How Follow-Up Period in Prospective Cohort Studies Affects the Relationship Between Baseline Serum 25(OH)D Concentration and Risk of Stroke and Major Cardiovascular Events

[William B. Grant](#) * and [Barbara J. Boucher](#)

Review

How Follow-Up Period in Prospective Cohort Studies Affects the Relationship between Baseline Serum 25(OH)D Concentration and Risk of Stroke and Major Cardiovascular Events

William B. Grant [1],* and Barbara J. Boucher [2]

[1] Sunlight, Nutrition and Health Research Center, 1745 Pacific Ave., Suite 504, San Francisco, CA 94109, USA

[2] The Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London E1 2AT, UK*

* Correspondence: wbgrant@infionline.net

Abstract: Background/Objectives: Prospective cohort studies are useful for studying how biomolecular status affects risk of adverse health outcomes. Less well known is that the longer the follow-up time, the lower the apparent effect due to "regression dilution." Here we evaluate how follow-up time affects the relationship between serum 25-hydroxyvitamin D [25(OH)D] concentration and incidence of stroke and major cardiovascular events (MCEs). **Methods:** Findings regarding the relative risk (RR) of stroke and MCEs with respect to serum 25(OH)D concentrations at baseline from prospective cohort studies were plotted against mean follow-up time. Fifteen studies from mainly European countries and the United States were used for stroke, with nine studies for MCEs. Linear regression analyses were performed for follow-up periods of up to 10 years. **Results:** For stroke, the linear regression fit for 1–10 years is RR = 0.34 + (0.065 × follow-up [years]), $r = 0.84$, adjusted $r^2 = 0.67$, $p < 0.001$. No significant change was evident for studies with follow-up periods of 10–20 years. For MCEs, the linear fir for 1–8.1 years is RR = 1.61 − (0.074 × follow-up [years]), $r = 0.75$, adjusted $r^2 = 0.49$, $p = 0.03$. **Discussion:** The shorter the follow-up period, the greater the apparent effect of vitamin D in reducing risk of stroke and MCEs. In addition, the apparent effect of higher 25(OH)D concentration found for the shortest follow-up time is more than twice as high as the estimate based on averaging the results for all studies without considering follow-up time. Mechanisms have been found to explain how higher serum 25(OH)D concentrations could reduce risk of stroke and MCEs. Randomized controlled trials have not shown that vitamin D supplementation significantly reduces risk of either stroke or MCE, probably because risk of both outcomes increases rapidly below 15 ng/mL (38 nmol/L) and it is difficult in Western developed countries to enroll enough participants with concentrations that low. Nonetheless, vitamin D's role in reducing risk of stroke and MCE can be considered causal on the basis of an evaluation of the evidence with respect to Hill's criteria for causality in a biological system. **Conclusions:** Serum 25(OH)D concentrations above 20 ng/mL are associated with significantly reduced risk of stroke and MCEs in an apparent causal manner. Raising serum 25(OH)D concentrations to >20 ng/mL should be recommended for everyone likely to be at risk for stroke or MCE and indeed in the general population.

Keywords: cardiovascular disease; causality; follow-up period/time; heart failure; hemorrhagic; hypertension; ischemic; prospective cohort study; stroke; vitamin D

1. Introduction

The prospective cohort study is a type of observational study commonly used to assess how dietary and lifestyle factors and biological variables affect health outcomes. Participants are recruited

and enrolled, information relevant to the study is obtained from each participant, and participants are followed up for some period during which various health outcomes of interest are recorded. Afterward, health outcome rates are compared statistically with data obtained at enrollment. Most such studies do not remeasure any variables assessed during the follow-up period. Therefore, an underestimation of risk associations due to "regression dilution" generally occurs in long-term follow-up of prospective studies, as outlined by Clarke and colleagues in 1999 [1]. Those authors reported repeated measurements over 25 years for systolic and diastolic blood pressure and blood cholesterol for participants in the Framingham Study. The researchers showed that the range from high to low for the first and fifth quantile shrank by 65%, 75%, and 57%, respectively. Although the article had 897 citations by August 31, 2024, according to Google Scholar, the research seems not to have had much effect on conduct of prospective cohort studies or, more important, on meta-analyses of such studies. The concentration of 25-hydroxyvitamin D [25(OH)D] in serum changes over time. For example, a study in Norway reported that the correlation coefficient, r, for serum 25(OH)D concentrations measured in 2668 participants in 1994 and again in 2008 and adjusted for season of measurement was 0.42 [2]. In comparison, the correlation coefficients for systolic blood pressure, diastolic blood pressure, pulse, body mass index (kilograms per square meter of body surface area), serum total cholesterol, and triglycerides between 1994 and 2008 values were 0.47, 0.42, 0.46, 0.83, 0.37, and 0.49, respectively. The effect of changes in serum 25(OH)D has been known for cancer since 2011 [3] and for all-cause mortality rate since 2012 [4], yet that effect was overlooked in a highly cited meta-analysis of risk of colorectal cancer with respect to serum 25(OH)D concentration in 2019 [5], as Muñoz and Grant pointed out in 2022 [6].

Recently, researchers showed that the same effect (of changes in vitamin D status over time) is found for risk of cognitive impairment, dementia, and Alzheimer's disease. The 2024 meta-analyses included 15 prospective studies regarding dementia and/or Alzheimer's disease and nine regarding cognitive impairment [7]. As shown in plots of risk ratio for low versus high 25(OH)D concentration with different follow-up periods, linear decreases emerged in the regression fit to the data from near 2.0 for the shortest follow-up periods (near 4–5 years) to near 1.0 for follow-up periods near 13 years [8].

Stroke is an important cause of disability and death. In 2016, an estimated 13.7 million new incident strokes occurred globally, of which about 87% were ischemic strokes [9]. In 2017, an estimated 1.12 million incident strokes occurred in the European Union, with 9.53 million stroke survivors and 7.06 million disability-adjusted life years lost because of stroke [10]. A prospective study of 418,329 participants in the European Prospective Investigation into Cancer and Nutrition (EPIC) included an analysis of dietary risk factors for stroke [11]. Risk of ischemic stroke was inversely associated with consumption of fruit and vegetables, dietary fiber, and dairy foods, whereas risk of hemorrhagic stroke was positively associated with egg consumption.

A 2021 meta-analysis of prospective cohort studies of risk of stroke with respect to serum 25(OH)D by Su and colleagues [12] suggested that a similar analysis now be repeated, as did a recent meta-analysis of relevance by Xiong and colleagues [13].

2. Materials and Methods

The data used here are from the prospective cohort studies in Su and colleagues [12] and Xiong and colleagues [13]. Tables 1 and 2 list the studies in ascending order of follow-up period. Table 1 includes the OR/RR from Su and colleagues [12] and Xiong and colleagues [13] and the follow-up period and 25(OH)D concentration comparison obtained from each article. We excluded four studies from the analysis: Two did not have enough information on follow-up period or on how 25(OH)D concentrations were compared, whereas two others were based on dietary vitamin D intake.

Table 1. Findings for stroke studies from data listed in Su and colleagues [12] and Xiong and colleagues [13].

Follow-up (yrs)	Type of stroke	Inc or mor	OR/RR (95% CI)	25(OH)D comparison (ng/mL)	Ref.
NA			1.41 (0.64–3.13)		Guo 2017* [14]
NA			1.19 (0.79–1.79)		Leu Agelii 2017* [15]
1		Inc	0.42 (0.14–1.28)	≥10 vs. <10	Zittermann 2016 [16]
1.3		Inc	0.56 (0.38–0.84)	>30 vs. ≤15	Anderson 2010 [17]
3.1		Inc	0.54 (0.34–0.85)	>30 vs. <20	Judd 2016 [18]
4		Inc	0.33 (0.15–0.73)	>30 vs. ≤10	Drechsler 2010 [19]
5		Inc	0.71 (0.40–1.25)	≥20 vs. <20	Bolland 2010 [20]
6.8		Inc + mor	0.91 (0.81–1.02)	per +10*	Perna 2013 [21]
			0.76 (0.55–1.05)	<12 vs. ≥20	
7.6		Inc	0.60 (0.59–1.09)	Q4 (27 median) vs. Q1 (12 median)*	Kuhn 2013 [22]
			0.65 (0.44–0.95)	≥20 vs. <10	
8.0		Inc	0.93 (0.46–1.85)	≥20 vs. <20	Welles 2014 [23]
9.3	I	Inc	0.81 (0.70–0.94)	≥20 vs. <10	Afzal 2017 [24]
10		Inc + mor	1.00 (0.51–1.94)	High vs. low tertile	Marniemi 2005 [25]
			0.88 (0.49–1.61)	Middle vs. low tertile	
10		Inc	1.13 (0.80–1.59)	Fourth vs. first quartile	Skaaby 2013 [26]
10.3	All		0.56 (0.36–0.86)	Lowest vs. highest quintile	Leung 2017 [27]
	I	Inc	0.55 (0.35–0.86)	Middle vs. highest quintile	
10.6		Inc	0.91 (0.75–1.11)	One 25(OH)D SD increase	Berghout 2019 [28]
16		Inc or mor	0.60 (0.39–0.91)	≥20 vs. <20	Schierbeck 2012 [29]
19.3			0.66 (0.49–0.89)	>440 vs. <110 IU/day vitamin D	Sheerah 2018* [30]

20	Inc	0.75 (0.58–0.94)	≥31 vs. <17	Schneider 2015 [31]
34		0.82 (0.68–0.99)	>4 vs. <1.1 µg/day	Kojima 2012* [32]

*, omitted from the analysis; 25(OH)D, 25-hydroxyvitamin D; 95% CI, 95% confidence interval; I, ischemic; Inc, incidence; mor, mortality; NA, not available; OR, odds ratio; RR, relative risk.

Table 2. Baseline data for stroke studies listed in Su and colleagues [12] and Xiong and colleagues [13] used in this study.

Country	Patient characteristics	Mean age (± SD) or range (yrs)	BMI (± SD) (kg/m^2)	M, F (%)	Stroke type	N_S	N_C	Ref.
Germany	Left ventricular assist device implants	62 (37–81)	23 ± 3	100, 0	All	25		Zittermann 2016 [16]
		57 (49–66)	26 ± 5	85, 15			129	
USA	Community hospital	55 ± 21	NA	25, 75	All	208	25,818	Anderson 2010 [17]
USA	B and W community dwellers				I	536	1069	Judd 2016 [18]
Germany	Diabetic and on hemodialysis	66 ± 8		60, 40	All	89	1019	Drechsler 2010 [19]
New Zealand	Healthy community-dwelling	74 ± 4	NA	0, 100	All	59	1412	Bolland 2010 [20]
Germany	Population-based	65% 50–65; 35% 65–74	27 ± 5	41, 59	All	353	7356	Perna 2013 [21]
Germany	Population-based	51	NA	42, 58	All	471	1661	Kuhn 2013 [22]
USA	Stable CVD	66 ± 11	29	81, 19	All	49	897	Welles 2014 [23]
Denmark	General population	58 (48–68)	26 ± 3	48, 52	I	960	~115,000	Afzal 2017 [24]
Finland	Population-based	65-99	NA	48, 52	All	70	685	Marniemi 2005 [25]
Denmark	General population	49 (41–73)	26	50, 50	All	316	8830	Skaaby 2013 [26]
Hong Kong	Osteoporosis study, Chinese	63 ± 10		37, 63	All	244	3214	Leung 2017 [27]
					I	205	3253	

Netherlands	Population-based	65 ± 10	27 ± 4	43, 57	All	735	8603	Berghout 2019 [28]
Denmark	Osteoporosis study	50 ± 2	25 ± 5	0, 100	All	89	1924	Schierbeck 2012 [29]
USA	Population-based	57	NA	43, 57	All	804	11,354	Schneider 2015 [31]

*, data for participants with incident stroke: B, Black; BMI, body mass index (kilograms per square meter of body surface area); CVD, cardiovascular disease; F, female; I, ischemic; M, male; NA, not available; N_C, number of controls; N_S, number with incident stroke; SE, standard error; W, white.

Tables 3 and 4 list information from eight studies used to investigate the effect of follow-up period on risk of incidence of MCEs. The tables do not include studies regarding cardiovascular disease (CVD) mortality rate because mortality can be significantly affected by treatment, thereby obscuring the effect of serum 25(OH)D concentration. MCE incidence can also be lowered through intervention. For example, lowering blood pressure pharmacologically by 5 mmHg can lower incidence of MCEs by 5%–15% depending on baseline blood pressure [33].

Table 3. Findings for MCE rates in prospective cohort studies from studies in Grandi and colleagues [34], and Zhang and colleagues [35].

Follow-up (yrs)	RR (95% CI)	25(OH)D comparison (ng/mL)	Ref.
1.0	1.85 (1.25–2.75)	<9 vs. >9	de Metrio 2015 [36]
1.0	1.20 (0.72–2.00)	<12 vs. >12	Beska 2019 [37]
1.5	1.61 (1.15–2.27)	<7.3 vs. >7.3	Ng 2013 [38]
2.7	1.32 (1.07–1.63)	<12.7; 12.7-21.59; ≥21.6	Verdoia 2021 [39]
5	1.2 (0.7–2.2)	<20 vs. ≥20	Bolland 2010 [20]
7.6	1.62 (1.11–2.36)	<15 vs. >15	Wang 2008 [40]
8.0	1.11 (0.85–1.44)	≥20 vs. <20	Welles 2014 [23]
8.1	0.83 (0.37–1.86)	Quartiles	Grandi 2010 [41]

25(OH)D, 25-hydroxyvitamin D; 95% CI, 95% confidence interval; MCE, major cardiovascular event; RR, relative risk.

Table 4. Participant information for MCEs in prospective cohort studies from studies in Grandi and colleagues [34] and Zhang and colleagues [35].

Country	Patient characteristics	Mean Age (± SD) or range (yrs)	BMI (± SD) (kg/m²)	M, F (%)	Type of event	N_{MCDE}	N_C	Ref.
Italy	ACS	67 ± 12	27 ± 4	72, 28	MCE	125	689	de Metrio 2015 [36]
UK	After non–ST elevation ACS	81 ± 5	27 ± 5	62, 38	MCE	76	224	Beska 2019 [37]

Country	Population	Age	BMI	M, F	Event	N_{MCDE}	N_C	Reference
UK	Acute MI	66 ± 13	NA	72, 28	Nonfatal MCE	224	1035	Ng 2013 [38]
Italy	CAD undergoing percutaneous coronary intervention	68 ± 11	28 ± 5	73, 27	MCE	174	531	Verdoia 2021 [39]
New Zealand	Healthy community-dwelling	74 ± 4	NA	0, 100	MI	52	1419	Bolland 2010 [20]
USA	General population				Incident CVD			Wang 2008 [40]
USA	Stable CVD	66 ± 11	29	81, 19	CVD events	49	897	Welles 2014 [23]
Germany	Stable CHD				CVD events	148	977	Grandi 2010 [41]

ACS, acute coronary syndrome; BMI, body mass index (kilograms per square meter of body surface area); CAD, coronary artery disease; CHD, coronary heart disease; CVD, cardiovascular disease; F, female; HF, heart failure; M, male; MCE, major cardiovascular event; MI, myocardial infarction; NA, not available; N_{MCDE}, number with a major cardiovascular disease event; N_C, number of controls.

Data were analyzed using SigmaStat 4.0 (Grafiti, Palo Alto, CA, USA). Data plots were made using KaleidaGraph 4.5.4 (Synergy Software, Reading, PA, USA).

3. Results

Figure 1 shows a plot of the data from Table 1 along with the linear regressions calculated from those data. The data were analyzed after division into two groups of 1–10 years or >10-year follow-up periods between baseline vitamin D and determination of the incident events. Determination of the dividing year was made by inspecting the data plot. The graph shows a good linear fit to the data for follow-up periods of 1–10 years: RR = 0.34 + (0.065 × follow-up [years]), $r = 0.84$, adjusted $r^2 = 0.67$, $p < 0.001$. The regression fit for data from >10-year follow-up periods shows no significant change with increasing follow-up periods. In addition, the 95% confidence interval (95% CI) values are smaller for shorter follow-up periods. That result may simply reflect the higher events-to-controls ratios in studies with shorter follow-up periods. Although most studies gave results for all strokes (hemorrhagic and ischemic), ischemic strokes are much more frequent than hemorrhagic strokes in the countries studied [9]. Some reports included in the present study, however, showed that hemorrhagic strokes had weaker associations with baseline serum 25(OH)D concentrations than did ischemic stokes [18,28].
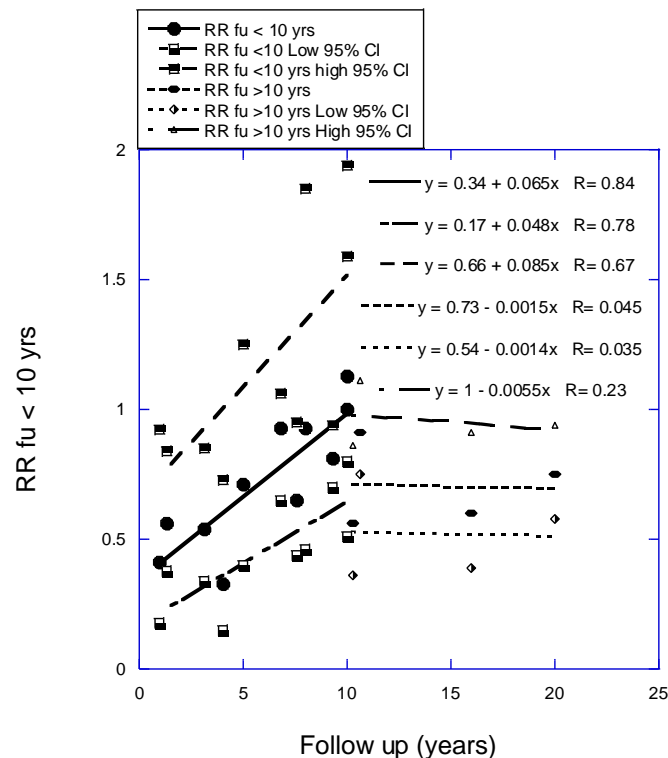
Figure 1. Plot of relative risk for stroke versus years of follow up (fu), with regression fits to studies of less than 10 years and for those carried out over more than 10 years. 95% CI, 95% confidence interval.

4. Discussion

The meta-analysis of RRs for stroke in relation to serum 25(OH)D concentration, based on 21 prospective observational studies by Su and colleagues [12], reported an average value of 0.78 (95% CI, 0.70–0.86), whereas that by Xiong and colleagues [13], based on a slightly different 21 prospective observational studies, showed an average value of 0.78 (95% CI, 0.70–0.87). Those values are approximately half the reduction seen for the 1-year follow-up period and 44% of the regression found for the zero follow-up period in the present study. That finding offers more evidence that not considering the effect of follow-up period in meta-analyses of observational studies with long follow-up times can greatly underestimate the effect of the variable studied. As a result, public policy recommendations are not as strong as they could be.

In a 2020 article, Shi and colleagues [42] calculated the dose–response relationship for 25(OH)D concentration and stroke risk by using mostly the same observational studies as Su and colleagues [12]. The result, shown in Figure 2 in ref. [42], was that risk decreased by ~20% with increasing vitamin D status between zero and ~20 ng/ml, with no further risk reduction between 20 and 40 ng/ml. However, as a result of not having accounted for variation in follow-up period, that analysis underestimates the reduction actually achievable with better vitamin D status. By contrast, the study does show that the main risk reduction occurs with increases in serum 25(OH)D up to 15 ng/mL. Very few randomized controlled trials (RCTs) could enroll participants with mean 25(OH)D concentrations that low, for ethical reasons, unless conducted in a country where such low 25(OH)D concentrations commonly persist. For example, many Middle Eastern countries have diets low in animal products and where people often wear clothing that covers most of the body and stay indoors during the hot summers [43]. Results of a stratified randomized field trial of vitamin D supplementation in pregnant women in Iran reported that the mean baseline 25(OH)D concentration was 11 ng/mL [44]. By supplementing the women at one hospital with enough vitamin D to raise 25(OH)D concentrations to above 20 ng/mL, significant reductions occurred for gestational diabetes,

preeclampsia, and preterm birth in comparison with outcomes in a comparable hospital where pregnant women stayed unsupplemented.

Comparing the differences in outcomes for stroke, CVD, and cancer is insightful. Cancer incidence and mortality rates used to have very large geographic variations in the United States [6], but stroke and CVD have not shown similar geographical variations. However, they do have more pronounced seasonal variations in mortality rates than cancer, with 20% higher mortality rates in winter than in summer in northern hemisphere countries [45]. The seasonal variation is due in part to the seasonal variations in serum 25(OH)D concentrations related to solar UVB doses [46,47]. Cancers have large geographical variations in midlatitude counties such as the United States [48] because serum 25(OH)D concentrations greater than 50 ng/mL reduce risk in comparison with lower concentrations, as shown for colorectal cancer [49] and breast cancer [50]. The differences in the 25(OH)D concentration–risk relationship also explain why the VITAL study—with a mean 25(OH)D concentration of 31 ng/mL for the participants in the vitamin D treatment arm whose serum 25(OH)D was measured—showed significantly reduced all-cancer incidence rates for participants with BMI < 25 kg/m^2 and reduced all-cancer mortality rates for the entire treatment group, but no significant effects for cardiovascular disease [51]. In that RCT, MCEs occurred in 3.1% of the vitamin D treatment arm and in 3.2% of the placebo arm, whereas strokes occurred in 1.1% of the vitamin D treatment arm and 1.2% of the placebo arm.

An important question is how rapidly vitamin D might reduce risk of adverse brain and other health outcomes. As discussed in the analysis of follow-up period for cognitive function, an RCT showed significant beneficial effects in improving cognitive function during 1 year of vitamin D supplementation [52]. To examine that question, we searched Google Scholar for representative RCTs that reported a beneficial effect on brain health in less than 1 year (Table 5). Significant benefits were found for depression and cognitive function within a year. Those studies show evidence that raising serum 25(OH)D concentrations can yield significant improvements in brain health in less than a year. Three of those papers dealt with studies from China, India, and/or Iran, whose serum 25(OH)D concentrations are generally low. That situation allows RCTs in those communities to be more likely to show health benefits from supplementation than studies done in countries with higher mean 25(OH)D concentrations.

Table 5. Results of short-term vitamin D supplementation on brain health.

Participants	Duration (wks)	Condition	Intervention	Outcomes	Ref.
Meta-analysis of 9 clinical trials, China and Iran	8–52	Mental health	50,000 IU/wk or 2 wks or higher single dose	Beck Depression Inventory, weighted mean difference, –3.9 (95% CI, –5.2 to –2.7)	[53]
46 patients, India; baseline 25(OH)D: N/A	12	Major depressive disorder	Usual treatment or usual treatment plus 3 million IU of vitamin D	Significantly greater improvement in depression score with vitamin D than placebo; also quality of life.	[54]
64 patients under methadone maintenance	24	Cognitive function	50,000 IU or placebo/2 wks	Vitamin D treatment resulted in significant improvement in Iowa Gambling Task,	[55]

treatment, Iran. Baseline 25(OH)D: 14 ± 4 ng/mL				Verbal Fluency Test, Reverse Digit Span, and visual working memory.	
42 women, USA mean age 58 ± 6 years, BMI, 30.0 ± 3.5 kg/m², baseline 25(OH)D: 23 ± 6 ng/mL	52	Cognitive outcome	600, 2000, or 4000 IU/day of vitamin D_3	2000-IU/day group had improved visual and working memory and learning; the 4000-IU/day group had slower attention reaction time.	[56]

25(OH)D, 25-hydroxyvitamin D; 95% CI, 95% confidence interval.

For many reasons, serum 25(OH)D concentrations change over time scales from months to years (Table 6).

Table 6. Why 25(OH)D concentrations may change over time.

Reason	Ref.
For increases in 25(OH)D concentrations	
Increased awareness of overall benefits of vitamin D	[57]
Increase amount of omega-3 fatty acid supplementation	[58]
Increased vitamin D supplementation after menopause	[59]
Vitamin D fortification of food instituted countrywide	[60]
For variable changes in 25(OH)D concentrations	
Change geographic location	[61]
Retire from work	
Change in physical activity	[2]
Change in season from winter/spring to summer/autumn	[46,47]
For reductions in 25(OH)D concentrations	
Decline with age due to reduced production from solar UVB	[62]
Change in diet with reduced meat, fish consumption	[63]
Increase in body mass	[64]
Increase in use of sunscreen/sunblock, clothing when in sunlight	[65]
Increased use of sunscreen in cosmetics	[66]
Moving into residential care	

25(OH)D, 25-hydroxyvitamin D; UVB, ultraviolet-B.

Several mechanisms have been identified that help explain how higher 25(OH)D concentrations can reduce risk of stroke. For example, a 2014 review [67] stated that vitamin D influences neuronal function by binding to vitamin D receptors that can act as transcription factors and regulate gene expression [68]. Particularly in the nervous system, vitamin D's biological effects appear to arise from stimulation of neurotrophic factors, quenching of oxidative hyperactivity, and regulation of autoimmune responses [69]. Increasing 25(OH)D concentrations through vitamin D supplementation causes more genes to be expressed (or, more rarely, fewer). Thirty healthy adults were randomized to receive 600, 4000, or 10,000 IU/day of vitamin D_3 for 6 months. The study showed a dose-dependent

25(OH)D alteration in broad gene expression with 162, 320, and 1289 genes, respectively, upregulated or downregulated in white blood cells [70].

Evidence also exists that vitamin D supplementation in vitamin D–deficient subjects can reduce serum concentrations of matrix metalloproteinases MMP-2 and MMP-9, as well as of the inhibitor, TIMP-1, and C-reactive protein [71]. That finding is relevant because people who develop MCEs have raised MMP-9 concentrations, and MMP-9 is an important risk factor for vulnerable atherosclerotic plaque [72].

Hypertension is a risk factor for stroke. A dose–response meta-analysis showed that higher adherence to antihypertension medications reduced the risk of hemorrhagic stroke by 45% (RR = 0.55 [95% CI, 0.42–0.72]) and ischemic stroke by 26% (RR = 0.74 [95% CI, 0.69–0.79]) [73]. A meta-analysis of serum 25(OH)D concentration on risk of hypertension in the general population based on cohort studies reported a significant increase of 38% (RR = 1.38 [95% CI = 1.14–1.64]) as 25(OH)D concentration decreased from 75 to 15 nmol/L [74]. The same paper showed no effect of vitamin D supplementation on systolic or diastolic blood pressure on the basis of 27 studies. However, three supplementation studies reported significant or near-significant reductions of systolic blood pressure [75–77]. Also, an open-label study in Canada in which participants took enough vitamin D_3 to increase serum 25(OH)D concentrations above 40 ng/mL reported significant reductions in the prevalence of hypertension [78]. Thus, some evidence shows that vitamin D does lower blood pressure and reduces the risk of established hypertension.

Table 7 lists more mechanisms by which vitamin D can reduce risk of stroke and MCEs.

Table 7. Mechanisms by which vitamin D can reduce risk of stroke and MCEs.

Mechanism	Ref.
Antifibrotic, antihypertrophic signaling	[79]
Anti-inflammatory, antioxidant effects	[80]
Atherosclerosis progression reduction	[81]
Reduces arterial stiffness and narrowing of the vessel lumen due to activation of the renin–angiotensin–aldosterone system	[81]
Cellular effects through effects on genes (cell cycle, proliferation, apoptosis, and angiogenesis)	[81]
Endothelial function maintenance	[82]
Insulin resistance risk reduction	[83]
Lipid metabolism regulation	[84]
MMP-2 and MMP-9 activity reduced, which reduces acute arterial event risk	[71,72]
Neuroprotective growth factor promotion	[85]
Reduced risk of plaque instability and acute arterial events	[86]
Reduction of blood pressure through vasodilation	[85]
Reduction of arterial pressure through effects on endothelial and muscle cells	[87]
Type 2 diabetes mellitus risk reduction	[88]

MCE, major cardiovascular event; MMP, matrix metalloproteinase.

RCTs have not supported the role of better vitamin D provision in reducing risk of stroke. A 2020 systematic review and meta-analysis of vitamin D supplementation and incidence of stroke included 13 RCTs [89]. The mean age was 66 years and the mean follow-up time was 3.1 years. The mean baseline 25(OH)D concentration for studies that reported values was 19.4 ng/mL (range, 8.8–25.4 ng/mL). The percentage of participants in those 13 trials who experienced a stroke was 2.1% in both the treatment and control arms, resulting in a RR for stroke of 1.00 (95% CI, 0.91–1.10). Inspecting the baseline characteristics of participants in those trials (in Table 1 in Nudy 2021 [89]) shows that

participants were being studied for various adverse health effects, including arthritis index pain, asthma exacerbations, progression to type 2 diabetes mellitus, falls and fractures, insulin sensitivity, and renal function. In other words, none of the trials was established specifically to evaluate the role of vitamin D supplementation, in deficiency, on the risk of stroke incidence.

A 2024 review included a different set of five vitamin D RCTs assessing the risk of ischemic strokes [90]. Mean baseline 25(OH)D concentrations were from 66 ± 23 to 77 ± 25 ng/mL in four trials and 38 ± 16 ng/mL in one trial. Follow-up duration ranged from 3.3 to 5.3 years. All those trials had CVD outcome as a primary outcome. Again, no significant difference in stroke risk was found between the vitamin D treatment and control arms, as could have been predicted from the baseline vitamin D status.

Even if the trials had been set up to test for stroke incidence, they probably would not have shown a beneficial effect for several reasons. First, nearly all vitamin D RCTs have been based on guidelines for pharmaceutical drugs. In such trials, the control arm does not receive the drug. That is not the case for vitamin D trials because vitamin D is a naturally occurring substance required for life. In 2014, Heaney outlined guidelines for trials regarding nutrients [91]. The main steps appropriate for vitamin D [92] are as follows:

1. Measure 25(OH)D concentrations and include participants with low concentrations appropriate for the outcome of interest.
2. Give a vitamin D dose large enough to raise 25(OH)D concentrations to levels at which beneficial effects are expected.
3. Measure achieved 25(OH)D concentrations.
4. Analyze results with respect to achieved vitamin D concentrations.

The second main reason for poor results from vitamin D RCTs is the enrollment of people with relatively high 25(OH)D concentrations. Other reasons include giving relatively low doses, too small to correct deficiency, and permitting participants in the control arms to take moderate vitamin D supplements; and analyzing results by intention to treat rather than by initial and achieved vitamin D status. Those common failures have been discussed in two 2022 reviews [93,94].

Risk of incidence of stroke and other CVD events increases rapidly as serum 25(OH)D concentrations fall below 20 ng/mL [23,95]. Thus, the large VITAL study [51], which enrolled participants whose mean baseline 25(OH)D concentration was 31 ng/ml in the vitamin D treatment arm, had no chance of finding any significant reduction of CVD from supplementation at 2000 IU/day of vitamin D_3. The failure to recruit participants with deficiency and permitting those in the placebo arm to take up to 600 or, for those over 70 years, 800 IU/day of vitamin D, reduced the possibility of any significant findings being able to emerge from the VITAL trial.

A stratified randomized field trial of vitamin D supplementation for pregnant women in Iran [44] shows how to design and carry out a vitamin D RCT more appropriately, here for CVD and stroke. First, participants had mean 25(OH)D concentrations of about 11 ng/mL. Second, serum 25(OH)D concentrations for the vitamin D treatment arm were measured during the trial. Those readings were used to adjust vitamin D doses to ensure that 25(OH)D concentrations greater than 20 ng/mL were achieved throughout the trial, as shown by follow-up 25(OH)D values, whereas participants in the control arm received no vitamin D supplements. Outcomes for gestational diabetes, preeclampsia, and preterm delivery were then evaluated with respect to achieved 25(OH)D concentrations, and significant reductions in those outcomes were found. Those findings were in contrast with results from standard vitamin D RCTs for pregnant women that were used to prepare a recent Cochrane review [96].

The allopathic medical system relies on RCTs to show effectiveness and limited adverse effects before approving pharmaceutical drugs for general use. As discussed, vitamin D RCTs based on guidelines for pharmaceutical drugs are not appropriate for vitamin D. In addition, Hill in 1965 proposed another way to ascertain causality in a biological system [97]. The criteria appropriate for vitamin D include strength of association, consistent findings in different populations, temporality, biological gradient, plausibility (e.g., mechanisms known), coherence with known science of the day, experiment, and analogy. Necessary adjustment for confounding factors and bias were added later

[98]. A 2024 review discussed how those considerations have influenced epidemiologic methods [99]. For vitamin D, examination of Hill's criteria for causality of inadequate vitamin D provision for various health outcomes (diseases) [94], including cardiovascular disease [100], suggested that the criteria were generally satisfied. The main limitation was in finding reliable evidence from experimentation owing to the problems with RCTs as discussed above and as recently reviewed [87].

Table 8 uses Hill's criteria to briefly outline the evidence that vitamin D reduces risk of stroke. As Hill noted, not all criteria need be satisfied for causality to be likely, though the more that are, the stronger the claim.

Table 8. Evaluation of findings regarding serum 25(OH)D concentration and risk of stroke and major cardiovascular disease events with respect to Hill's criteria for causality in a biological system [97].

Criterion	Strength of finding	Ref.
Strength of association	"Strong" as suggested by Figures 1 and 2.	
Consistency	"Strong," i.e., results from various European countries as well as the United States and Hong Kong were in general agreement with each other.	
Temporality	"Strong" as all the prospective cohort studies agreed.	
Biological gradient	"Strong" as the serum 25(OH)D concentration–risk relationship is well known across the literature on human health.	[42]
Plausibility	"Strong" as many mechanisms have been shown (see Table 7 and preceding text)	
Coherence	"Strong" because vitamin D has many mechanisms for maintaining good health, including affecting gene expression and downregulating adverse effects on immune and inflammatory processes in vivo.	[70]
Experiment	"Weak" as RCTs have not found that vitamin D supplementation reduces risk of stroke or CVD event, probably from not conducting RCTs of appropriate design (see Discussion).	[93,94,101]
Analogy	Similar findings are seen for Alzheimer's disease, dementia, and cognitive decline.	[8]
Confounding factors	A possible confounding factor is release of nitric oxide from subcutaneous nitrate stores through the action of UV irradiation, though strong evidence for that effect is lacking.	[102]

25(OH)D, 25-hydroxyvitamin D; CVD, cardiovascular disease; RCT, randomized controlled trial.
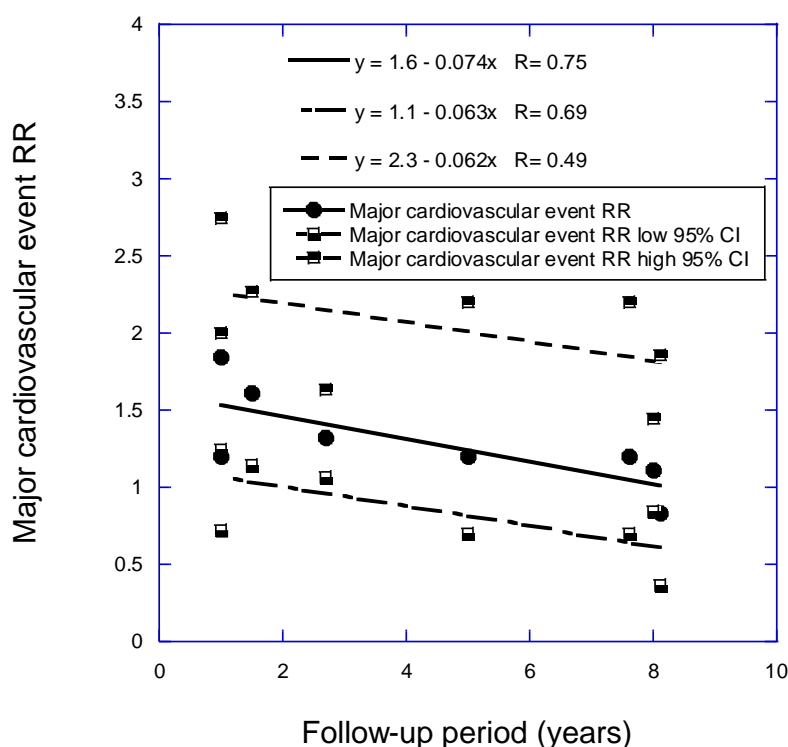
Figure 2. Plot of relative risk of a major cardiovascular event (MCE) versus mean follow-up period. The regression for risk of MCEs versus baseline serum 25-hydroxyvitamin D is relative risk (RR) = 1.61 – (0.074 × follow-up [years]), $r = 0.75$, adjusted $r^2 = 0.49$, $p = 0.03$. 95% CI, 95% confidence interval.

5. Conclusions

From our findings, the risks of stroke and of acute cardiovascular events could be minimized by keeping serum 25(OH)D concentrations above 20–30 ng/mL in the population. In normal-weight people, that level can be achieved with supplementation of at least 1000 IU/day of vitamin D3. A more reliable dose would be 2000 IU/day [103]. Obese people, however, should take 2–3 times higher doses and overweight individuals should take 1.5 times as much [104].

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