Mendelian Randomization Studies Do Not Support a Role for Vitamin D in Coronary Artery Disease

Despoina Manousaki, MD; Lauren E. Mokry, MSc; Stephanie Ross, PhD; David Goltzman, MD; J. Brent Richards, MD, MSc

Background—Observational studies support a possible association between decreased vitamin D levels and risk of coronary artery disease (CAD); however, it remains unclear whether this relationship is causal. We aimed to evaluate whether genetically lowered vitamin D levels influence the risk of CAD using a Mendelian randomization approach.

Methods and Results—In this 2-stage Mendelian randomization study, we first identified single-nucleotide polymorphisms associated with 25-hydroxyvitamin D (25OHD) levels in the SUNLIGHT consortium (n=33 996), then tested them for possible violation of Mendelian randomization assumptions. A count of risk alleles was tested for association with 25OHD levels in a separate cohort (n=2347). Alleles were weighted by their relative effect on 25OHD and tested for their combined effect on CAD in the Coronary Artery Disease Genome-Wide Replication and Meta-Analysis (CARDIoGRAM) study (22 233 cases/64 762 controls). Four single-nucleotide polymorphisms were identified to be associated with 25OHD levels, all in or near genes implicated in 25OHD synthesis, transport or metabolism. A count of these risk alleles was strongly associated with 25OHD (n=2347, *F*-test statistic=49.7, $P=2\times10^{-12}$). None of the single-nucleotide polymorphisms associated with 25OHD levels were associated with CAD (all *P* values >0.6). The Mendelian randomization odds ratio (OR) for CAD was 0.99 (95% confidence interval, 0.84–1.17; *P*=0.93; $I^2=0$) per SD decrease in log-transformed 25OHD levels. These results persisted after sensitivity analyses for population stratification and pleiotropy.

Conclusions—Genetically lowered 25OHD levels were not associated with increased risk of CAD in a large, well-powered study, suggesting that previous associations between circulating 25OHD levels and CAD are possibly confounded or due to reverse causation. **(*Circ Cardiovasc Genet*. 2016;9:349-356. DOI: 10.1161/CIRCGENETICS.116.001396.)**

Key Words: coronary artery disease ■ Genome-Wide Association Study ■ Mendelian randomization analysis ■ single-nucleotide polymorphism ■ vitamin D

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Despite increasing public awareness and major therapeutic progress, coronary artery disease (CAD) remains the international leading cause of morbidity and mortality.[1] Growing evidence suggests that vitamin D deficiency is associated with CAD development.[2–4] Specifically, the results from many,[5–8] although not all,[9–12] observational studies investigating this relationship suggest that low levels of vitamin D, as measured by serum total 25-hydroxy vitamin D (25OHD) concentrations, are associated with increased risk of CAD-related outcomes. These findings may have important public health impact because vitamin D insufficiency and deficiency are common (affecting one third of Americans in 2001–2006),[13] and vitamin D replacement therapy is relatively safe and inexpensive.

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Such findings have motivated inquiry into the biological plausibility of this relationship, and some evidence has suggested that vitamin D may influence CAD directly through the vitamin D receptor in smooth muscle cells of the cardiac vasculature or indirectly by promoting calcium absorption at the expense of lipid absorption or excretion in the gut.[14] Other mechanistic explanations have been proposed, including endothelial dysfunction from the lack of adequate vitamin D, vascular compliance impairment because of smooth muscle changes, enhanced inflammation, effects related to high levels of PTH, or the renin–angiotensin system.[15–17] Finally, some studies have suggested that vitamin D supplementation could lower vascular risk by improving glucose tolerance or by inhibiting inflammatory components in the metabolic syndrome.[18–20]

Recent meta-analyses of observational studies have reported, on balance, a significant relationship between 25OHD levels and composite cardiovascular events.[21–23] Meta-analyses of the few randomized controlled trials that have assessed the effect of vitamin D administration on risk of CAD have not produced conclusive results, possibly because of heterogeneity of outcomes and interventions.[20,21,23]

Given this uncertainty, clinical practice guidelines do not support vitamin D supplementation for CAD risk reduction and

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conclude that additional research, particularly from randomized trials, is needed.[24,25] Given the high prevalence of low vitamin D levels, such randomized controlled trials would be relevant, yet large, long-term randomized, controlled trials (RCTs) are difficult to fund because vitamin D therapy cannot be patented.

In the absence of high-quality RCT data and given the inconclusive results from the existing observational studies, the principles of Mendelian randomization (MR) can be applied to strengthen, or refute, the causality of biomarkers in disease pathogenesis.[26] MR analysis uses genetic associations to test the relationship between biomarkers, such as 25OHD, and risk of disease, such as CAD. This approach, which is conceptually similar to an RCT, is based on the principle that genetic variants are randomly allocated at gamete formation and consequently these genetic variants are independent of confounding factors that bias observational studies. Furthermore, MR is free of reverse causation because genotypes are assigned before the onset of CAD. A recent MR study found no relationship between 25OHD levels and CAD risk, but only used 2 of 4 well-validated 25OHD genetic loci in a smaller sample size of 14 455 cases.[27]

In this study, we adopted an MR design to estimate the effect of genetically lowered 25OHD levels on CAD susceptibility combining data from several large-scale studies. We first selected genome-wide significant single-nucleotide polymorphisms (SNPs) as identified by the Study of Underlying Genetic Determinants of Vitamin D and Highly Related Traits (SUNLIGHT) consortium, the largest genome-wide association study (GWAS) published to date for 25OHD levels (n=33 995).[28] Next, we used the estimates of the effect of each of these SNPs on 25OHD levels in the Canadian Multicentre Osteoporosis Study (CaMos),[29] which we had generated in a previous MR study.[30] The CaMos cohort was used because effect sizes could not be estimated from the SUNLIGHT consortium because of different 25OHD measurement methods used in SUNLIGHT cohorts. Finally, we applied the principles of MR to test whether a lifetime of genetically lowered 25OHD levels influence CAD risk using data from the largest meta-analysis of GWAS studies assessing the CAD risk, the Coronary Artery Disease Genome-Wide Replication and Meta-Analysis (CARDIoGRAM, n=86 995).[31]

Methods

SNP Selection and Data Sources

We used the SUNLIGHT consortium,[28] a GWAS study consisting of 33 996 individuals of European descent from 15 cohorts, to obtain genetic variants associated with 25OHD levels at a genome-wide significant level ($P<5\times10^{-8}$). 25-Hydroxyvitamin D levels in this study were measured by radioimmunoassay, chemiluminescent assay, ELISA, or mass spectrometry. Given that different cohorts used different methods to measure 25OHD levels, results were combined across cohorts in the SUNLIGHT consortium using *Z* score–weighted meta-analysis. Although other GWAS for 25OHD have been reported,[32–34] the SUNLIGHT consortium offers the largest sample size and no other loci have been genome-wide significant, to our knowledge.

Data from the CaMos[29] were used to estimate the effect of each genome-wide significant SNP on 25OHD levels because the effect of each SNP on 25OHD levels could not be used from the SUNLIGHT consortium because of the *Z* score meta-analytic approach used. CaMos is a large population-based cohort and was among the largest included in the replication phase of the SUNLIGHT consortium. It includes 2347 individuals who were genotyped using TaqMan genotyping at the same genome-wide significant vitamin D loci found in the SUNLIGHT consortium. Serum total 25OHD was measured using chemiluminescent immunoassay technology. We have previously reported the effects of these SNPs in CaMos.[30]

To obtain precise estimates for the association of 25OHD with CAD, we tested the effect of each genome-wide significant SNP for vitamin D levels in CARDIoGRAM,[31] a meta-analysis of 22 GWAS studies of European descent imputed to HapMap 2 involving 22 233 cases and 64 762 controls. CAD outcomes were defined as one of the following: myocardial infarction, >50% stenosis in at least 1 coronary vessel at angiography, history of percutaneous transluminal coronary angioplasty or coronary artery bypass graft surgery, and angina or death due to CAD. Genotyping in individual discovery GWAS was carried out on Affymetrix or Illumina platforms. Data from the more recent and larger CARDIoGRAMplusC4D Metabochip meta-analysis,[35] involving 63 746 cases and 130 681 controls, could not be used in the present MR analysis because no estimates for the SNPs of interest for vitamin D levels were found on the Metabochip, whereas all 4 SNPs were present in the original CARDIoGRAM GWAS. Moreover, no proxies of the 4 SNPs with an appropriate level of r^2 (>0.8) were found in Metabochip.

SNP Validation

MR analysis requires genetic variants used as instrumental variables to be evaluated for the several MR assumptions: linkage disequilibrium, population stratification, and pleiotropy. For the 4 SUNLIGHT 25OHD SNPs, linkage disequilibrium and population stratification have been already tested in our previous MR study.[30] Our population stratification assessment showed that only the *DHCR7* SNP was strongly associated with non-European ancestry in the CaMos cohort ($P=2.7\times10^{-13}$). In this study, we performed an assessment for pleiotropy to assure that the chosen SNPs do not exert effects on CAD through biological pathways independent of 25OHD levels. Although findings from the 1958 British Birth Cohort[36] did not support any association between the SUNLIGHT 25OHD SNPs and relevant pleiotropic pathways, such as sun exposure, time outside, physical activity, fish oil consumption, smoking, alcohol consumption, and body mass index (BMI), some of these factors could act at least partially through the vitamin D pathway. To assess for additional possible pleiotropy, we looked at the association between the vitamin D–related SNPs and major risk factors for CAD: low-density lipoprotein cholesterol, systolic and diastolic blood pressures, diagnosis of type 2 diabetes mellitus, and BMI in 4 large GWAS consortia (the Global Lipids Genetic Consortium,[37] the International Consortium for Blood Pressure [ICBP] consortium,[38] the Diabetes Genetics Replication and Meta-Analysis DIAGRAM] consortium,[39] and the Genetic Investigation of Anthropometric Traits [GIANT],[40] respectively). These CAD risk factors may represent possible pleiotropic pathways if the 25OHD-associated SNPs influence CAD through these risk factors, independently of 25OHD. We further explored for pleiotropy by conducting a literature search of gene name and gene mutation to identify published possible pleiotropic mechanisms for any of the selected SNPs and CAD.

Association of 25OHD-Associated SNPs With CAD Susceptibility

To increase study power and to obtain the most precise estimates of the association of 25OHD-associated SNPs on risk of CAD, we used summary-level data from the CARDIoGRAM study (as described above). We assessed whether each SNP was associated with the risk of CAD, applying a Bonferroni correction, where statistical significance was declared at $P\leq0.05/4$ because 4 SNPs were associated with 25OHD levels from the SUNLIGHT consortium.

MR Estimates

We assessed the effects of the SNPs on risk of CAD, weighting the effect of each SNP by the magnitude of its effect on 25OHD levels using a 2-sample MR approach.[41] According to this study design, the independent SNPs were used to evaluate the association of exposure to genetically lowered 25OHD based on data from one study (CaMos) with CAD risk from another study (CARDIoGRAM). The SNP alleles were aligned in the 2 studies to ensure that the estimates represent the effects of 25OHD-decreasing alleles for each SNP. These individual

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estimates were then pooled using statistically efficient estimators formally analogous to those of inverse-variance weighted meta-analysis.[42] We next meta-analyzed the estimates obtained from individual 25OHD-decreasing alleles, using a fixed-effects model with an I^2 estimate to account for heterogeneity in the effect size.[43,44] The effect-size for the meta-analysis is reported in the main results as the effect of an SD change in natural log-transformed 25OHD levels, since this metric is more interpretable than an arbitrary difference. Next, we undertook power calculations[45] to test whether our study was adequately powered to detect clinically relevant changes in CAD risk.

To better understand the meaning of a 1 SD change in natural log-transformed 25OHD levels, we report data from a previous MR study published from our group.[30] In this study,[30] the effect of 1 SD increase in log-transformed 25OHD levels on 25OHD levels in vitamin D sufficient individuals (defined as individuals with 25OHD levels between 50 and 75 nmol/L) was 35.6 nmol/L. The same effect on vitamin D insufficient individuals (25OHD levels between 25 and 50 nmol/L) was 23.72 nmol/L and on vitamin D–deficient individuals (25OHD levels <25 nmol/L) was 11.86 nmol/L. Therefore, in the vitamin D insufficient and sufficient groups, a 1 SD increase in log-transformed 25OHD levels results in normalization of 25OHD levels, effect comparable to that achieved with supplementation.[46]

Sensitivity Analyses

We then recalculated our MR estimates after exclusion of SNPs potentially influenced by pleiotropy or population stratification. Because SNPs associated with 25OHD levels in the SUNLIGHT consortium influence either 25OHD synthesis or 25OHD metabolism,[47] we elected to perform a stratified MR analysis where SNPs involved in either 25OHD synthesis or metabolism were analyzed separately.

Results

SNP Selection and Validation

SNP Selection

All the data sources used in this study are generated from populations of European descent (Figure 1). The SUNLIGHT consortium identified 4 SNPs as genome-wide significant for 25OHD levels[28]: rs2282679 in *GC* (association with 25OHD $P=1.9\times10^{-109}$), rs12785878_near_*DHCR7* ($P=2.1\times10^{-27}$), rs10741657 near *CYP2R1* ($P=3.3\times10^{-20}$), and rs6013897 in *CYP24A1* ($P=6.0\times10^{-10}$). In addition to this strong statistical evidence of association, all SNPs map to genes implicated in the modulation of 25OHD levels through distinct mechanisms, and more specifically transport (*GC*), synthesis (*DHCR7*), hepatic hydroxylation (*CYP2R1*), and catabolism (*CYP24A1*).[47] Notably, all 4 SNPs lie in intergenic or intronic regions, yet the exact effect of each SNP on these enzymes is unknown. Nevertheless, all SNPs reside near genes strongly implicated in vitamin D synthesis or metabolism.[47]

Linkage Disequilibrium (LD) and *Pleiotropy Assessment*

We found no evidence of LD between any of these SNPs (all pairwise $r^2\leq0.01$) in the 1000 Genomes Project European samples.[48] Of note, only 2 of our SNPs, rs10741657 and rs12785878, were located on the same chromosome, which greatly decreases the risk of confounding by LD. Among the 5 vitamin D–associated SNPs reported in the GWAS catalog, only the rs3829251 on chromosome 11 was not reported as genome-wide significant in the SUNLIGHT study, thus not included as an instrumental variable in our MR analysis. We did not find evidence of LD between this SNP and our 2 chromosomes, 11 SNPs rs10741657 and rs12785878 ($r^2<0.2$ for both).

We undertook a literature review for possible pleiotropic pathways influencing cardiometabolic traits, assessing associations between the 4 SNPs with known CAD risk factors, such as hypertension, hyperlipidemia, type 2 diabetes mellitus, and obesity. We found no evidence for pleiotropic mechanisms for the vitamin D metabolism SNPs: rs10741657 (*CYP2R1*) and rs6013897 (*CYP24A1*). Interestingly, a GWAS study by Shen et al[49] has demonstrated a marginal association between another SNP (rs2762939) in the *CYP24A1* gene and coronary artery calcification with a P value of 2.9×10^{-6}, but there was no correlation between this SNP and 25OHD levels in the SUNLIGHT consortium.[50] Also, we did not detect any LD between the rs2762939 SNP and the 25OHD-associated *CYP24A1* SNP, rs10741657. A recent MR study using rs12785878 (*DHCR7*) and rs10741657 (*CYP24A1*) as instrumental variables, along with two other SNPs on the *GC* and *CYP24A1* genes, did not demonstrate any association with type 2 diabetes mellitus.[51]

Although it has been argued that vitamin D–binding protein, encoded by *GC*, can act independently of vitamin D to produce clinical phenotypes, this does not seem to be the case for CAD.[52] For rs12785878 (*DHCR7*), a large MR study on vitamin D levels and blood pressure showed a small but significant association with hypertension[53] (OR, 0.92; 95% confidence interval [CI], 0.87–0.97; P=0.001), a major risk factor for CAD.[54] By querying the results for rs12785878 in the ICBP consortium,[38] a large GWAS meta-analysis of 200 000 subjects of European descent, we found that this SNP was not associated with systolic or diastolic blood pressure (P=0.703 and P=0.121, respectively). Genetic variation in *DHCR7* also seems to cause Smith–Lemli–Opitz syndrome, a clinical phenotype relating to cholesterol deficiency. Given that several studies suggest an interdependence of cholesterol and vitamin D pathways in the pathogenesis of CAD,[55] we queried

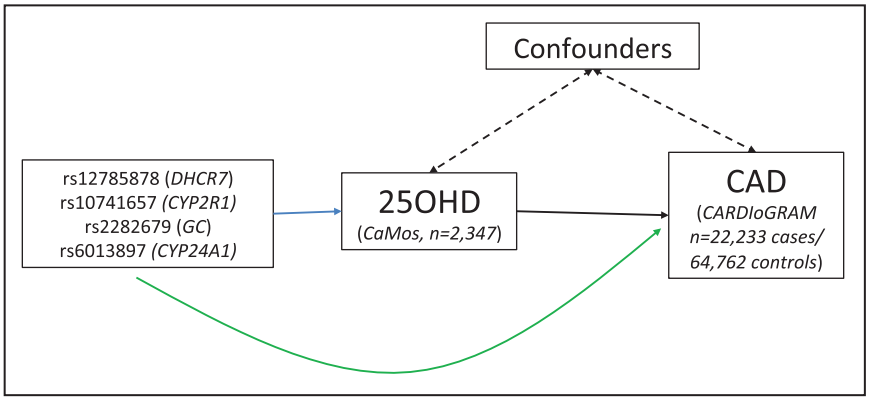

Figure 1. Schematic representation of Mendelian Randomization analysis. The box on the left describes single-nucleotide polymorphisms (SNPs), which were genome-wide significant for 25-hydroxyvitamin D (25OHD) levels in the SUNLIGHT consortium. The blue arrow represents the effect of the SNPs on multiply-adjusted, natural log-transformed 25OHD levels using data from the Canadian Multicentre Osteoporosis Study (CaMos). The green arrow represents the causal effect of decreased 25OHD levels on the risk of coronary artery disease (CAD) using data from the Coronary Artery Disease Genome-Wide Replication and Meta-Analysis (CARDIoGRAM) consortium.

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Table 1. *P* Values of the Association of the SNPs Used As Instrumental Variables With the Cardiometabolic Outcomes

SNP (gene)	LDL Cholesterol (Global Lipids Consortium[37])	Systolic/Diastolic Blood Pressure (ICBP[38])	BMI (GIANT[40])	Type 2 Diabetes Mellitus (DIAGRAM[39])
rs2282679 (*GC*)	0.29	0.47/0.64	0.91	0.76
rs6013897 (*CYP24A1*)	0.53	0.05/0.02	0.61	0.06
rs10741657 (*CYP2R1*)	0.16	0.99/0.59	0.29	0.23
rs12785878 (*DHCR7*)	0.04	0.70/0.12	0.78	0.14

BMI indicates body mass index; DIAGRAM, Diabetes Genetics Replication and Meta-Analysis Consortium; GIANT, Genetic Investigation of Anthropometric Traits; ICBP, International Consortium for Blood Pressure; LDL, low-density lipoprotein; and SNP, single-nucleotide polymorphism.

the association of rs12785878, in the largest publically available GWAS consortium results for lipids, the Global Lipids Genetics Consortium,[37] and found that this SNP was associated with a minimum *P* value of 0.043 across all lipid traits, suggesting that the SNP is weakly associated with cholesterol.

Finally, as part of our assessment for pleiotropy, we queried all 4 SNPs from the SUNLIGHT consortium in the Global Lipids Genetics Consortium,[37] the ICBP,[38] DIAGRAM consortium[39] and the GIANT[40] consortium (Table 1). Given the threshold of a *P* value of ≤0.0125 (0.05/4) set in the context of our study, we found no significant association between the *GC, DHCR7, CYP24A1*, and *CYP2R1* SNPs and the clinical outcomes of the 4 consortia (low-density lipoprotein cholesterol, systolic/diastolic blood pressure, type 2 diabetes mellitus, and BMI).

Population Stratification Assessment

Given that CAD varies by geographical location and such location might be a surrogate for ancestry, we assessed whether any of the 25OHD SNPs was associated with non-European ancestry. We have previously demonstrated that only rs1278578 may be associated with non-European ancestry,[30] thus undertook sensitivity analyses excluding this SNP.

Association of SUNLIGHT SNPs With 25OHD Levels

The association of the 4 SNPs that achieved genome-wide significance for 25OHD levels in the SUNLIGHT consortium with 25OHD levels is described in Table 2. Each of these SNPs explained an important proportion of the population-level variance in 25OHD levels, as reflected by the *F* statistics. As already shown in our previous study,[30] the count of 25OHD-decreasing alleles across these 4 SNPs was strongly associated with lower total 25OHD levels in the 2347 CaMos participants (Table 2).

Association of SUNLIGHT SNPs With CAD Susceptibility

Summary statistics for the 4 25OHD-associated SNPs (rs2282679 at *GC*, rs10741657 at *CYP2R1*, rs12785878 at *DHCR7*, and rs6013897 at *CYP24A1*) and their association with CAD was taken from the CARDIoGRAM study. All 4 25OHD-decreasing alleles were not associated with the risk of CAD (Table 2), and the 95% CIs were tight around the null.

MR Analysis for the Association of 25OHD With CAD Risk

To estimate the association of genetically lowered 25OHD on CAD, we used a fixed-effects model in which all 4 25OHD-decreasing alleles of the MR set were included. A decrease in 25OHD levels by 1 SD on the natural log scale was not associated with CAD, and the 95% CI limits were close to the null (OR, 0.99; 95% CI, 0.84–1.17; P=0.93; I^2=0; Table 3; Figure 2). We note that as our model included only 4 SNPs, the 95% CIs of the I^2 statistic are wide and consequently heterogeneity cannot be accurately measured using this parameter. In addition, due to potential effects of population stratification, we

Table 2. Characteristics of SNPs Used as Instrumental Variables

Vitamin D Results										CAD Results (CARDIoGRAM)			
Locus	Chr	25OHD-Associated SNP	25OHD Decreasing Allele	Allele Frequency	Effect on 25OHD*	SE of the Effect on 25OHD*	*P* Value for Association With 25OHD*	*F* Statistic for 25OHD*	SUNLIGHT Value for Association With 25OHD†	OR	95% CI	*P* Value for Association With CAD	Sample Size (Cases)
CYP2R1	11	rs10741657	C	0.62	−0.052	0.012	1.6×10^{-5}	18.78	3.3×10^{-20}	1.00	0.97–1.03	0.90	80 677 (19 739)
DHCR7	11	rs12785878	G	0.27	−0.056	0.013	2.0×10^{-5}	18.29	2.1×10^{-27}	0.99	0.96–1.02	0.53	83 295 (21 369)
GC	4	rs2282679	C	0.30	−0.047	0.013	2.6×10^{-4}	13.38	1.9×10^{-109}	0.98	0.96–1.01	0.31	82 323 (20 728)
CYP24A1	20	rs6013897	A	0.19	−0.027	0.015	7.7×10^{-2}	3.13	6.0×10^{-10}	0.99	0.96–1.02	0.60	84 099 (21 840)

25OHD indicates 25-hydroxyvitamin D; CAD, coronary artery disease; CI, confidence interval; OR, odds ratio; and SNP, single-nucleotide polymorphism.

*Effect on multiply adjusted natural log-transformed 25OHD levels, SE, *P* value, and F statistic of the association in the Canadian Multicentre Osteoporosis Study (CaMos) Cohort (n=2347).

†*P* values derived from the SUNLIGHT consortium (n=33 996).

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Table 3. MR Estimate of the Association of Decreased 25OHD With the Risk of CAD

Model	OR (95% CI)*	*P* Value	I^2 (95% CI)
Fixed effects (including the *DHCR7* locus)	0.99 (0.84–1.17)	0.93	0 (0–84.7)
Fixed effects (excluding the *DHCR7* locus)	1.04 (0.85–1.27)	0.72	0 (0–84.7)
Fixed effects (excluding the *CYP24A1* locus)	1.00 (0.85–1.19)	0.96	0 (0–84.7)

25OHD indicates 25-hydroxyvitamin D; CAD, coronary artery disease; CARDIoGRAM, Coronary Artery Disease Genome-Wide Replication and Meta-Analysis; CI, confidence interval; MR, Mendelian randomization; and OR, odds ratio.

*OR is expressed as the odds of CAD for a 1 SD decrease in natural log-transformed 25OHD levels. Estimates of the effect of the SNPs on 25OHD were derived from the Canadian Multicentre Osteoporosis Study (CaMos) Cohort, and estimates of the effect of the SNPs on CAD were derived from the CARDIoGRAM consortium.

undertook a sensitivity analysis by excluding the rs12785878 SNP (*DHCR7*). Despite removal of this variant, we again observed no association of genetically lowered 25OHD levels with the risk of CAD (OR, 1.04; 95% CI, 0.85–1.27; P=0.724; I^2=0%; 95% CI, 0%–84.7%; Table 3; Figure I in the Data Supplement). To further assess the effect of the independent vitamin D pathways on risk of CAD, we analyzed SNPs near genes implicated in 25OHD synthesis (*DHCR7* and *CYP2R1*) and metabolism (*GC* and *CYP24A1*) separately and found that both were again not associated with increased risk of CAD (Table I in the Data Supplement). Finally, as the *CYP24A1* SNP has a low F statistic for 25OHD and may lead to weak instrument bias, and therefore mask a true causal effect,[56] we undertook further sensitivity analyses after removing this variant. The results were again consistent (Table 3). Given these null results, we undertook a power calculation.[45] On the basis of our sample size of 86995 individuals (22233 cases and 64762 controls from the CARDIoGRAM study) and setting α to 0.05, our study had 98% power to detect an OR of 1.02 for the effect of 25OHD on CAD risk and 100% power to detect and OR of 1.2.

Discussion

Using summary level data for CAD and total 25OHD levels from a large population of European descent, our study provides evidence against a causal role for vitamin D in CAD susceptibility. These findings suggest that previous observational epidemiological associations may have been influenced by confounding or reverse causation. The 95% CIs of our summary estimates do not include clinically relevant effects of vitamin D on CAD, despite the large change in vitamin D levels. These results provide no rationale for the use of vitamin D to prevent CAD.

The discrepancy between the findings of many observational studies and of our MR study is likely due to residual confounding. Adiposity predisposes to CAD and lowers 25OHD levels.[57] Indeed, although most observational studies adjust for BMI, few studies adjust for dual energy X-ray absorptiometry (DXA) measured percent fat mass; contrary to body fat, BMI is not specific for adiposity, thus residual confounding cannot be eliminated.[58] This concept is supported by a recent observational study in a lean and physically active population,[59] where although a strong association between serum 25OHD and DXA-assessed body fat in both sexes was found, no association between 25OHD concentrations and CV indexes was demonstrated after adjusting for body fat. Physical activity might be another strong confounder in observational studies because it is associated with both CAD risk and sunlight exposure, which in turn influences vitamin D status. Most of the existing observational studies accounted for self-reported physical activity, which is, in general, a poor measure of physical activity.[60]

Our results are in accordance with the most recent meta-analysis of randomized trials,[23] which showed no association between 25OHD and 3 different cardiovascular outcomes (death, MI, and stroke). They are also in agreement with an

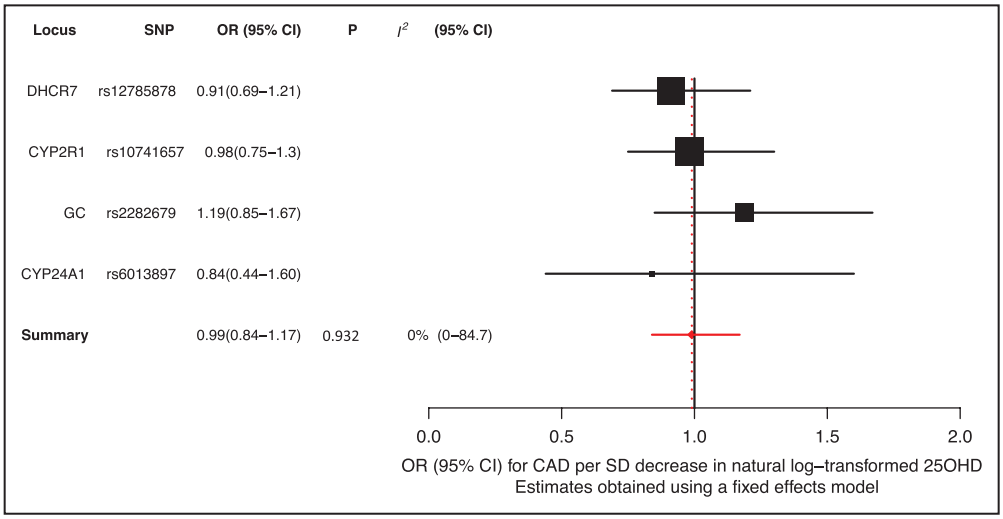

Figure 2. Mendelian randomization estimate of the association of 25-hydroxyvitamin D (25OHD) levels with the risk of coronary artery disease (CAD). Estimates obtained from using a fixed-effects model. Estimates of the effect of the single-nucleotide polymorphisms (SNPs) on 25OHD were derived from the Canadian Multicentre Osteoporosis Study (CaMos) Cohort, and estimates of the effect of the SNPs on CAD were derived from the Coronary Artery Disease Genome-Wide Replication and Meta-Analysis (CARDIoGRAM) consortium. CI indicates confidence interval; and OR, odds ratio.

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MR analysis published by our group studying the association of vitamin D–binding protein, a key determinant of 25OHD levels, with the risk of CAD.[52] Using the single polymorphism rs2282679 near the *GC* gene as an instrumental variable (whose effect allele was associated with an age- and sex-adjusted decrease in vitamin D–binding protein level of 27.4 mg/L), this study investigated the relationship of vitamin D–binding protein with multiple cardiometabolic outcomes in the CARDIoGRAM consortium and found no association (OR, 1.02; 95% CI, 0.99–1.05; *P*=0.31). Using an approach related to MR, Jorde et al[61] examined causal associations of 25OHD with the risk of CAD on 9528 subjects, but could not establish or exclude any causal relationship, possibly because of their relatively small sample size. In agreement with our findings, a recent MR study using 14 455 ischemic heart disease Danish cases[27] showed that genetically lowered 25OHD concentrations were not associated with increased myocardial infarction. Another MR study used 3231 cases of death from cardiovascular disease[62] from the same Danish population and reported no association between low 25OHD levels and cardiovascular mortality.[62] The instrumental variables used for both studies were only SNPs near the *DHCR7* and *CYP2R1* genes, involved only in vitamin D synthesis, but did not include either *GC* SNPs, which have been shown to have the largest impact on 25OHD levels,[28] or those at *CYPR24A1*. Our study, thus, provides a more thorough examination of the effects of 25OHD on CAD risk by using a substantially larger sample size, including all 25OHD-associated loci, in a general European-ancestry population.

MR analyses assessing the effect of 25OHD on cardiometabolic outcomes, other than CAD, have also been described. We and others have recently provided evidence from MR that low vitamin D levels do not increase insulin resistance or the risk of type 2 diabetes mellitus,[51] but do increase the risk of type 1 diabetes mellitus[63] and blood pressure.[53] Interestingly, MR evidence has shown that 25OHD levels are directly influenced by BMI, and converse effects are likely to be small.[57]

Our analysis has several strengths. First, using data from a large genetic consortium for 25OHD levels (n=33 996) and CAD risk (22 233 cases and 64 762 controls) has enabled us to more precisely test our study hypothesis than if we had used individual-level data from a small study. The null association between 25OHD and CAD, as reflected in an OR close to 1 and with a tight CI, indicates that our study had enough power to rule out clinically relevant effects of a large change in 25OHD levels. This observation is also supported by the high statistical power of our study to detect a potential effect. Second, previous work has shown that the use of estimates from meta-analytic data for uncorrelated genetic variants are similarly efficient to individual-level data in MR studies.[41] Finally, the findings from this study represent the association of a life-long exposure to reduced vitamin D levels in the general European population and in the absence of large-scale, long-term RCT data, our findings provide strong evidence against a causal role for low vitamin D levels in CAD susceptibility.

Our study also has limitations. Pleiotropy is difficult to exclude in any MR study; however, our sensitivity analyses demonstrated no evidence of pleiotropic effects. The null result could also be explained by canalization, which is defined as compensatory feedback interactions.[26,64,65] Similar to previous studies, our MR analysis might be limited in its ability to elucidate the causal role of biologically active vitamin D, reflected by the levels of the active metabolite 1,25-dihydroxyvitamin D ($1,25[OH]_2D$). Thus, although genetically lowered total 25OHD levels do not seem to be associated with increased risk of CAD, our study still leaves open the possibility that reduced lifelong total 25OHD is not associated with reduced production of $1,25[OH]_2D$. In this respect, concentrations of total 25OHD and $1,25[OH]_2D$ are weakly correlated ($r<0.3$).[66] However, $1,25[OH]_2D$ remains understudied because of its short half-life and its low concentration in blood.[66] Furthermore, although developmental differences in prenatal and postnatal expression of the vitamin D receptor through which $1,25[OH]_2D$ acts have been well-demonstrated and reflect developmental differences in vitamin D function,[67] developmental differences in synthesis of $1,25[OH]_2D$ have not been well studied. Consequently, it is also possible that in lifelong low total 25OHD states, a compensatory increase in conversion of free 25OHD to $1,25[OH]_2D$ could result in a new steady state with low free 25OHD but normal $1,25[OH]_2D$; however, the same compensation might not occur with acquired 25OHD deficiency later in life or the mechanism of this compensation may be different and more deleterious to the vasculature. Finally, the results of our MR study are generalizable only in population of European ancestry and only in generally healthy adults.

In conclusion, evidence from the largest existing genetic consortia provides no support for a causal role for 25OHD levels in the risk of CAD in individuals of European descent. Instead, association of 25OHD levels with CAD may be attributable to confounding by lifestyle factors such as obesity and physical inactivity, which may provide more fruitful targets for cardiovascular disease prevention than vitamin D supplementation.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Observational epidemiological studies have associated vitamin D deficiency with the risk of Coronary Artery Disease (CAD); however, these studies are susceptible to confounding and reverse causation, thus it remains unclear whether this association is causal. The few small randomized controlled trials published to date on this topic have been inconclusive. If vitamin D deficiency did cause CAD, this would be of tremendous clinical relevance, since vitamin D deficiency is common and safely correctable. To investigate this, in an analysis not biased by confounding or reverse causation, we undertook a Mendelian randomization study to understand whether genetically lowered vitamin D is associated with a higher risk of CAD. Using large sample sizes, we identified genetic variants strongly associated with vitamin D levels from the SUNLIGHT consortium (n=33 998). We first tested the validity of these genetic variants as instrumental variables and then undertook a Mendelian randomization analysis to test whether vitamin D levels influence the risk of CAD in 22 233 cases and 64 762 healthy controls included in Coronary Artery Disease Genome-Wide Replication and Meta-Analysis, one of the largest genetics consortia for CAD. We found that the odds ratio for CAD was 0.99 (95% confidence interval, 0.84–1.17, P=0.93) per each genetically determined SD decrease in log-transformed 25-hydroxyvitamin D levels. Thus, our study showed that there is no evidence of a causal association between genetically lowered vitamin D and CAD susceptibility in a large population, suggesting that previous associations between 25-hydroxyvitamin D levels and CAD are possibly confounded or due to reverse causation.

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Mendelian Randomization Studies Do Not Support a Role for Vitamin D in Coronary Artery Disease

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SUPPLEMENTAL MATERIAL

Supplemental Table 1: MR estimate of the association of decreased 25OHD on the risk of CAD, stratified by SNPs near genes involved in 25OHD synthesis or metabolism using a fixed effects model

Model	OR (95% CI)*	P-Value
Synthesis	0.95 (0.78-1.15)	0.59
Metabolism	1.10 (0.82-1.49)	0.51

*OR is expressed as the odds of CAD, for a one standard deviation decrease in natural log transformed 25OHD levels. Note that the 95% CI for the I^2 cannot be properly estimated given that there are only two SNPs per model. Estimates of the effect of the SNPs on 25OHD were derived from the CaMos Cohort, and estimates of the effect of the SNPs on CAD were derived from the CARDIOGRAM consortium.

Supplemental Fig. 1: Mendelian Randomization Estimate of the Association of 25OHD Levels with Risk of CAD Excluding the *DHCR7* Locus

Estimates obtained using a fixed-effects model. Estimates of the effect of the SNPs on 25OHD were derived from the CaMos Cohort, and estimates of the effect of the SNPs on CAD were derived from the CARDIOGRAM consortium.