adequate vitamin D status for patients and populations based on current evidence are necessary, although they may need to be modified based on future studies. Based on the findings from the Harvard cohorts, as well as many other studies, the risk of various chronic disease endpoints is minimized at a circulating vitamin D level of at least 30 ng/mL.

The current adequate intakes for vitamin D (200 IU/day for young adults <51 years, 400 IU/day for adults 51–70 years, and 600 IU/day for those aged >71 years) were based on recommendations from the Institute of Medicine and Food and Nutrition Board established in 1997 [57]. These recommendations have not changed despite substantial growing evidence that higher intakes are necessary to achieve optimal circulating 25(OH)D levels for the majority of US adults. For adults who receive minimal sun exposure (UV-B radiation), much higher intakes of vitamin D are required to achieve levels in the range of 30–40 ng/mL. For most individuals, 1000–2000 IU/day may be sufficient, although those with very minimal sun exposure over prolonged periods may require even greater intake.

Vitamin D intake of 2000 IU/day is the current upper tolerated dose for adults [57], but systematic and comprehensive reviews of the entire vitamin D literature, including a risk assessment review of rigorous human clinical trials of vitamin D (using the same methodology as the Food and Nutrition Board), indicate that the upper limit for vitamin D for adults should probably be at least 10 000 IU/day [58]. Vitamin D synthesis in the magnitude of 10 000–20 000 IU/day occurs physiologically through solar UV-B exposure with mechanisms to protect against intoxication. Moreover humans express 25(OH)D-24-hydroxylase (CYP24) to catabolize excess 25(OH)D and $1,25(OH)_2D_3$ to the inactive, water-soluble vitamin D metabolite calcitroic acid that is then excreted [59]. Thus, daily doses of vitamin D in the range of 1000–2000 IU/day are likely safe and efficacious [2, 5, 60–62], and some studies additionally report vitamin D_3 is safe at doses of $\geq$10 000 IU daily [63], weekly, or every other week [4, 5, 64].

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