Estimating dose—response relationships for vitamin D with coronary heart disease, stroke, and all-cause mortality: observational and revised Mendelian randomization analyses

Emerging Risk Factors Collaboration/EPIC-CVD/Vitamin D Studies Collaboration

SUPPLEMENTARY METHODS

The stepwise selection method for selecting genetic variants to include in the genetic analyses had two components: a series of forward steps and then a backward step. In each forward step, genetic variants were ranked based on their associations with 25(OH)D concentrations conditional on variants selected at any previous step. At each step, the variant having the lowest p-value was selected. The process was repeated for each locus until no further variants were conditionally associated with 25(OH)D concentrations at a genome-wide level of significance ($p < 5\times10^{-8}$). Finally, a backward step was applied to omit any variant failing to meet the genome-wide level of significance for association with 25(OH)D concentrations in a joint model including all selected variants.

The adjusted analyses presented in Figure 1 include data on up to 431,489 individuals, comprising 67,992 from VitDSC, 14,941 from EPIC-CVD, and 348,556 from UK Biobank with complete data on all covariates. Associations for progressive levels of covariate adjustment are provided in Supplementary Table 8; each analysis is presented for the same complete data sample. Analysis for the full sample of 500,962 individuals with adjustment for age and sex only are presented in Supplementary Figure 4.

Outcome definitions for the Copenhagen studies were defined using ICD codes and manual validation for some outcomes as described previously [S1, S2, S3]. Only a limited number of genetic variants were available in the Copenhagen studies as these datasets have never been fully genotyped using a modern genetic chip.

To account for the case-cohort study design in EPIC-CVD, Cox models were adapted using Prentice weights and stratified by centre [S4]. To avoid overfitting models, studies contributing fewer than ten incident events to the analysis of a particular outcome were excluded from that analysis.

The primary dose—response analyses assessed the continuous shape of association of 25(OH)D and outcomes by meta-analysis of fractional polynomials adjusted for the conventional risk factors [S5]. First, the best-fitting fractional polynomial of degree 2 was estimated for each outcome using a Cox regression model fitted to the combined dataset stratified by study, centre, sex, and trial arm. Next, the coefficients for the best fitting fractional polynomial powers were estimated separately within each study, and then pooled across studies by random effects meta-analysis [S6]. The pooled coefficients were used to plot the continuous shape of association relative to the reference value of 50 nmol/L.

The rationale for the stratified genetic analyses ("non-linear Mendelian randomization") has been explained at length previously [S7, S8]: briefly, exposure measurements are affected by the genetic variants used in the analyses as instrumental variables, so this represents a "post-randomization" covariate. Stratifying on the exposure directly would lead to collider bias, as the exposure is a collider (a common effect) of the genetic variants and confounders of the exposure—outcome association.

The doubly-ranked non-linear Mendelian randomization method first constructs subgroups of the population ("pre-strata") that have similar levels of the instrument by ranking participants according to their level of the instrument, and then forms strata based on ranking participants according to their level of the exposure within each pre-stratum. The method assumes that the population ranking of individuals according to their exposure values would be similar at all values of the instrument (the "rank preserving assumption"). That is, the counterfactual values of the exposure for each individual would be at the same percentile of the exposure distribution (say, the 10th percentile) whatever their value of the instrument. This assumption is strictly weaker than the

"constant genetic effect" assumption made by the residual stratification method, as the constant genetic effect assumption is a specific case of the rank preserving assumption. However, the rank preserving assumption is more flexible, as it allows the effect of the genetic variant to be stronger or weaker at different levels of the exposure.

As stratum-specific estimates from the doubly-ranked method can be sensitive to specification of the analytic sample, we repeated analyses 100 times for each dataset omitting a small number of individuals in each iteration (12 individuals were removed at random in each iteration), and then combined estimates across iterations using Rubin's rules.

In UK Biobank, after correcting for season of blood draw to convert all values to an autumn measurement, 16% of individuals had a 25(OH)D concentration below 35 nmol/L, and 25% below 40 nmol/L. A more detailed assessment of the distribution of 25(OH)D concentrations throughout the calendar year is provided in Supplementary Table 7.

Season correction in the genetic analyses was performed as follows. First, we calculated the average 25(OH)D measurement in each season, separately in each study (and centre for EPIC-CVD). Secondly, for measurements not taken in autumn, we subtracted the study-specific mean of measurements taken in that season, and added the study-specific mean of measurements taken in autumn. Spring is defined as March to May, summer is June to August, autumn is September to November, and winter is December to February. For illustration, let us assume that the mean value of 25(OH)D in a particular study is 50 nmol/L for participants measured in autumn, 70 nmol/L for participants measured in summer, and 40 nmol/L for participants measured in winter. To convert a winter measurement into an autumn measurement, we would add 10 nmol/L (50-40 = 10). To convert a summer measurement into an autumn measurement, we would subtract 20 nmol/L (50-70 = -20). So an individual with a summer measurement of 65 nmol/L would have a season-corrected value of 45 nmol/L.

The genome-wide score was derived as follows: from variants reported in Supplementary Table S2 of Manousaki *et al* [S9], we took one variant from each linkage disequilibrium block, selecting in each case the variant with the lowest p-value. Weights were taken as the beta-coefficients from the BOLT-LMM analysis in UK Biobank provided by the authors. In total, 71 variants were included in the genome-wide score. The genome-wide score explained 4.5% of the variance in 25(OH)D levels.

Some participants in the Copenhagen studies are included in the observational analyses as part of the Vitamin D Studies Collaboration. While there is overlap in participants between the observational and genetic analyses, we have been careful to avoid including participants twice within each study in either the observational analysis or the genetic analysis.

Derivation of the analytic sample for UK Biobank of individuals of European ancestries followed quality control steps described previously [S10]: after filtering genetic variants (call rate ≥ 99%, info score > 0.9, Hardy-Weinberg equilibrium p-value ≥ 10^{-5}) and participants (removal of genetic sex mismatches), we excluded participants having non-European ancestries (self-report or inferred by genetics) or excess heterozygosity (>3 standard deviations from the mean), and included only one of each set of related participants (third-degree relatives or closer).

Estimates from the non-linear observational and genetic analyses have somewhat different interpretations. The observational analyses include incident events only. Estimates are hazard ratios relative to a common reference value – in main analyses, this is 50 nmol/L. The genetic analyses include both incident and prevalent events. Estimates are odds ratios and represent the association between genetically-predicted levels of the exposure (in our case, 25(OH)D concentrations) and the

outcome. We scale estimates to correspond to a 10 nmol/L increase in genetically-predicted 25(OH)D concentration. Under the instrumental variable assumptions, overall estimates represent the population-averaged effect of a shift in the distribution of the exposure [S11]. In the non-linear Mendelian randomization analyses, estimates are odds ratios per 10 nmol/L increase in genetically-predicted 25(OH)D concentration calculated within a stratum of the population. Under the instrumental variable assumptions, estimates represent the stratum-averaged effect of a shift in the distribution of the exposure. While we use the term "non-linear Mendelian randomization" to connect to previous presentations of the methodology in the literature [S12], the term "stratified Mendelian randomization" may be more understandable, as the stratum-specific estimates are linear estimates, but estimated in a specific stratum of the population.

There are several differences between observational and genetic estimates: two key differences are that the observational estimates represent the association of current levels of 25(OH)D concentrations with disease risk, whereas genetic estimates represent the association of genetically-predicted levels of 25(OH)D concentrations with disease risk, hence reflecting the impact of long-term differences in 25(OH)D levels. Another difference is that the genetic analyses are conducted separately within each stratum, and so there is no common reference category; estimates represent the impact of 10 nmol/L higher genetically-predicted 25(OH)D concentrations in each stratum.

For the genetic analyses, all 25(OH)D measurements were standardized by quality control as certified by the Vitamin D Standardization-Certification Program of the Centers for Disease Control and Prevention (VDSP) for UK Biobank, or the Vitamin D External Quality Assessment Scheme (DEQAS) for EPIC-CVD and the Copenhagen studies. Several cohorts in VitDSC also measured 25(OH)D in accredited laboratories.

Our literature search terms were as follows:

PubMed, search through 16 April 2021

("Vitamin D"[Mesh] OR "Vitamin D" OR "25-hydroxyvitamin D" OR "25(OH)D" OR "Calciferol" OR "Vitamin D2" OR "Vitamin D3" OR "Cholecalciferol" OR "Ergocalciferol" OR "Alphacalcidol" OR "Alfacalcidol" OR "Calcitriol" OR "Paricalcitol" OR "Doxerocalciferol")

AND ("Cardiovascular Diseases"[Mesh] OR "Cardiovascular Disease" OR "All-cause Mortality" OR "Mortality" OR "Survival")

AND ("Randomized Controlled Trial" [Publication Type] OR "Randomized Controlled Trials as Topic"[Mesh] OR "Randomized Controlled Trial" OR "Controlled Clinical Trial" [Publication Type] OR "Controlled Clinical Trials as Topic"[Mesh] OR "Controlled Clinical Trial" OR "Random Allocation" OR "Trial")

Scientific Citation Index Expanded, search through 16 April 2021

TS= ("Vitamin D" OR "25-hydroxyvitamin D" OR "25(OH)D" OR "Calciferol" OR "Vitamin D2" OR "Vitamin D3" OR "Cholecalciferol" OR "Ergocalciferol" OR "Alphacalcidol" OR "Alfacalcidol" OR "Calcitriol" OR "Paricalcitol" OR "Doxerocalciferol") AND TS= ("Cardiovascular Disease" OR "All-cause Mortality" OR "Mortality" OR "Survival")

AND TS= ("Randomized Controlled Trial" OR "Controlled Clinical Trial" OR "Random Allocation" OR "Trial")

EMBASE, search through 15 April 2021

("Vitamin D" OR "25-hydroxyvitamin D" OR "25(OH)D" OR "Calciferol" OR "Vitamin D2" OR "Vitamin D3" OR "Cholecalciferol" OR "Ergocalciferol" OR "Alphacalcidol" OR "Alfacalcidol" OR "Calcitriol" OR "Paricalcitol" OR "Doxerocalciferol").af. AND ("Cardiovascular Disease" OR "All-cause Mortality" OR "Mortality" OR "Survival").af. AND ("Randomized Controlled Trial" OR "Controlled Clinical Trial" OR "Random Allocation" OR "Trial").af. (Limited to Embase Status)

In total, we identified 90 relevant articles, including 79 studies of all-cause mortality and 41 studies of cardiovascular outcomes.

Supplementary references:

S1. Afzal S, Brøndum-Jacobsen P, Bojesen SE, Nordestgaard BG. Genetically low vitamin D concentrations and increased mortality: mendelian randomisation analysis in three large cohorts. British Medical Journal 2014. **349**:g6330.

S2. Riis J, Nordestgaard BG, Jensen BG, Afzal S. Secular trends in risk of stroke according to body mass index and blood pressure, 1976–2017. Neurology 2019. **93**(14):e1397-e1407.

S3. Brøndum-Jacobsen P, Benn M, Jensen GB, Nordestgaard BG. 25-hydroxyvitamin d levels and risk of ischemic heart disease, myocardial infarction, and early death: population-based study and meta-analyses of 18 and 17 studies. Arteriosclerosis, Thrombosis, and Vascular Biology 2012. **32**(11):2794-802.

S4. Prentice RL. A case-cohort design for epidemiologic cohort studies and disease prevention trials. Biometrika, 1986. **73**(1):1-11.

S5. White IR, Kaptoge S, Royston P, Sauerbrei W, ERFC. Meta-analysis of non-linear exposure-outcome relationships using individual participant data: a comparison of two methods. Statistics in Medicine, 2019. **38**(3):326-338.

S6. Thompson SG, Kaptoge S, White I, Wood A, Perry P, Danesh J, ERFC. Statistical methods for the time-to-event analysis of individual participant data from multiple epidemiological studies. International Journal of Epidemiology, 2010. **39**(5):1345-1359.

S7. Tian H, Burgess S. Relaxing parametric assumptions for non-linear Mendelian randomization using a doubly-ranked stratification method. PLOS Genetics 2023 (in press).

S8. Burgess S. Violation of the constant genetic effect assumption can result in biased estimates for non-linear Mendelian randomization. Human Heredity 2023 (in press).

S9. Manousaki D, Mitchell R, Dudding T, et al. Genome-wide association study for vitamin D levels reveals 69 independent loci. The American Journal of Human Genetics 2020; **106**(3):327-337.

S10. Astle WJ, Elding H, Jiang T, et al. The allelic landscape of human blood cell trait variation and links to common complex disease. Cell 2016; **167**(5):1415-1429.

S11. Burgess S, Davies NM, Thompson SG. Instrumental variable analysis with a nonlinear exposure–outcome relationship. Epidemiology 2014; **25**(6):877-885.

S12. Sun Y-Q, Burgess S, Staley JR, et al. Body mass index and all cause mortality in HUNT and UK Biobank studies: linear and non-linear mendelian randomisation analyses. British Medical Journal 2019; **364**:l1042.S12.

SUPPLEMENTARY TABLES AND FIGURES

Supplementary Table 1: Non-linear Mendelian randomization analyses of vitamin D performed using the residual stratification method

Lead author	Journal and year	Link
Sofianopoulou	Lancet Diabetes Endocrinol 2021	https://pubmed.ncbi.nlm.nih.gov/34717822/
Zhou	Euro Heart J 2022	https://pubmed.ncbi.nlm.nih.gov/34891159/
Sutherland	Ann Intern Med 2022	https://pubmed.ncbi.nlm.nih.gov/36279545/
Navale	Am J Clin Nutr 2022	https://pubmed.ncbi.nlm.nih.gov/35451454/
Zhou	Int J Epidemiol 2023	https://pubmed.ncbi.nlm.nih.gov/35579027/
Sutherland*	Nutrients 2023	https://pubmed.ncbi.nlm.nih.gov/37375607/
Sha	JAMA Network Open 2023	https://pubmed.ncbi.nlm.nih.gov/37647062/

* Sutherland used both the residual and doubly-ranked stratification methods, obtaining markedly different results from the two methods.

Supplementary Table 2: Methods for measurement of 25(OH)D concentration in studies of the Vitamin D Studies Collaboration

Method	Number of studies
Radioimmunoassay (RIA)	11
Automated immunoassay	4
Competitive protein binding (CPB)	1
Immunometric assay (IMA)	1
High-performance liquid chromatography mass spectrometry (HPLC-MS)	13
Electro-chemiluminescence immunoassay (ECLIA)	1

A list of the methods used in each study is provided in Supplementary Table 5.

Supplementary Table 3: ICD-10 codes for cause-specific mortality outcomes

Cause-specific mortality	ICD-10 codes
Cardiovascular	G45, I01, I03-I82, I87, I95-I99, F01, Q20-Q28, R96
Cancer	C00-C97, D00-D48
Non-cardiovascular non-cancer	All others

Supplementary Table 4: List of genetic variants for genetic risk score in UK Biobank and EPIC-CVD

Chromosome: Position (hg19)	rsID	Effect allele	Other allele	Conditional association with 25(OH)D (nmol/L)
4:72617775	rs1352846	G	A	0.172
4:72618334	rs7041	C	A	-0.045
4:72634343	rs4694431	T	C	-0.034
4:72770563	rs139148694	GTGCTTTTATCAA	G	0.028
11:14339328	rs16913816	A	G	-0.031
11:14900931	rs117913124	A	G	0.503
11:14912573	rs117576073	T	G	0.246
11:14913575	rs12794714	A	G	0.139
11:14913645	rs202122669	A	G	-0.615
11:14913900	rs187639972	C	G	-0.360
11:14941652	rs117115472	G	C	0.148
11:71157867	rs139168803	A	G	-0.188
11:71158672	rs12573951	G	A	-0.045
11:71161063	rs7928249	G	A	-0.131
11:71180762	rs549000212	A	C	-0.364
11:71290740	rs4081429	C	A	0.017
20:52714706	rs6123359	G	A	-0.026
20:52731402	rs6127099	T	A	0.013
20:52735238	rs35870583	GT	G	0.027
20:52737123	rs2585442	G	C	-0.025
20:52788925	rs2762942	A	G	-0.053

In the Copenhagen studies, rs12794714 (11:14913575) and rs117913124 (11:14900931), and rs7944926 (11:71165625) were used for genetic analyses. The rs7944926 variant is in high linkage disequilibrium with rs7928249 (r^2 = 0.986 in European ancestry 1000 Genomes participants).

Supplementary Table 5: Baseline characteristics of participants in the observational analyses

Characteristic*	Cohorts	N	Mean (SD) or %
25(OH)D (nmol/L)	40	500,962	52.0 (21.7)
<25 nmol/L, Deficient	40	65,313	13%
25-49 nmol/L, Insufficient	40	208,223	42%
50-74 nmol/L, Sufficient	40	165,162	33%
≥75 nmol/L, Adequate	40	62,264	12%
Age and physical measures			
Age at survey (yrs)	40	500,962	60.7 (8.7)
Height (cm)	40	499,369	167 (9)
Weight (kg)	40	498,356	74.0 (15.4)
Body mass index [BMI] (kg/m^2)	40	498,019	26.6 (4.7)
Waist circumference (cm)	29	464,017	89.0 (13.2)
Hip circumference (cm)	29	463,946	102 (9)
Waist:Hip circumference ratio	29	463,881	0.87 (0.09)
Systolic blood pressure [SBP] (mmHg)	38	488,928	138 (19)
Diastolic blood pressure [DBP] (mmHg)	37	486,117	80.2 (10.3)
Lipids			
Total cholesterol (mmol/l)	37	489,120	5.92 (1.02)
Friedewald LDL cholesterol (mmol/l)	32	431,296	3.80 (0.89)
Measured LDL cholesterol (mmol/l)	3	388,309	3.37 (0.76)
Non-HDL cholesterol (mmol/l)	35	452,863	4.49 (1.00)
HDL-cholesterol (mmol/l)	35	453,646	1.40 (0.38)
Log Triglycerides (mmol/l)	34	476,028	0.34 (0.52)
Apolipoprotein A1 (g/l)	9	374,898	1.51 (0.27)
Apolipoprotein B (g/l)	9	407,933	1.05 (0.22)
Log Lipoprotein a [Lp(a)] (mg/dl)	9	329,672	3.18 (1.11)
Glycaemia markers			
Log Glucose (mmol/l)	29	426,373	1.65 (0.19)
Log Fasting glucose (mmol/l)	16	39,327	1.64 (0.19)
Glycated haemoglobin [HbA1c] (%)	9	389,733	5.52 (0.86)
Inflammation markers			
Fibrinogen (µmol/l)	12	24,246	9.15 (2.27)
Log C-reactive protein [CRP] (mg/l)	29	447,320	0.43 (1.06)
Log White cell count (x10^9/l)	13	400,868	1.85 (0.26)
Albumin (g/l)	25	411,035	43.7 (2.8)
Kidney function			
Log Creatinine (µmol/l)	30	446,152	4.40 (0.20)
Log eGFR by MDRD (ml/min/1.73m^2)	30	446,152	4.31 (0.20)
Bone-related markers			
Calcium (mmol/l)	21	401,249	2.38 (0.10)
Log Parathyroid hormone (ng/L)	14	21,379	1.30 (0.44)
Log Phosphate (mmol/L)	13	365,621	0.13 (0.14)
Log Calcitriol [1,25$(OH)_2$D] (pmol/L)	7	3,160	4.38 (0.42)
Log Alkaline Phosphatase (IU/L)	10	419,940	4.16 (0.28)
Categorical variables			
Sex	40	500,962	
Male	32	225,383	45%
Female	36	275,579	55%
Ethnic group (4 groups)	36	489,927	
White	36	463,935	95%
Asian	7	8,504	2%
Black	8	10,749	2%
Other	8	6,739	1%
Smoking status	40	499,495	
Other	40	431,683	86%
Current	40	67,812	14%
Alcohol status	34	481,755	
Other	34	54,161	11%
Current	31	427,594	89%
History of diabetes	40	488,586	
No	39	465,183	95%
Yes	40	23,403	5%
Season of 25(OH)D blood draw	40	500,962	
Winter	36	99,694	20%
Spring	35	138,518	28%
Summer	39	142,646	28%
Autumn	38	120,104	24%
Highest level of education reached	28	456,164	
Primary	25	25,375	6%
Secondary	28	176,416	39%
Vocational/University	24	254,373	56%

* Common abbreviations are shown in square brackets: LDL low density lipoprotein cholesterol; HDL high-density lipoprotein cholesterol; eGFR estimated glomerular filtration rate using the Modification of Diet in Renal Disease (MDRD) equation. For the purposes of this table, EPIC-CVD countries are enumerated as separate cohorts as covariate information differed by centre.

Supplementary Table 6: Details of studies contributing to the observational analyses

Index	Dataset	Cohort abbreviation	Study design	Population type	Country	Median year of baseline	Maximum year of follow up	Total participants, n	25(OH)D (nmol/L), mean (sd)	Age at survey (yrs), mean (sd)	Male sex, n (%)	Median follow-up (5th & 95th percentiles)	Non-fatal MI and CHD death	Stroke	All CVD	Person-years of first event follow up	CVD mortality	Cancer mortality	Non-CVD non-cancer mortality	Unknown mortality	All-cause mortality	Person-years of mortality follow up
1	VITDSC	4D	Clinical trial	Diabetes	Germany	1999	2004	656	45 (24)	66 (8)	359 (55)	2.7 (0.5 to 5.6)	80	54	213	1,894	133	19	126	0	278	2,026
2	VITDSC	AUCKLAND	Clinical trial	General	New Zealand	1999	2005	1300	53 (19)	74 (4)	0 (0)	5.1 (0.0 to 5.5)	41	41	94	5,891	26	12	10	2	50	6,036
3	VITDSC	BRUN	Cohort	General	Italy	1990	2010	794	80 (32)	57 (11)	388 (49)	20.2 (4.9 to 20.5)	62	57	141	13,548	84	83	79	3	249	13,981
4	VITDSC	BWHHS	Cohort	General	UK	2000	2014	2741	44 (20)	68 (5)	0 (0)	12.2 (3.5 to 13.3)	119	116	275	30,377	208	281	242	10	741	35,645
5	VITDSC	CAIFOS	Clinical trial	General	Australia	1998	2008	1383	67 (29)	75 (3)	0 (0)	10.0 (2.5 to 10.0)	104	128	232	12,219	96	0	36	134	266	12,853
6	VITDSC	CAPS	Cohort	General	UK	1991	2000	1220	47 (22)	62 (5)	1220 (100)	3.1 (1.8 to 3.3)	35	0	39	3,565	28	26	8	0	62	3,820
7	VITDSC	CCHS	Cohort	General	Denmark	1982	2013	8250	45 (24)	56 (12)	4043 (49)	20.3 (2.9 to 29.0)	1197	1047	2825	155,796	1840	1063	2039	737	5679	173,614
8	VITDSC	DOPS	Cohort	General	Denmark	1992	2008	1990	63 (31)	50 (3)	0 (0)	16.5 (8.6 to 17.5)	47	89	143	31,378	23	76	33	0	132	32,185
9	VITDSC	EPICBMD	Cohort	General	UK	1996	2012	575	58 (21)	70 (3)	0 (0)	12.1 (3.4 to 13.8)	38	49	97	6,349	78	0	0	0	225	7,718
10	VITDSC	EPICNOR	Cohort	General	UK	1999	2015	12630	57 (23)	61 (9)	5441 (43)	15.4 (7.0 to 16.8)	0	174	349	184,307	349	960	737	306	2352	184,307
11	VITDSC	ESTHER	Cohort	General	Germany	2001	2015	2692	54 (24)	54 (3)	1191 (44)	5.0 (1.9 to 6.0)	23	31	54	12,573	52	123	54	20	249	37,660
12	VITDSC	HCS	Cohort	General	UK	2000	2012	1053	47 (24)	65 (3)	502 (48)	10.0 (6.5 to 11.8)	17	3	32	10,460	32	63	20	1	116	10,460
13	VITDSC	HDZNRW	Cohort	Other	Germany	2005	2006	124	54 (49)	52 (8)	39 (31)	1.0 (1.0 to 1.0)	0	0	0	124	0	0	0	0	0	124
14	VITDSC	INTER99	Cohort	General	Denmark	1999	2012	6318	51 (27)	46 (8)	3082 (49)	12.0 (9.5 to 12.7)	120	94	231	73,692	37	118	44	73	272	80,360
15	VITDSC	LASA	Cohort	General	Netherlands	1996	2020	839	55 (24)	75 (6)	369 (44)	10.8 (1.3 to 24.0)	73	78	190	9,700	116	103	124	399	742	10,998
16	VITDSC	LURIC	Cohort	Other	Germany	1998	2009	578	44 (23)	61 (11)	353 (61)	10.2 (2.5 to 11.5)	7	3	55	5,441	55	22	38	1	116	5,441
17	VITDSC	MESA	Cohort	General	USA	2001	2018	1388	54 (28)	65 (10)	635 (46)	15.7 (3.9 to 17.1)	57	55	117	18,653	82	0	0	17	369	20,100
18	VITDSC	MIDSPAN	Cohort	General	UK	1996	2013	1999	50 (24)	45 (6)	884 (44)	17.4 (10.7 to 17.8)	58	34	95	33,454	21	55	41	5	122	33,981
19	VITDSC	MINIFIN	Cohort	General	Finland	1979	2006	6200	43 (20)	49 (14)	2805 (45)	27.1 (5.4 to 28.8)	639	290	929	141,224	929	0	0	0	2490	141,224
20	VITDSC	MONICA10	Cohort	General	Denmark	1994	2012	2488	65 (27)	55 (11)	1226 (49)	17.3 (4.0 to 18.4)	131	276	446	37,452	154	225	190	133	702	41,050
21	VITDSC	MROS	Cohort	General	USA	2004	2013	1878	72 (22)	76 (5)	1878 (100)	8.1 (2.7 to 8.7)	42	89	185	13,816	115	101	128	13	357	14,106
22	VITDSC	NHANESIII	Cohort	General	USA	1993	2013	13898	64 (28)	47 (18)	6434 (46)	19.1 (4.8 to 22.6)	616	265	1344	243,301	1344	938	1452	56	3790	243,301
23	VITDSC	PROSPER	Clinical trial	Other	Scotland/Ireland/N	1999	2002	2816	43 (26)	75 (3)	1175 (42)	2.8 (1.2 to 3.3)	186	91	287	7,557	74	83	34	0	191	7,815
24	VITDSC	SHIP-1	Cohort	General	Germany	2004	2011	2426	48 (23)	57 (13)	1160 (48)	5.5 (0.0 to 6.9)	19	12	31	10,131	51	58	40	16	165	14,577
25	VITDSC	SOF1	Cohort	General	USA	1987	2010	473	64 (29)	72 (5)	0 (0)	12.9 (1.8 to 22.3)	72	38	153	5,999	153	84	97	0	334	5,999
26	VITDSC	SOF4	Cohort	General	USA	1993	2011	4299	59 (29)	76 (4)	0 (0)	13.3 (3.0 to 16.9)	435	256	965	50,937	965	432	822	0	2219	50,937
27	VITDSC	STENO	Cohort	Diabetes	Denmark	1987	2010	225	43 (25)	53 (9)	138 (61)	18.9 (3.7 to 22.9)	0	0	28	3,724	28	0	0	47	139	3,732
28	VITDSC	TURKUFIN	Cohort	General	Finland	1987	1995	458	32 (20)	77 (6)	213 (47)	7.5 (0.6 to 9.3)	65	53	139	2,797	126	35	93	1	255	2,852
29	VITDSC	TWINSUK	Cohort	General	UK	1998	2013	3274	75 (40)	50 (12)	224 (7)	15.6 (8.0 to 18.3)	2	12	28	47,420	28	74	47	4	153	47,420
30	VITDSC	ULSAM	Cohort	General	Sweden	1993	2008	936	69 (19)	71 (2)	936 (100)	13.8 (2.1 to 16.8)	142	105	298	10,611	177	171	103	6	457	11,634
31	VITDSC	WHITEI	Cohort	General	UK	1997	2010	4014	58 (19)	76 (5)	4014 (100)	11.9 (2.0 to 13.3)	335	253	810	39,624	810	625	703	30	2168	39,624
	VITDSC	SUBTOTAL				1997	2011	89915	55 (25)	62 (11)	38709 (43)	14.0 (2.5 to 27.7)	4762	3793	10825	1,224,014	8214	5830	7340	2014	25440	1,295,580
32.1	EPICCVD	EPICCVD_DNK	Case-cohort	General	Denmark	1996	2009	5193	41 (18)	57 (4)	3161 (61)	10.3 (1.6 to 14.6)	1766	1658	3419	48,849	339	-	-	-	-	66,316
32.2	EPICCVD	EPICCVD_FRA	Case-cohort	General	France	1997	1999	579	40 (18)	57 (7)	0 (0)	0.0 (0.0 to 0.0)	40	0	0	2	0	-	-	-	-	2
32.3	EPICCVD	EPICCVD_DEU	Case-cohort	General	Germany	1996	2008	2958	39 (17)	52 (8)	1505 (51)	8.4 (1.6 to 11.4)	589	445	988	22,679	137	-	-	-	-	24,557
32.4	EPICCVD	EPICCVD_ITA	Case-cohort	General	Italy	1995	2009	3130	37 (16)	52 (8)	1256 (40)	10.1 (2.3 to 14.1)	464	321	1155	29,505	66	-	-	-	-	36,559
32.5	EPICCVD	EPICCVD_NLD	Case-cohort	General	Netherlands	1995	2007	3324	42 (18)	55 (10)	784 (24)	10.2 (1.1 to 13.9)	473	528	1965	29,478	222	-	-	-	-	39,127
32.6	EPICCVD	EPICCVD_SPA	Case-cohort	General	Spain	1994	2012	5175	38 (18)	51 (8)	2456 (47)	13.5 (3.3 to 15.6)	691	570	1652	62,286	188	-	-	-	-	73,356
32.7	EPICCVD	EPICCVD_SWE	Case-cohort	General	Sweden	1994	2006	1954	55 (17)	50 (10)	1144 (59)	10.9 (2.4 to 13.9)	527	494	928	18,947	186	-	-	-	-	22,828
32.8	EPICCVD	EPICCVD_GBR	Case-cohort	General	UK	1995	2012	4023	42 (17)	61 (10)	1950 (48)	8.5 (2.1 to 12.9)	875	674	2751	32,283	639	-	-	-	-	43,105
32	EPICCVD	SUBTOTAL				1995	2009	26336	42 (17)	54 (8)	12256 (47)	9.9 (1.1 to 14.8)	5425	4690	12858	244,029	1777	-	-	-	-	305,850
33	UKBIOBANK	UKBB	Cohort	General	UK	2009	2020	384711	49 (21)	56 (8)	174418 (45)	10.9 (7.8 to 12.5)	6373	5091	12225	4,068,589	3284	11211	4893	139	19527	4,270,585
	OVERALL	TOTAL				1996	2010	500962	52 (22)	61 (9)	225383 (45)	11.0 (4.9 to 17.2)	16560	13574	35908	5,536,632	13275	17041	12233	2153	44967	5,872,015

Supplementary Table 6 (continued): List of acronyms of studies contributing to the observational analyses.

Cohort abbreviation	Cohort name	25(OH)D assay method
4D	The German Diabetes & Dialysis Study	Automated immunoassay
AUCKLAND	Auckland Calcium Study	RIA
BRUN	Bruneck Study	Automated immunoassay
BWHHS	British Women's Heart and Health Study	HPLC-MS
CAIFOS	Calcium Intake Fracture Outcome Study	HPLC-MS
CAPS	Caerphilly Prospective Study	HPLC-MS
CCHS	Copenhagen City Heart Study	RIA
DOPS	Danish Osteoporosis Prevention Study	RIA
EPICBMD	European Prospective Investigation of Cancer - Norfolk Study (Bone Mineral Density sub-study)	IMA
EPICNOR	European Prospective Investigation of Cancer - Norfolk Study	HPLC-MS
ESTHER	Epidemiologische Studie zu Chancen der Verhütung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung	RIA
HCS	Hertfordshire Cohort Study	RIA
HDZNRW	The Heart and Diabetes Center NRW Study	RIA
INTER99	Inter99 Study	HPLC-MS
LASA	Longitudinal Aging Study Amsterdam	CPB
LURIC	Ludwigshafen Risk and Cardiovascular Health	RIA
MESA	Multi-Ethnic Study of Atherosclerosis	HPLC-MS
MIDSPAN	MIDSPAN Family Study	HPLC-MS
MINIFIN	Mini-Finland Health Survey	RIA
MONICA10	Monitoring of Trends and Determinants in Cardiovascular Disease	ECLIA
MROS	Osteoporotic Fractures in Men	HPLC-MS
NHANESIII	Third National Health and Nutrition Examination Survery	RIA
PROSPER	Prospective Study of Pravastatin in the Elderly at Risk	HPLC-MS
SHIP-1	Study of Health in Pomerania-1	Automated immunoassay
SOF1	Study of Osteoporotic Fractures (visit 1)	HPLC-MS
SOF4	Study of Osteoporotic Fractures (visit 4)	HPLC-MS
STENO	The Steno Diabetes Study	HPLC-MS
TURKUFIN	Turku-Finland Elderly Study	RIA
TWINSUK	Twins UK Study	RIA
ULSAM	The Uppsala Longitudinal Study of Adult Men	HPLC-MS
WHITEI	Whitehall I	Automated immunoassay
EPICCVD_Denmark	EPIC-CVD Denmark (Aarhus, Copenhagen)	HPLC-MS
EPICCVD_France	EPIC-CVD France (France)	HPLC-MS
EPICCVD_Germany	EPIC-CVD Germany (Heidelberg, Potsdam)	HPLC-MS
EPICCVD_Italy	EPIC-CVD Italy (Florence, Varese, Ragusa, Turin, Naples)	HPLC-MS
EPICCVD_Netherlands	EPIC-CVD Netherlands (Bilthoven, Utrecht)	HPLC-MS
EPICCVD_Spain	EPIC-CVD Spain (Asturias, Granada, Murcia, Navarra, San Sebastian)	HPLC-MS
EPICCVD_Sweden	EPIC-CVD Sweden (Umea)	HPLC-MS
EPICCVD_UK	EPIC-CVD UK (Cambridge, Oxford)	HPLC-MS
UKBIOBANK	UK Biobank	CLIA

The EPIC-CVD study was specifically designed as a case-cohort study of CVD outcomes therefore does not contribute to the analysis of non-CVD outcomes nor all-cause mortality.

Supplementary Table 7: Distribution of 25(OH)D measurements and proportion of those with low 25(OH)D status in different seasons and months of blood draw in UK Biobank.

Season	Winter	Spring	Summer	Autumn
Mean 25(OH)D (nmol/L)	41.0	42.9	58.2	54.5
Participants below 25 nmol/L (%)	22.2	19.1	2.9	6.0
Participants below 40 nmol/L (%)	54.2	49.9	17.4	25.6

	Winter			Spring			Summer			Autumn		
Month	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Mean 25(OH)D	44.4	40.1	39.4	39.5	42.0	47.0	55.1	59.7	60.7	60.0	55.4	48.5
% below 25 nmol/L	15.7	23.1	25.8	26.1	20.5	11.2	3.9	2.5	2.1	2.6	4.8	10.3
% below 40 nmol/L	46.5	56.0	58.0	58.1	52.7	39.8	21.8	15.4	13.8	15.5	23.3	37.1

Supplementary Table 8: Progressively adjusted observational associations of 25(OH)D concentrations with outcomes by clinical categories. The adequate stratum is the reference group. Confidence intervals are calculated using the floating variance method. Each analysis is performed for the same data sample with complete data on all covariates.

Outcome / Progressive adjustment variables	**Hazard ratio (95% confidence interval)**			
(N = Cohorts / Participants / Outcomes)	Deficient (< 25 nmol/L)	Insufficient (25-49 nmol/L)	Sufficient (50-74 nmol/L)	Adequate [Ref] (≥ 75 nmol/L)
Coronary heart disease (N = 31 / 417,937 / 12,818)				
Adjusted for sex and age	1.38 (1.22, 1.55)	1.11 (1.05, 1.18)	1.02 (0.96, 1.08)	1.00 (0.91, 1.10)
Plus month of recruitment	1.48 (1.32, 1.66)	1.16 (1.09, 1.23)	1.04 (1.01, 1.08)	1.00 (0.91, 1.10)
Plus smoking status	1.34 (1.22, 1.47)	1.12 (1.09, 1.14)	1.03 (0.97, 1.09)	1.00 (0.88, 1.13)
Plus total cholesterol	1.37 (1.24, 1.52)	1.12 (1.08, 1.17)	1.03 (0.97, 1.09)	1.00 (0.89, 1.12)
Plus HDL cholesterol	1.25 (1.12, 1.39)	1.04 (0.98, 1.10)	0.99 (0.94, 1.04)	1.00 (0.89, 1.12)
Plus systolic blood pressure	1.21 (1.07, 1.36)	1.02 (0.96, 1.09)	0.98 (0.94, 1.01)	1.00 (0.90, 1.11)
Plus history of diabetes	1.17 (1.05, 1.30)	1.00 (0.95, 1.06)	0.97 (0.93, 1.02)	1.00 (0.89, 1.12)
Plus body mass index	1.16 (1.04, 1.29)	1.00 (0.94, 1.06)	0.97 (0.93, 1.01)	1.00 (0.89, 1.12)
Stroke (N = 30 / 427,698 / 9947)				
Adjusted for sex and age	1.48 (1.37, 1.61)	1.17 (1.09, 1.27)	1.01 (0.95, 1.07)	1.00 (0.94, 1.07)
Plus month of recruitment	1.58 (1.44, 1.73)	1.20 (1.10, 1.31)	1.02 (0.95, 1.09)	1.00 (0.94, 1.07)
Plus smoking status	1.50 (1.37, 1.63)	1.17 (1.07, 1.27)	1.01 (0.94, 1.07)	1.00 (0.94, 1.07)
Plus total cholesterol	1.50 (1.38, 1.64)	1.17 (1.08, 1.27)	1.01 (0.95, 1.08)	1.00 (0.94, 1.07)
Plus HDL cholesterol	1.45 (1.33, 1.58)	1.14 (1.05, 1.24)	1.00 (0.93, 1.06)	1.00 (0.93, 1.07)
Plus systolic blood pressure	1.40 (1.28, 1.53)	1.12 (1.03, 1.22)	0.99 (0.92, 1.06)	1.00 (0.93, 1.08)
Plus history of diabetes	1.36 (1.25, 1.49)	1.10 (1.01, 1.19)	0.97 (0.91, 1.04)	1.00 (0.93, 1.08)
Plus body mass index	1.36 (1.24, 1.49)	1.10 (1.01, 1.19)	0.97 (0.90, 1.04)	1.00 (0.93, 1.08)
All-cause mortality (N = 26 / 416,548 / 36,949)				
Adjusted for sex and age	1.76 (1.60, 1.93)	1.24 (1.19, 1.29)	1.03 (1.00, 1.07)	1.00 (0.98, 1.02)
Plus month of recruitment	1.89 (1.71, 2.09)	1.28 (1.24, 1.34)	1.04 (1.01, 1.08)	1.00 (0.98, 1.02)
Plus smoking status	1.73 (1.57, 1.91)	1.24 (1.20, 1.29)	1.04 (1.00, 1.08)	1.00 (0.98, 1.02)
Plus total cholesterol	1.75 (1.58, 1.93)	1.26 (1.21, 1.31)	1.05 (1.01, 1.09)	1.00 (0.98, 1.02)
Plus HDL cholesterol	1.74 (1.57, 1.92)	1.25 (1.20, 1.30)	1.05 (1.01, 1.08)	1.00 (0.98, 1.02)
Plus systolic blood pressure	1.72 (1.55, 1.91)	1.24 (1.19, 1.30)	1.04 (1.01, 1.08)	1.00 (0.98, 1.02)
Plus history of diabetes	1.68 (1.51, 1.86)	1.22 (1.16, 1.28)	1.03 (1.00, 1.07)	1.00 (0.98, 1.02)
Plus body mass index	1.66 (1.50, 1.83)	1.21 (1.15, 1.26)	1.03 (0.99, 1.06)	1.00 (0.98, 1.02)
Cardiovascular mortality (N = 33 / 431,489 / 9953)				
Adjusted for sex and age	1.92 (1.72, 2.15)	1.36 (1.33, 1.40)	1.05 (1.00, 1.11)	1.00 (0.93, 1.07)
Plus month of recruitment	2.07 (1.83, 2.35)	1.42 (1.38, 1.46)	1.07 (1.03, 1.12)	1.00 (0.94, 1.06)
Plus smoking status	1.92 (1.70, 2.17)	1.37 (1.34, 1.41)	1.07 (1.02, 1.11)	1.00 (0.94, 1.06)
Plus total cholesterol	1.94 (1.72, 2.19)	1.39 (1.35, 1.43)	1.07 (1.03, 1.12)	1.00 (0.94, 1.06)
Plus HDL cholesterol	1.87 (1.65, 2.12)	1.35 (1.31, 1.39)	1.06 (1.01, 1.11)	1.00 (0.94, 1.06)
Plus systolic blood pressure	1.80 (1.59, 2.04)	1.31 (1.28, 1.35)	1.05 (1.00, 1.09)	1.00 (0.94, 1.06)
Plus history of diabetes	1.74 (1.54, 1.98)	1.27 (1.23, 1.31)	1.03 (0.99, 1.07)	1.00 (0.94, 1.06)
Plus body mass index	1.67 (1.48, 1.88)	1.22 (1.19, 1.26)	1.01 (0.97, 1.05)	1.00 (0.94, 1.07)
Cancer mortality (N = 22 / 407,567 / 14,581)				
Adjusted for sex and age	1.38 (1.26, 1.52)	1.21 (1.15, 1.26)	1.01 (1.00, 1.03)	1.00 (0.95, 1.05)
Plus month of recruitment	1.50 (1.39, 1.63)	1.24 (1.19, 1.30)	1.02 (1.00, 1.04)	1.00 (0.95, 1.05)
Plus smoking status	1.40 (1.31, 1.49)	1.20 (1.14, 1.26)	1.01 (0.99, 1.03)	1.00 (0.95, 1.05)
Plus total cholesterol	1.42 (1.33, 1.51)	1.21 (1.15, 1.27)	1.02 (0.99, 1.04)	1.00 (0.95, 1.05)
Plus HDL cholesterol	1.39 (1.30, 1.48)	1.19 (1.13, 1.24)	1.01 (0.99, 1.04)	1.00 (0.95, 1.05)
Plus systolic blood pressure	1.37 (1.27, 1.47)	1.18 (1.13, 1.23)	1.01 (0.98, 1.03)	1.00 (0.95, 1.05)
Plus history of diabetes	1.37 (1.29, 1.46)	1.17 (1.12, 1.23)	1.00 (0.98, 1.03)	1.00 (0.95, 1.05)
Plus body mass index	1.38 (1.31, 1.45)	1.15 (1.11, 1.20)	1.00 (0.98, 1.03)	1.00 (0.95, 1.05)
Non-cardiovascular/cancer mortality (N = 23 / 408,536 / 9662)				
Adjusted for sex and age	2.10 (1.80, 2.45)	1.29 (1.21, 1.38)	0.99 (0.91, 1.08)	1.00 (0.96, 1.04)
Plus month of recruitment	2.31 (1.93, 2.77)	1.36 (1.25, 1.48)	0.99 (0.92, 1.08)	1.00 (0.97, 1.03)
Plus smoking status	2.09 (1.76, 2.48)	1.31 (1.21, 1.43)	0.99 (0.91, 1.07)	1.00 (0.97, 1.03)
Plus total cholesterol	2.14 (1.78, 2.57)	1.34 (1.22, 1.47)	1.00 (0.92, 1.09)	1.00 (0.97, 1.03)
Plus HDL cholesterol	2.20 (1.81, 2.67)	1.38 (1.25, 1.52)	1.03 (0.95, 1.10)	1.00 (0.97, 1.03)
Plus systolic blood pressure	2.18 (1.79, 2.67)	1.37 (1.24, 1.52)	1.03 (0.95, 1.10)	1.00 (0.97, 1.03)
Plus history of diabetes	2.12 (1.75, 2.57)	1.34 (1.21, 1.50)	1.01 (0.93, 1.09)	1.00 (0.98, 1.02)
Plus body mass index	2.11 (1.75, 2.54)	1.34 (1.20, 1.49)	1.01 (0.93, 1.09)	1.00 (0.98, 1.03)

Supplementary Table 9: Study-specific Mendelian randomization estimates for main outcomes in overall population and divided into clinical strata by residual concentration of 25(OH)D.

Study and stratum	Mean 25(OH)D (nmol/L)	Coronary heart disease	Stroke	All cause mortality
UK Biobank				
Overall		0.98 (0.95, 1.01)	1.01 (0.96, 1.05)	1.00 (0.96, 1.03)
Stratum 1	28.6	1.32 (0.98, 1.77)	1.10 (0.75, 1.63)	1.06 (0.81, 1.39)
Stratum 2	35.7	0.94 (0.74, 1.19)	1.24 (0.90, 1.73)	1.06 (0.84, 1.34)
Stratum 3	41.1	0.98 (0.80, 1.20)	1.06 (0.81, 1.39)	1.09 (0.89, 1.33)
Stratum 4	46.0	1.02 (0.86, 1.22)	1.04 (0.82, 1.33)	1.12 (0.94, 1.34)
Stratum 5	50.7	0.93 (0.81, 1.07)	0.94 (0.77, 1.14)	0.95 (0.82, 1.10)
Stratum 6	55.5	0.96 (0.85, 1.09)	0.92 (0.77, 1.09)	0.98 (0.86, 1.11)
Stratum 7	60.7	0.97 (0.86, 1.08)	0.91 (0.79, 1.06)	0.93 (0.83, 1.04)
Stratum 8	66.5	0.95 (0.87, 1.04)	0.95 (0.84, 1.08)	0.97 (0.88, 1.07)
Stratum 9	73.8	0.97 (0.89, 1.05)	1.02 (0.91, 1.14)	0.97 (0.89, 1.05)
Stratum 10	86.2	1.00 (0.94, 1.07)	1.07 (0.97, 1.17)	1.01 (0.94, 1.08)
EPIC-CVD				
Overall		1.00 (0.91, 1.10)	1.10 (0.96, 1.26)	
Stratum 1	25.3	0.97 (0.45, 2.10)	1.17 (0.57, 2.39)	
Stratum 2	31.9	0.86 (0.48, 1.54)	1.18 (0.66, 2.10)	
Stratum 3	36.4	1.38 (0.86, 2.22)	1.08 (0.65, 1.77)	
Stratum 4	40.3	0.91 (0.60, 1.39)	0.96 (0.61, 1.49)	
Stratum 5	44.1	1.04 (0.70, 1.55)	0.95 (0.66, 1.37)	
Stratum 6	47.9	0.97 (0.70, 1.35)	1.03 (0.72, 1.48)	
Stratum 7	51.8	0.93 (0.69, 1.24)	0.96 (0.70, 1.32)	
Stratum 8	56.3	0.81 (0.62, 1.08)	0.83 (0.64, 1.08)	
Stratum 9	62.2	1.02 (0.79, 1.31)	0.91 (0.73, 1.15)	
Stratum 10	72.7	0.89 (0.74, 1.07)	1.02 (0.85, 1.22)	
Copenhagen studies				
Overall		0.95 (0.86, 1.05)	0.99 (0.90, 1.09)	0.89 (0.80, 0.99)
Stratum 1	25.3	0.94 (0.69, 1.28)	0.93 (0.59, 1.45)	0.95 (0.66, 1.37)
Stratum 2	33.6	0.91 (0.64, 1.30)	0.91 (0.56, 1.48)	0.92 (0.60, 1.41)
Stratum 3	40.1	0.98 (0.68, 1.41)	0.97 (0.56, 1.67)	0.91 (0.60, 1.38)
Stratum 4	46.0	0.99 (0.67, 1.45)	1.00 (0.59, 1.70)	0.93 (0.61, 1.42)
Stratum 5	51.6	0.93 (0.64, 1.36)	1.15 (0.63, 2.09)	0.93 (0.60, 1.44)
Stratum 6	57.4	0.92 (0.63, 1.35)	1.27 (0.72, 2.24)	0.90 (0.57, 1.43)
Stratum 7	63.7	1.04 (0.70, 1.55)	1.35 (0.74, 2.45)	0.88 (0.57, 1.38)
Stratum 8	71.1	1.10 (0.73, 1.67)	1.29 (0.70, 2.37)	0.81 (0.51, 1.28)
Stratum 9	81.1	1.14 (0.76, 1.73)	1.09 (0.63, 1.89)	0.81 (0.52, 1.27)
Stratum 10	100.0	1.11 (0.76, 1.61)	1.31 (0.75, 2.28)	0.83 (0.56, 1.23)

Estimates (95% confidence intervals) represent odds ratio per 10 nmol/L higher genetically-predicted concentration of 25(OH)D.

Supplementary Table 10: Mendelian randomization estimates for stroke in UK Biobank divided into overall stroke (10,489 events), incident-only stroke (excluding those with prevalent stroke at baseline, 5044 events), ischaemic stroke (including unknown, 4164 events), and haemorrhagic stroke (intracerebral plus subarachnoid haemorrhage, 1194 events): odds ratios (95% confidence intervals) per 10 nmol/L higher genetically-predicted concentration of 25(OH)D.

Stroke	**Overall**	**Incident only**	**Ischaemic**	**Haemorrhagic**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Overall	1.01 (0.96-1.05) p=0.81	0.98 (0.91-1.04) p=0.49	1.01 (0.94-1.09) p=0.73	0.91 (0.80-1.04) p=0.19
Women	0.99 (0.92-1.07) p=0.85	0.94 (0.85-1.04) p=0.24	1.00 (0.88-1.12) p=0.95	0.85 (0.70-1.03) p=0.09
Men	1.01 (0.96-1.08) p=0.64	1.00 (0.92-1.09) p=0.95	1.02 (0.93-1.12) p=0.64	0.99 (0.82-1.19) p=0.88
Stratifying on 25(OH)D:				
Stratum 1	1.10 (0.75, 1.63)	1.02 (0.56, 1.86)	0.89 (0.47, 1.67)	1.73 (0.49, 6.17)
Stratum 2	1.24 (0.90, 1.73)	1.45 (0.90, 2.35)	1.29 (0.77, 2.17)	1.93 (0.72, 5.21)
Stratum 3	1.06 (0.81, 1.39)	1.05 (0.71, 1.55)	1.08 (0.70, 1.66)	1.07 (0.48, 2.37)
Stratum 4	1.04 (0.82, 1.33)	0.90 (0.65, 1.25)	0.94 (0.65, 1.34)	0.76 (0.39, 1.50)
Stratum 5	0.94 (0.77, 1.14)	0.99 (0.75, 1.32)	1.07 (0.79, 1.47)	0.70 (0.38, 1.27)
Stratum 6	0.92 (0.77, 1.09)	0.93 (0.73, 1.20)	0.97 (0.73, 1.28)	1.03 (0.62, 1.70)
Stratum 7	0.91 (0.79, 1.06)	0.85 (0.69, 1.04)	0.91 (0.72, 1.15)	0.81 (0.54, 1.22)
Stratum 8	0.95 (0.84, 1.08)	0.91 (0.76, 1.09)	0.99 (0.80, 1.21)	0.83 (0.58, 1.18)
Stratum 9	1.02 (0.91, 1.14)	1.01 (0.86, 1.17)	1.06 (0.89, 1.27)	0.81 (0.59, 1.10)
Stratum 10	1.07 (0.97, 1.17)	1.01 (0.88, 1.15)	1.04 (0.89, 1.20)	0.92 (0.72, 1.19)

Supplementary Table 11: Mendelian randomization estimates for coronary heart disease in UK Biobank divided into overall (22,363 events) and incident-only CHD (excluding those with prevalent CHD at baseline, 5447 events): odds ratios (95% confidence intervals) per 10 nmol/L higher genetically-predicted concentration of 25(OH)D.

CHD	Overall	Incident only
	OR (95% CI)	**OR (95% CI)**
Overall	0.98 (0.95-1.01) p=0.25	1.00 (0.94-1.07) p=0.98
Women	0.98 (0.92-1.04) p=0.54	1.01 (0.89-1.15) p=0.85
Men	0.98 (0.94-1.02) p=0.34	1.00 (0.93-1.07) p=0.92
Stratifying on 25(OH)D:		
Stratum 1 (lowest)	1.32 (0.98, 1.77)	1.37 (0.79, 2.39)
Stratum 2	0.94 (0.74, 1.19)	1.03 (0.66, 1.62)
Stratum 3	0.98 (0.80, 1.20)	1.16 (0.79, 1.70)
Stratum 4	1.02 (0.86, 1.22)	1.11 (0.80, 1.54)
Stratum 5	0.93 (0.81, 1.07)	0.94 (0.72, 1.24)
Stratum 6	0.96 (0.85, 1.09)	0.95 (0.76, 1.20)
Stratum 7	0.97 (0.86, 1.08)	0.94 (0.77, 1.16)
Stratum 8	0.95 (0.87, 1.04)	0.92 (0.77, 1.10)
Stratum 9	0.97 (0.89, 1.05)	1.00 (0.85, 1.17)
Stratum 10 (highest)	1.00 (0.94, 1.07)	1.00 (0.87, 1.15)

Supplementary Table 12: Mendelian randomization estimates for main outcomes in UK Biobank in overall population and divided into clinical strata by residual concentration of 25(OH)D for pleiotropic genome-wide score.

Study and stratum	Mean 25(OH)D (nmol/L)	Coronary heart disease	Stroke	All cause mortality
UK Biobank				
Overall		0.92 (0.89, 0.95)	0.96 (0.92, 1.01)	0.99 (0.96, 1.03)
Stratum 1	28.7	0.96 (0.78, 1.19)	0.81 (0.61, 1.07)	0.98 (0.81, 1.19)
Stratum 2	35.8	1.04 (0.86, 1.25)	1.07 (0.83, 1.38)	1.12 (0.93, 1.36)
Stratum 3	41.1	0.93 (0.78, 1.11)	1.01 (0.81, 1.27)	0.94 (0.79, 1.11)
Stratum 4	45.9	0.82 (0.72, 0.95)	1.00 (0.81, 1.24)	0.92 (0.79, 1.07)
Stratum 5	50.6	0.86 (0.76, 0.97)	0.96 (0.80, 1.15)	1.02 (0.89, 1.16)
Stratum 6	55.4	0.92 (0.83, 1.03)	0.87 (0.73, 1.02)	1.01 (0.89, 1.15)
Stratum 7	60.5	0.93 (0.84, 1.04)	0.99 (0.85, 1.16)	0.99 (0.88, 1.12)
Stratum 8	66.3	0.86 (0.78, 0.95)	0.99 (0.86, 1.14)	1.03 (0.93, 1.14)
Stratum 9	73.8	0.97 (0.89, 1.05)	1.01 (0.89, 1.15)	0.96 (0.88, 1.05)
Stratum 10	86.5	0.95 (0.88, 1.02)	0.94 (0.85, 1.04)	0.99 (0.92, 1.07)

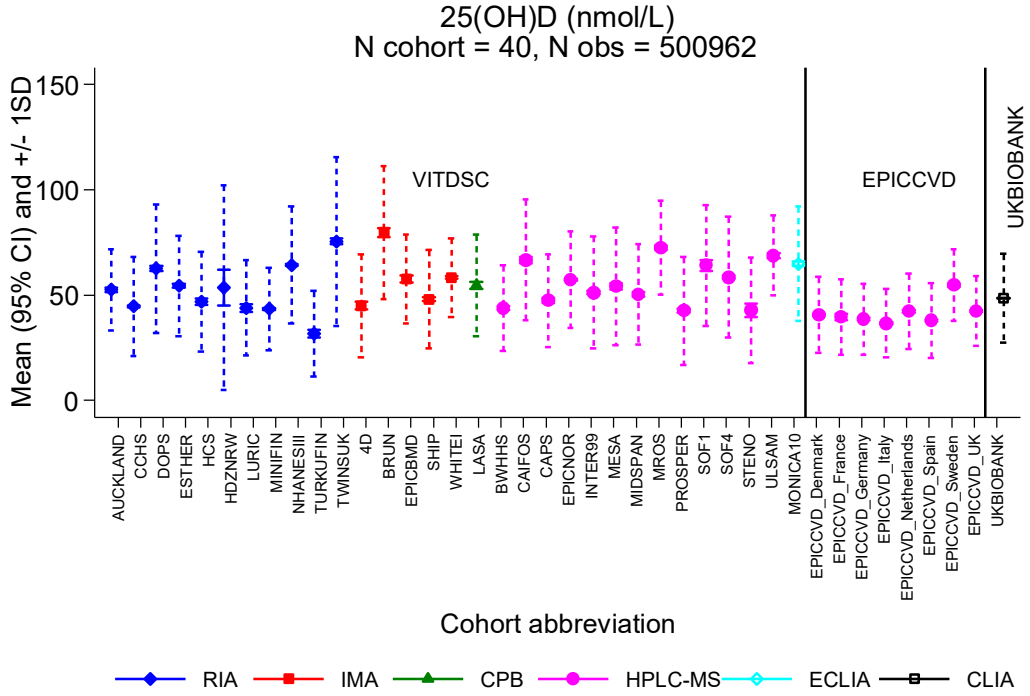
Mendelian randomization estimates using the genome-wide score cannot reliably be attributed to 25(OH)D levels due to pleiotropic associations of the genome-wide score with LDL-cholesterol and triglycerides. These estimates should therefore not be considered as reliable Mendelian randomization estimates.

Supplementary Table 13: Study-specific Mendelian randomization estimates for cause-specific mortality for overall population and divided into clinical strata by residual concentration of 25(OH)D.

Study and stratum	All-cause mortality	Cardiovascular mortality	Cancer mortality	Non-cancer non-cardiovascular mortality
UK Biobank				
Overall	1.00 (0.96, 1.03)	0.99 (0.92, 1.07)	0.99 (0.94, 1.03)	1.02 (0.95, 1.09)
Stratum 1	1.06 (0.81, 1.39)	1.30 (0.77, 2.22)	0.85 (0.58, 1.25)	1.23 (0.78, 1.93)
Stratum 2	1.06 (0.84, 1.34)	1.33 (0.80, 2.20)	0.90 (0.66, 1.24)	1.16 (0.75, 1.79)
Stratum 3	1.09 (0.89, 1.33)	1.22 (0.79, 1.89)	1.12 (0.86, 1.48)	0.90 (0.61, 1.31)
Stratum 4	1.12 (0.94, 1.34)	1.07 (0.74, 1.54)	1.15 (0.91, 1.46)	1.08 (0.77, 1.52)
Stratum 5	0.95 (0.82, 1.10)	0.93 (0.67, 1.27)	0.94 (0.77, 1.15)	1.00 (0.74, 1.34)
Stratum 6	0.98 (0.86, 1.11)	0.91 (0.69, 1.21)	1.00 (0.84, 1.18)	1.03 (0.79, 1.34)
Stratum 7	0.93 (0.83, 1.04)	0.97 (0.75, 1.26)	0.91 (0.79, 1.06)	0.97 (0.77, 1.21)
Stratum 8	0.97 (0.88, 1.07)	0.85 (0.68, 1.05)	1.03 (0.91, 1.18)	0.95 (0.78, 1.16)
Stratum 9	0.97 (0.89, 1.05)	0.94 (0.78, 1.14)	0.97 (0.87, 1.08)	0.99 (0.84, 1.18)
Stratum 10	1.01 (0.94, 1.08)	0.95 (0.82, 1.11)	1.01 (0.92, 1.10)	1.06 (0.92, 1.22)
Copenhagen studies				
Overall	0.89 (0.80, 0.99)	1.12 (0.95, 1.31)	0.88 (0.77, 1.01)	0.93 (0.81, 1.06)
Stratum 1	0.95 (0.66, 1.37)	1.04 (0.65, 1.65)	1.06 (0.68, 1.66)	0.96 (0.65, 1.41)
Stratum 2	0.92 (0.60, 1.41)	0.92 (0.52, 1.63)	0.90 (0.53, 1.53)	0.98 (0.61, 1.59)
Stratum 3	0.91 (0.60, 1.38)	1.05 (0.57, 1.90)	0.87 (0.51, 1.48)	0.88 (0.52, 1.49)
Stratum 4	0.93 (0.61, 1.42)	1.13 (0.61, 2.10)	0.90 (0.51, 1.59)	0.87 (0.50, 1.51)
Stratum 5	0.93 (0.60, 1.44)	1.34 (0.70, 2.57)	0.82 (0.48, 1.39)	0.89 (0.51, 1.55)
Stratum 6	0.90 (0.57, 1.43)	1.34 (0.67, 2.68)	0.84 (0.47, 1.52)	0.89 (0.50, 1.61)
Stratum 7	0.88 (0.57, 1.38)	1.31 (0.66, 2.63)	0.85 (0.46, 1.55)	0.92 (0.52, 1.65)
Stratum 8	0.81 (0.51, 1.28)	1.17 (0.56, 2.47)	0.79 (0.42, 1.45)	0.98 (0.53, 1.80)
Stratum 9	0.81 (0.52, 1.27)	1.10 (0.54, 2.25)	0.84 (0.45, 1.55)	0.92 (0.50, 1.71)
Stratum 10	0.83 (0.56, 1.23)	0.99 (0.49, 1.98)	0.95 (0.54, 1.68)	0.99 (0.58, 1.69)

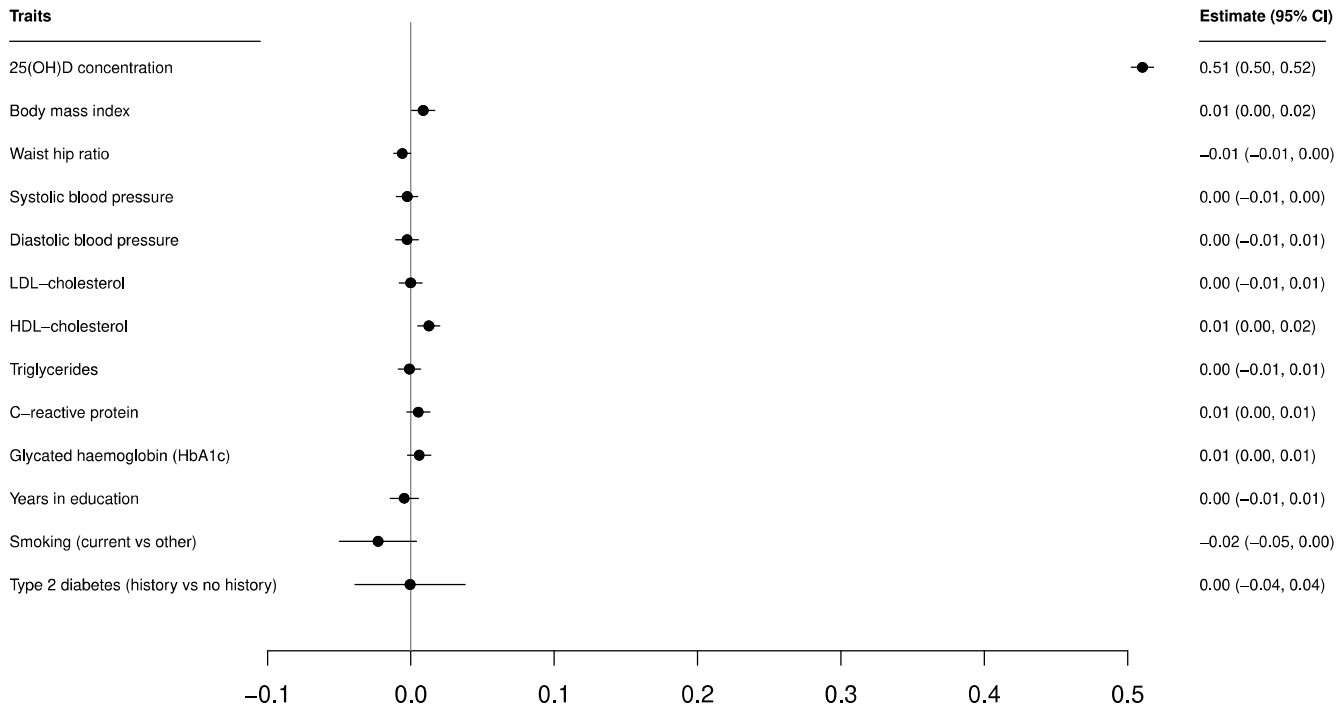
Estimates (95% confidence intervals) represent odds ratio per 10 nmol/L higher genetically-predicted concentration of 25(OH)D.

Supplementary Figure 1: Mean and spread of 25(OH)D measurements divided by data source and assay type. Solid error bars represent 95% confidence intervals (CI) for the mean, dashed error bars represent +/- 1 standard deviation (SD).



Assay abbreviations: RIA, Radioimmunoassay; IMA, Immunometric assay; CPB, Competitive protein binding; HPLC-MS, High-performance liquid chromatography mass spectrometry; ECLIA, Electro-chemiluminescence immunoassay; CLIA, Chemiluminescence immunoassay.

Supplementary Figure 2: Associations of the focused (primary) genetic risk score for 25(OH)D concentrations with cardiovascular traits in UK Biobank

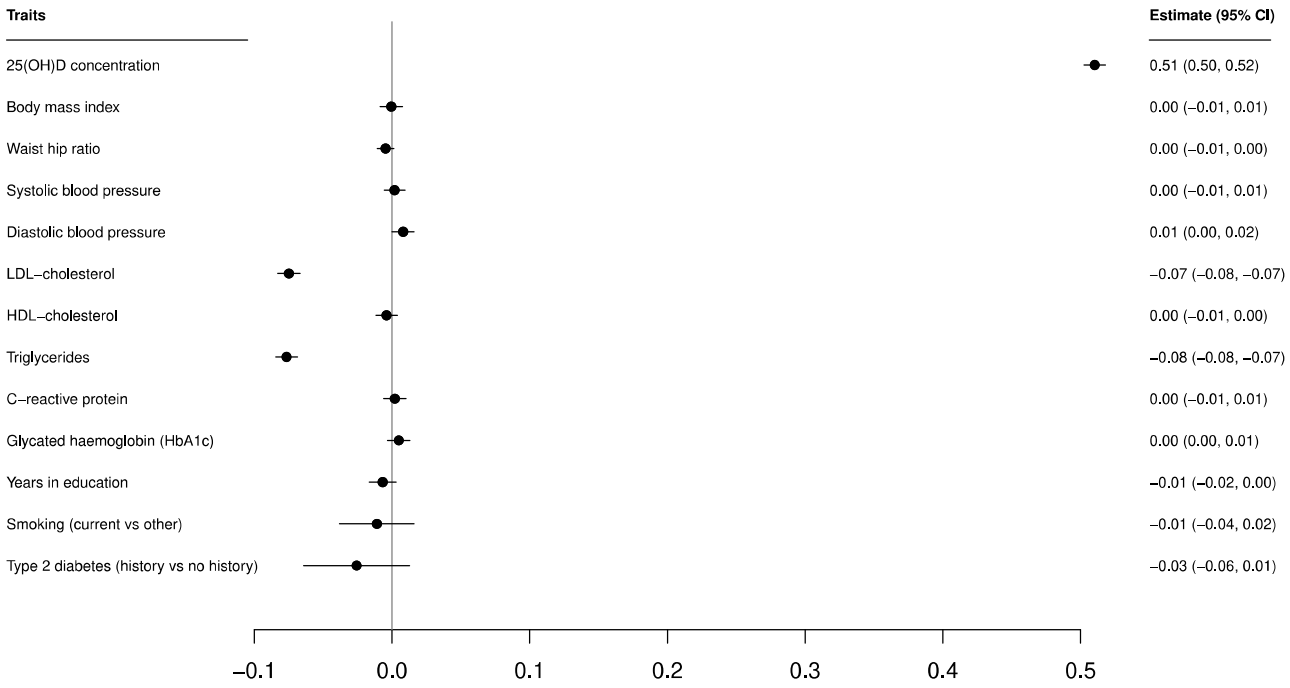


Estimates for all continuous traits expressed in standard deviation units. Estimates for the binary traits (smoking and Type 2 diabetes status) are log odds ratios. Associations are scaled to a 10 nmol/L increase in genetically-predicted 25(OH)D concentrations (10 nmol/L = 0.51 standard deviations).

Associations were estimated in UK Biobank with adjustment for age at baseline, sex, centre, and 10 genomic principal components.

The association with body mass index represents a 0.017 kg/m^2 increase per 1 standard deviation increase in the GRS (p = 0.035). The association with HDL-cholesterol represents a 0.002 mmol/L increase per 1 standard deviation increase in the GRS (p = 0.001).

Supplementary Figure 3: Associations of the genome-wide score with cardiovascular traits in UK Biobank



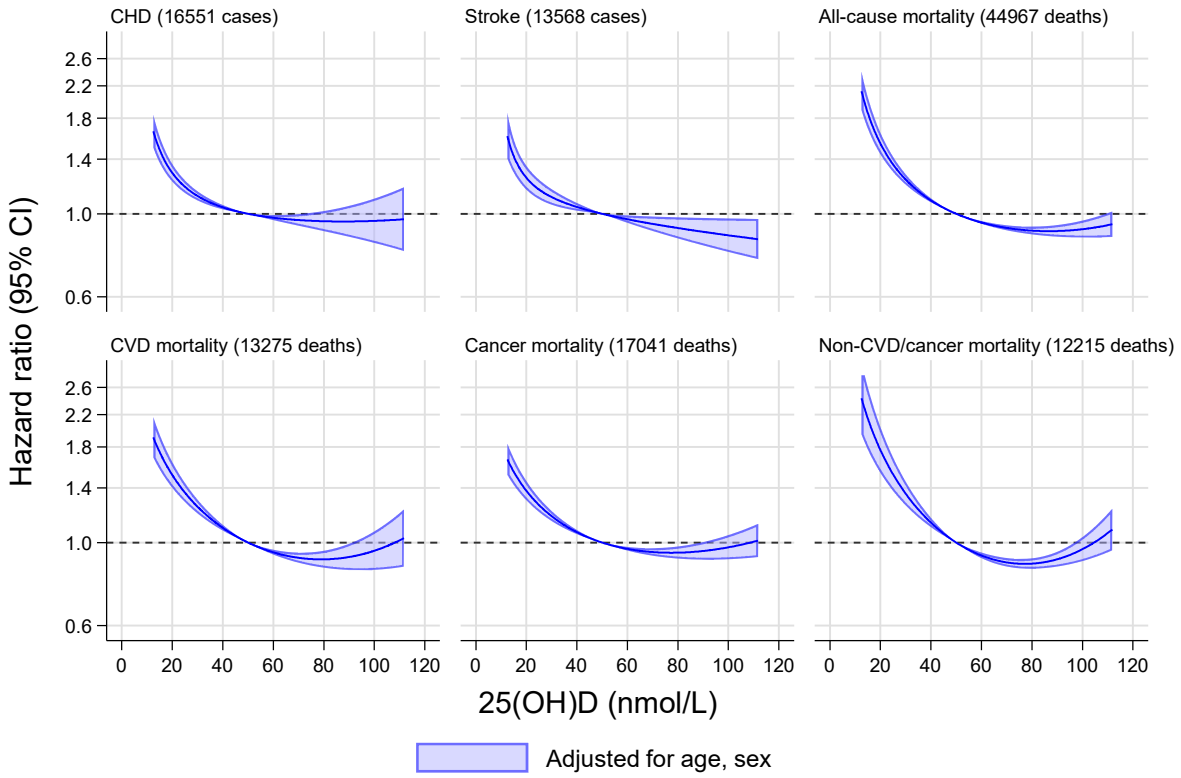
Estimates for all continuous traits expressed in standard deviation units. Estimates for the binary traits (smoking and Type 2 diabetes status) are log odds ratios. Associations are scaled to a 10 nmol/L increase in genetically-predicted 25(OH)D concentrations (10 nmol/L = 0.51 standard deviations).

Associations were estimated in UK Biobank with adjustment for age at baseline, sex, centre, and 10 genomic principal components.

The association with LDL-cholesterol represents a 0.024 mmol/L (0.94 mg/dL) decrease in LDL-cholesterol per 1 standard deviation increase in the GRS ($p = 1\times10^{-72}$). The association with triglycerides represents a 0.032 mmol/L (2.83 mg/dL) decrease in triglycerides per 1 standard deviation increase in the GRS ($p=3\times10^{-80}$). A one standard deviation increase in the GRS corresponds to a 4.2 nmol/L increase in 25(OH)D concentrations.

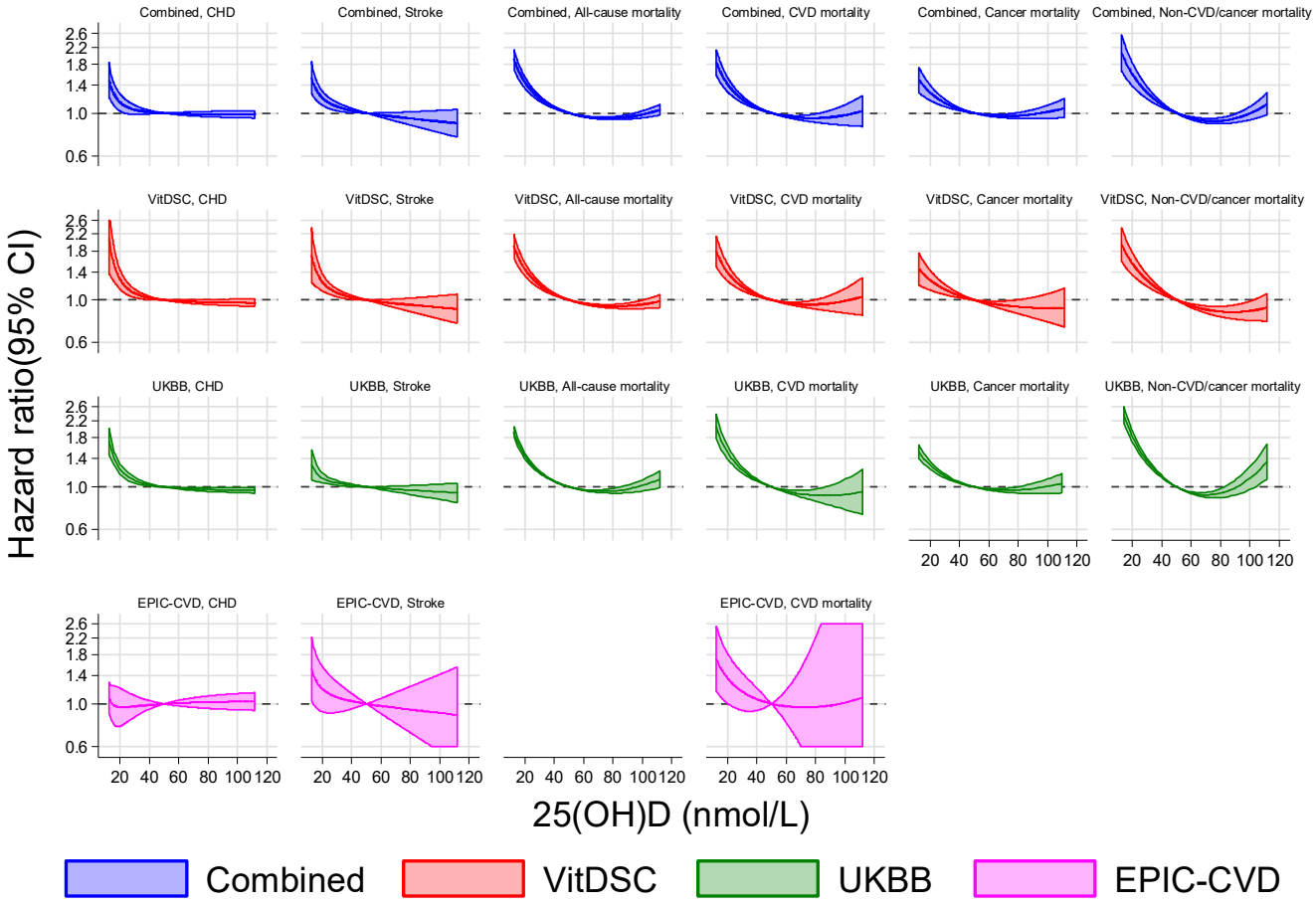
As the genome-wide score is strongly associated with LDL-cholesterol and triglycerides, and as these are strong risk factors for cardiovascular disease and mortality, Mendelian randomization estimates based on this choice of variants are not reliable.

Supplementary Figure 4: Observational associations of 25(OH)D concentrations with outcomes (age and sex adjusted only).



Reference value is 50 nmol/L. The shaded area represents the 95% confidence interval for the dose—response curve. Study-specific analyses involved fractional polynomial modelling of continuous associations of 25(OH)D and outcomes using Cox regression stratified by sex, and (where appropriate) trial arm and centre, and adjusted for age at blood draw for 25(OH)D measurement, followed by random effects meta-analysis (see **Methods**).

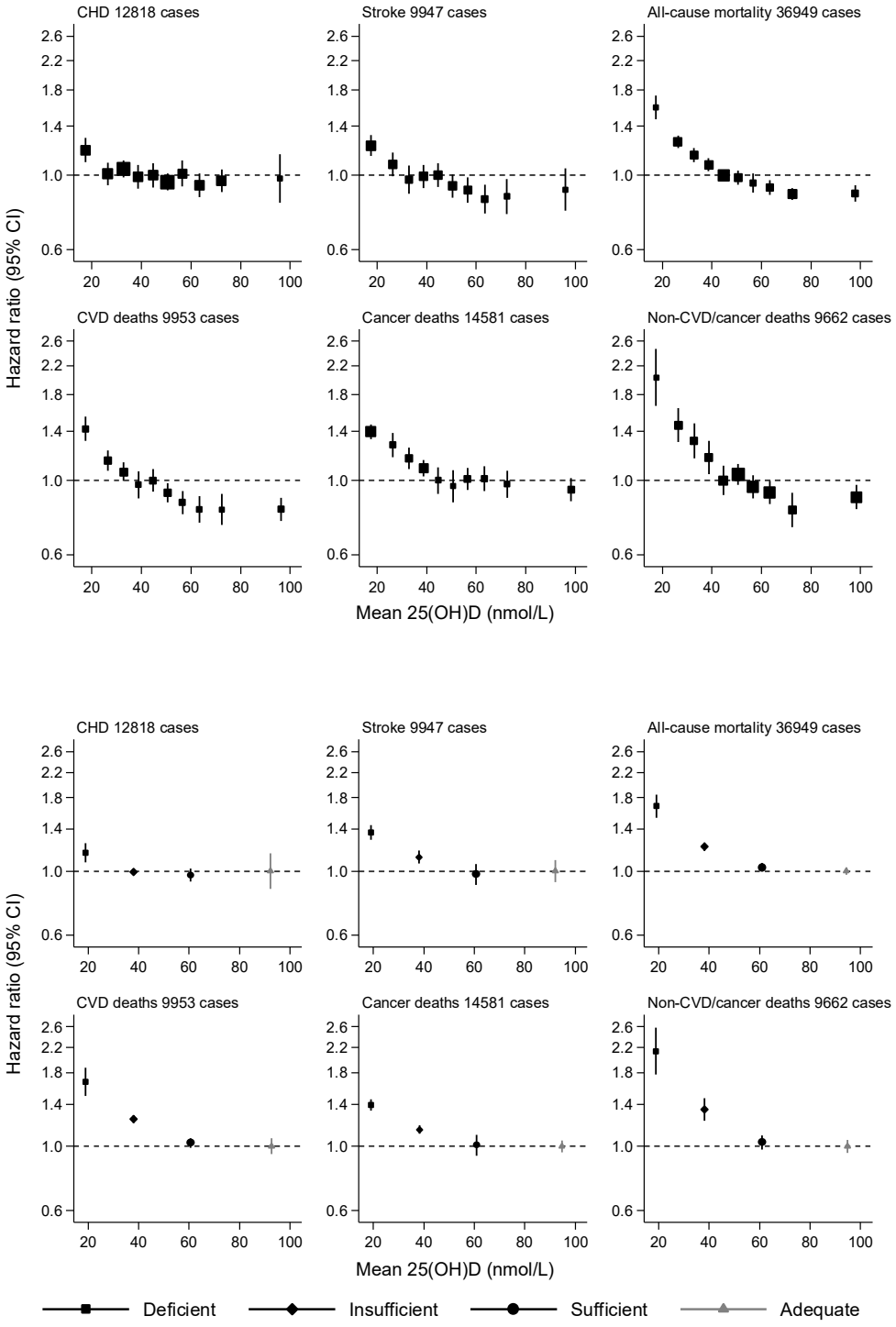
Supplementary Figure 5: Study-specific dose—response curves for associations of 25(OH)D concentrations with outcomes combined across data sources (top) and separated by data source (bottom) adjusted for conventional cardiovascular risk factors.



Reference value is 50 nmol/L. The shaded area represents the 95% confidence interval for the dose—response curve. Study-specific analyses involved fractional polynomial modelling of continuous associations of 25(OH)D and outcomes using Cox regression stratified by sex and (where applicable) centre or trial arm, and adjusted for conventional cardiovascular risk factors, namely: age at blood draw for 25(OH)D measurement, calendar month of blood draw, smoking status (current versus other), total cholesterol, high-density lipoprotein (HDL) cholesterol, systolic blood pressure, known history of diabetes, and body mass index, followed by random effects meta-analysis (**see Methods**).

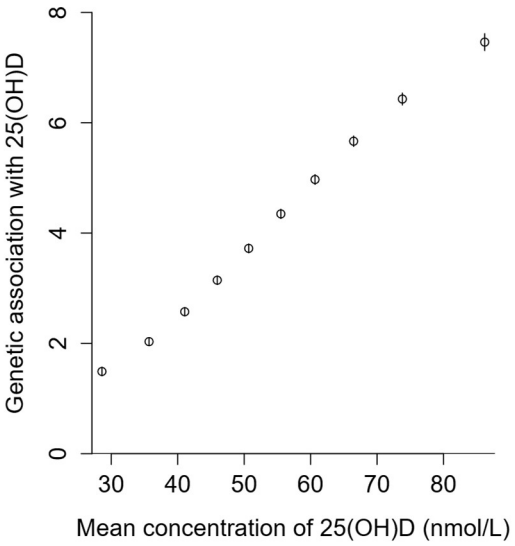
The EPIC-CVD study was specifically designed as a case-cohort study of CVD outcomes therefore does not contribute to analysis of non-CVD outcomes nor all-cause mortality.

Supplementary Figure 6: Dose—response association of 25(OH)D concentrations with outcomes by deciles (top) and categories (bottom).

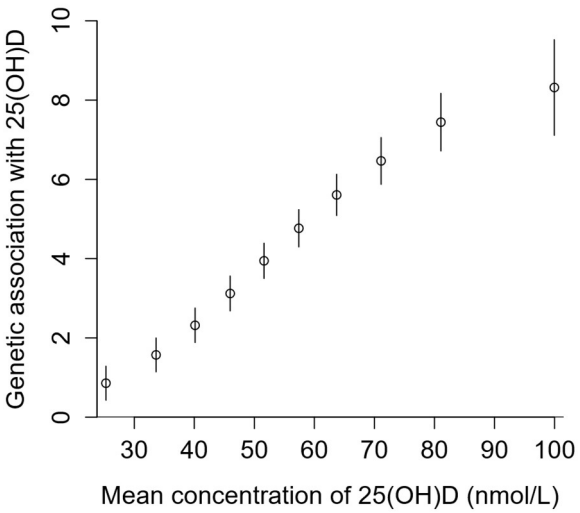


Reference value is decile 5 (close to 50 nmol/L) in top figure and is the adequate clinical subgroup (>75 nmol/L) in the bottom figure. The associations of 25(OH)D and outcomes were modelled using Cox regression stratified by sex and (where applicable) centre or trial arm, and adjusted for conventional cardiovascular risk factors, namely: age at blood draw for 25(OH)D measurement, calendar month of blood draw, smoking status (current versus other), total cholesterol, high-density lipoprotein (HDL) cholesterol, systolic blood pressure, known history of diabetes, and body mass index, followed by random effects meta-analysis (**see Methods**).

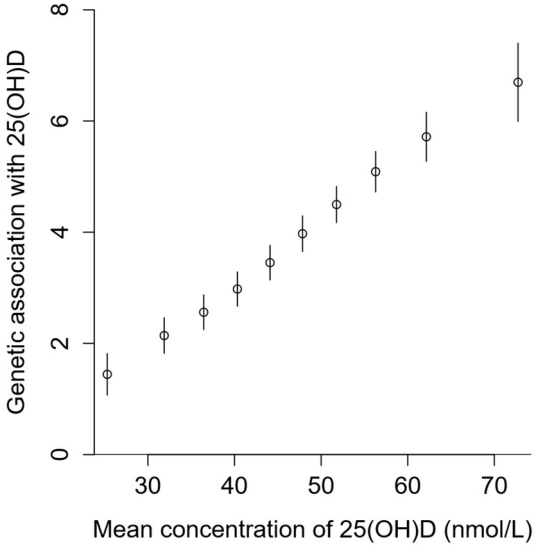
Supplementary Figure 7: Associations between the genetic risk score and 25(OH)D concentrations in strata of 25(OH)D concentrations in: a) UK Biobank, b) Copenhagen studies, and c) EPIC-CVD.



a.



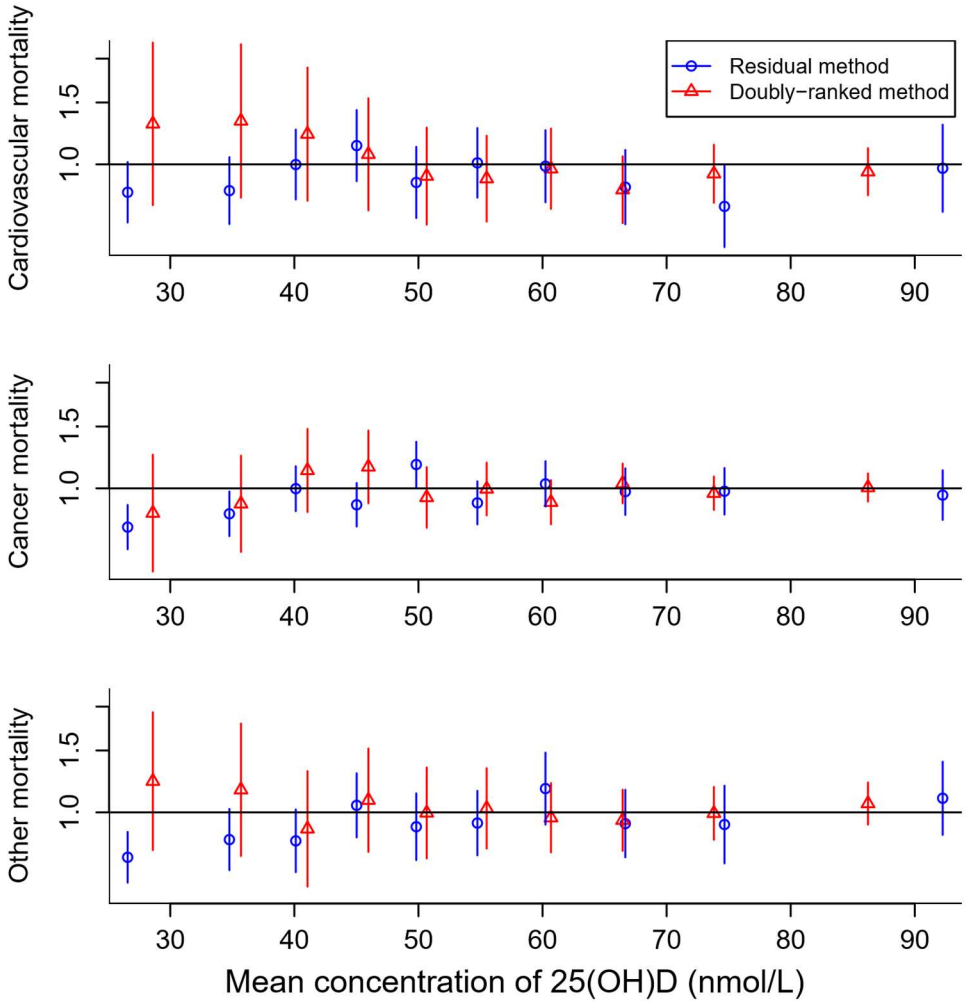
b.



c.

Estimates (95% confidence intervals) represent the association with 25(OH)D in nmol/L per 1 standard deviation increase in the genetic risk score (GRS). For UK Biobank, the genetic association with 25(OH)D in the lowest decile is 1.50 nmol/L; the genetic association with 25(OH)D in the highest decile is 7.51 nmol/L. The ratio between these values is 5.0. Corresponding values are 0.89 nmol/L and 8.59 nmol/L (ratio 9.7) in the Copenhagen studies; and 1.46 nmol/L and 6.74 nmol/L (ratio 4.6) in EPIC-CVD.

Supplementary Figure 8: Stratified Mendelian randomization estimates for mortality outcomes in UK Biobank from residual and doubly-ranked methods.



Estimates (95% confidence intervals) represent odds ratios per 10 nmol/L higher genetically-predicted concentration of 25(OH)D in strata of the population defined by residual concentration of 25(OH)D. Estimates from the residual method (not reliable when the genetic effect on the exposure vary in the population) are shown as blue triangles. Estimates from the doubly-ranked method (more reliable when the genetic effect on the exposure vary in the population) are shown as red triangles.

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