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CARDIOVASCULAR DISEASE

Potential causal associations of serum 25-hydroxyvitamin D with lipids: a Mendelian randomization approach of the HUNT study

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Abstract

Observational studies have shown consistent associations between higher circulating 25-hydroxyvitamin D [25(OH)D] levels and favorable serum lipids. We sought to investigate if such associations were causal. A Mendelian randomization (MR) study was conducted on a population-based cohort comprising 56,435 adults in Norway. A weighted 25(OH)D allele score was generated based on vitamin D-increasing alleles of rs2282679, rs12785878 and rs10741657. Linear regression analyses of serum lipid levels on the allele score were performed to assess the presence of causal associations of serum 25(OH)D with the lipids. To quantify the causal effects, the inverse-variance weighted method was used for calculating MR estimates based on summarized data of individual single-nucleotide polymorphisms. The MR estimate with 95% confidence interval (CI) represents percentage difference in the lipid level per genetically determined 25 nmol/L increase in 25(OH)D. The 25(OH)D allele score demonstrated a clear association with high-density lipoprotein (HDL) cholesterol ($p = 0.007$) but no association with total or non-HDL cholesterol or triglycerides ($p \geq 0.27$). The MR estimate showed 2.52% (95% CI 0.79–4.25%) increase in HDL cholesterol per genetically determined 25 nmol/L increase in 25(OH)D, which was stronger than the corresponding estimate of 1.83% (95% CI 0.85–2.81%) from the observational analysis. The MR estimates for total cholesterol (0.60%, 95% CI −0.73 to 1.94%), non-HDL cholesterol (0.04%, 95% CI −1.79 to 1.88%) and triglycerides (−2.74%, 95% CI −6.16 to 0.67%) showed no associations. MR analysis of data from a population-based cohort suggested a causal and positive association between serum 25(OH)D and HDL cholesterol.

Keywords Cholesterol · High-density lipoprotein (HDL) · Lipid · Low-density lipoprotein (LDL) · Mendelian randomization · Serum 25-hydroxyvitamin D [25(OH)D] · Single-nucleotide polymorphisms · Triglycerides · Vitamin D

Introduction

Vitamin D insufficiency is common globally [1, 2]. Insufficient vitamin D levels may contribute to many chronic diseases in addition to adverse bone health [3]. Low vitamin D may have an unfavorable effect on body lipids, since it may increase intestinal absorption and synthesis of lipids, reduce lipolysis and lipid metabolism, and reduce secretion and sensitivity of insulin and thereby increase the risks of diabetes and metabolic syndrome [4, 5]. Population studies have largely shown associations between higher levels of serum 25-hydroxyvitamin D [25(OH)D] and favorable lipid profiles [6–8]. Unfavorable circulating levels of major lipids have been associated with an increased risk of cardiovascular diseases [9, 10]. Hence, sufficient circulating vitamin D is anticipated to exert a preventive effect on cardiovascular diseases partially via promoting favorable lipid profiles [11].

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Observational epidemiological studies have limited ability to infer a causal relationship between circulating vitamin D and lipids due to residual confounding, other biases and reverse causation [12, 13]. It is also difficult to draw a solid conclusion about the effect of vitamin D supplementation on lipids from meta-analyses of clinical trials owing to large heterogeneity across the included individual studies [4, 11]. Mendelian randomization (MR) studies, using single nucleotide polymorphisms (SNPs) that are associated with the exposure of interest as instruments, have been applied to overcome confounding and reverse causation to assist in making causal inferences [12, 13]. This is because the SNPs are randomly assigned at meiosis, independent of non-genetic confounding and are not modified by disease processes. A genetic variant used as an instrumental variable in an MR study should satisfy three core conditions, i.e. it should be: (1) associated with the exposure; (2) independent of confounding factors of the observational association of the exposure with the outcome; and (3) associated with the outcome only via the exposure [12, 13].

MR analysis has been performed in two previous studies to assess the potential causal associations between circulating vitamin D and lipid levels, using different vitamin D SNPs and demonstrating inconclusive results [14, 15]. Therefore, we performed an MR study applying the vitamin D synthesis and metabolism SNPs derived from a genome-wide association study (GWAS) [16] to assess the causation in a Norwegian adult population, in which 60% of the cohort individuals were reported to have an insufficient serum 25(OH)D level of less than 50 nmol/L [17].

Materials and methods

Study population

We used the Nord-Trøndelag Health Study (HUNT) that was a population-based study in Norway, including three completed surveys: HUNT1 (1984–1986), HUNT2 (1995–1997) and HUNT3 (2006–2008) [18]. The current study was based on data from HUNT2, in which 65,227 subjects 20 years or older living in the county of Nord-Trøndelag participated (participation rate 70%). At baseline, all participants completed a general questionnaire regarding health, lifestyle and socio-economic status, and blood samples were drawn at a clinical examination. From this cohort a 10% random sample (n = 6613) was selected as a subcohort for serum 25(OH)D measurement.

Measurement and standardization of serum 25(OH) D levels

Blood from the HUNT2 participants was stored at − 80 °C for later use. Serum 25(OH)D levels were measured at HUNT Biobank using LIAISON 25-OH Vitamin D TOTAL (DiaSorin, Saluggia, Italy), a fully automated, antibody-based, chemiluminescence assay. The detection range of the assay for total serum 25(OH)D is 10–375 nmol/L. A cosinor model based on month of blood draw was used to calculate season-standardized 25(OH)D levels to correct the seasonal fluctuation of the levels owing to the high latitude of Norway [19]. Season-standardized 25(OH)D levels represent the annual average value of 25(OH)D for each individual.

Vitamin D SNPs and allele score as instrumental variables

DNA was isolated and stored at − 20 °C at HUNT Biobank. Genotyping was performed using Illumina HumanCoreExome arrays as described elsewhere [20]. Four SNPs located in or near vitamin D synthesis and metabolism genes were selected as instrumental variables based on the GWAS conducted by Wang et al. [16], including rs2282679 (*GC*), rs10741657 (*CYP2R1*), rs12785878 (*NADSYN1*/*DHCR7*), and rs6013897 (*CYP24A1*). As HUNT did not have information on rs6013897 or its proxy SNPs (R^2 of linkage disequilibrium > 0.8), the remaining three SNPs were used in the current study. Rs12785878 is located in the upstream of gene *DHCR7* (7-dehydrocholesterol reductase). It may have an effect on cholesterol synthesis in the skin. However, rs12785878 is not directly associated with blood cholesterol levels. Rs12785878 and its proxy SNPs (66 SNPs based on R^2 of linkage disequilibrium > 0.8) are not in the list of 444 variants that are associated with plasma lipids [21], and they are not among the 118 novel blood lipid SNPs found in a latest GWAS either [22]. Thus, using rs12785878 as an instrumental variable of 25(OH)D does not violate the third core condition. The 25(OH)D increasing allele was designated as the effect allele and coded as 1 (rs2282679: T = 1; rs10741657: A = 1 and rs12785878: T = 1). An externally weighted allele score was calculated by multiplying the number of the effect allele for each SNP by its weight and summing across the three SNPs. Weight was calculated as dividing the effect of the individual SNP on 25(OH)D by the sum of effects of these three SNPs derived from a large consortium study of 35,000 individuals by Vimaleswaran et al. [23].

Measurement of lipid levels

Non-fasting levels of total cholesterol, HDL cholesterol and triglycerides were measured in fresh serum at the time of sample collection at Levanger Hospital, Nord-Trøndelag Hospital Trust on a Hitachi 911 Auto-analyzer (Hitachi, Mito, Japan) with reagents from Boehringer Mannheim (Mannheim, Germany). Lipids were quantified using enzymatic colorimetric methods. We calculated non-HDL cholesterol as a measure of the atherogenic lipoproteins by

subtracting HDL cholesterol from the total cholesterol [24]. There are two reasons to calculate non-HDL cholesterol instead of LDL cholesterol: (1) there are minimal differences between the fasting and non-fasting levels for total and HDL cholesterol; and (2) non-HDL cholesterol is a better predictor for the risk of cardiovascular disease compared with LDL [25, 26].

Statistical analyses

We performed a one-sample MR study in which the SNP-outcome association was assessed in a total cohort and the SNP-exposure association was assessed in a subcohort [12]. The analysis total cohort consisted of 56,435 individuals who had complete information on the three vitamin D SNPs and all the lipid levels. The analysis subcohort included 5724 individuals who had complete information on the vitamin D SNPs, serum 25(OH)D and lipid levels.

The current MR study should meet the following specific assumptions [12, 13]: (1) the three vitamin D SNPs and allele score should be associated with the serum 25(OH)D levels; (2) the three vitamin D SNPs or allele score should not be associated with potential confounders of the observational associations; and (3) there should be no horizontally pleiotropic effects of the vitamin D SNPs on the lipids.

We tested the first assumption in the subcohort. Linear regression models were applied to calculate coefficients, F-statistic and R^2 values on serum 25(OH)D levels per effect allele or allele score. An F-statistic value greater than 10 suggests the genetic variant being a valid instrument [13], but this is an arbitrary cut-point and weak instrument bias is on a continuum. Linear regression analyses of the lipid levels on the three SNPs and allele score were also applied to assess the presence of causal associations of serum 25(OH)D with lipids in the total cohort. MR estimates were calculated to represent quantified causal effects using the inverse-variance weighted (IVW) method, which combined ratio estimates derived from each genetic variant in a fixed-effect meta-analysis model [27]. The ratio estimate was calculated as the effect estimate of each SNP on the ln(lipid) level obtained from the total cohort divided by the effect estimate of the same SNP on the serum 25(OH)D level obtained from the subcohort [28]. Linearity of all relationships and no interactions were assumed for these estimates [13]. An MR estimate was interpreted as percentage difference in each lipid per genetically determined 25 nmol/L increase in 25(OH)D levels, as the lipid levels were natural logarithm transformed due to non-normality of the distribution [29]. We also calculated coefficients for each lipid per 25 nmol/L increase in 25(OH)D in observational associations after adjustment for age (years), sex, pack-years of smoking, alcohol consumption, body mass index (kg/m^2), physical activity, education years and economic difficulties as a marker of socio-economic status. The classification of each covariate was consistent with a previous HUNT study [30].

To investigate the second assumption, we used linear or logistic regression to analyze associations between the vitamin D allele score and measured potential confounding variables in the total cohort. To test the third assumption of MR regarding possible pleiotropic effect of the vitamin D SNPs, we used the MR-Egger method to calculate values of intercepts and their p values [31]. If the intercepts do not deviate markedly from zero, substantial horizontal pleiotropy of the SNPs is less likely. Additionally, we tested for heterogeneity among the SNPs using I^2 and Cochran's Q statistic in the IVW method [32].

As secondary analyses, we applied the two-stage-least-square (2SLS) method, which is commonly used for a one-sample MR, to calculate the MR estimates using the externally weighted allele score [23]. A two-sample MR analysis was also performed using summarized data of SNPs-25(OH)D association derived from the study of about 35,000 individuals by Vimaleswaran et al. [23] and summarized data of SNPs-ln(lipid) association derived from the current HUNT study (n = 56,435). For one-sample MR using the IVW method, we also performed leave-one-out sensitivity analysis to ascertain if an association was disproportionately influenced by a single SNP.

Due to four outcome measures, multiple testing was taken into account and a Bonferroni adjusted $p = 0.0125$ (0.05/4) was considered as the statistical significance level. Sample size was calculated to estimate the number of individuals needed to detect a true causal association between 25(OH)D and each lipid in the 2SLS MR analysis [33]. MR analyses were carried out using the packages Mendelian randomization (version 0.2.2) and TwoSampleMR (version 0.4.12) in R (version 3.4.2). All other statistical analyses were performed with Stata/SE 14.2 (College Station, TX, USA).

Results

A similar distribution of characteristics including lipid levels was found in the total cohort and subcohort of the HUNT2 study (Table 1). Supplementary Table 1 shows the characteristics of the three vitamin D SNPs from the HUNT2 study. The effect allele frequency of each SNP was similar to that of the 1000 Genomes Phase 3 data (http://www.ensembl.org/index.html).

In the subcohort both the effect allele of the SNPs and the allele score were associated with an increase in 25(OH)D levels (Table 2), which satisfied the first assumption of MR. The F-statistic and R^2 values of the allele score were 219 and 3.7% respectively. We also investigated the second assumption of MR in the total cohort (Supplementary Table 2) and

Table 1 Baseline characteristics in the analysis total cohort and subcohort of the HUNT2 study, 1995–1997

	Cohort	Subcohort[a]
Number of subjects	56,435	5724
Age (years)	49.7 ± 16.7	49.5 ± 16.7
Sex, % (women/men)	53.0/47.0	53.1/46.9
Season-standardized 25(OH)D level (nmol/L)	–	48.2 ± 17.1
Total cholesterol (mmol/L)[†]	5.8 (5.0–6.7)	5.8 (5.0–6.7)
HDL cholesterol (mmol/L)[†]	1.3 (1.1–1.6)	1.3 (1.1–1.6)
Non-HDL cholesterol (mmol/L)[†]	4.4 (3.6–5.3)	4.4 (3.5–5.3)
Triglycerides (mmol/L)[†]	1.5 (1.0–2.2)	1.5 (1.0–2.2)
Smoking, % (never/ever/missing)	42.3/55.8/1.9	42.5/55.4/2.1
Alcohol consumption (times/month), % (never/≥ 1/missing)	34.1/57.4/8.5	33.4/58.3/8.3
Body mass index (BMI, kg/m^2)	26.3 ± 4.1	26.3 ± 4.1
Physical activity, % (inactive/active/missing)	21.5/48.2/30.2	21.3/48.5/30.3
Education (years), % (< 10/≥ 10/missing)	34.2/61.1/4.7	33.3/61.8/4.9
Economic difficulties, % (no/yes/missing)	49.6/21.5/28.8	50.6/20.8/28.6

Data are given as number of subjects, percentage of subjects, mean ± standard deviation, or [†]median (interquartile range)

25(OH)D 25-hydroxyvitamin D, *HDL* high-density lipoproteins, *HUNT2* The Nord-Trøndelag health study survey 2

[a]Those with measured serum 25(OH)D and lipid levels as well as genotype information

found that the allele score was not associated with the measured confounders of the observational associations between 25(OH)D and lipids ($p \geq 0.11$). Although not a guarantee, this provides some reassurance of no association with the unmeasured confounders.

The allele score demonstrated a clear association with HDL cholesterol in the total cohort (coefficient 0.26%, 95% CI 0.07–0.44%, $p = 0.007$) (Table 2) indicating the presence of a causal effect of 25(OH)D levels on HDL cholesterol, but there were no associations of the allele score with total cholesterol, non-HDL cholesterol and triglycerides ($p \geq 0.27$). As shown for the MR estimates in Table 3, each genetically determined 25 nmol/L increase in 25(OH)D was associated with 2.52% (95% CI 0.79–4.25%, $p = 0.004$) increase in HDL cholesterol. This was stronger than the estimate of 1.83% (95% CI 0.85–2.81%) per 25 nmol/L increase in 25(OH)D from the observational analysis. The MR estimates per genetically determined 25 nmol/L increase in 25(OH)D for total cholesterol (0.60%, 95% CI − 0.73 to 1.94%), non-HDL cholesterol (0.04%, 95% CI − 1.79 to 1.88%) or triglycerides (− 2.74%, 95% CI − 6.16 to 0.67%) showed no associations. Figure 1A–D displays the MR estimates summarized from each individual SNP for total, HDL and non-HDL cholesterol as well as triglycerides, respectively.

The intercepts and the corresponding *p* values by the MR-Egger method for testing the third assumption suggested no evidence of substantial horizontal pleiotropy of the three SNPs (Table 3), and the I^2 and Cochran's Q statistics using the IVW method did not show substantial heterogeneity of the SNPs on the lipids apart from triglycerides.

Because lipid measurement was performed in non-fasting blood samples, we adjusted for duration between blood draw and the latest meal in the models and repeated the analyses of the observational and MR estimates in sensitivity analyses (Supplementary Table 3). Similar results were obtained; per genetically determined 25 nmol/L increase in 25(OH)D was associated with 2.31% (95% CI 0.58–4.05%, $p = 0.01$) increase in HDL cholesterol, but it was not associated with other lipids.

Results from the 2SLS method using the allele score were similar to those from the IVW method (Supplementary Table 4). Results from the two-sample MR analysis in general supported our original results from the one-sample analysis (Supplementary Table 4). The leave-one-out sensitivity analysis showed that in general the relations were not disproportionately influenced by a single SNP except for triglycerides (Supplementary Fig. 1).

Discussion

Main findings

Our study demonstrated that each genetically determined 25 nmol/L increase in 25(OH)D was associated with 2.52% (95% CI 0.79–4.25%) increase in HDL cholesterol. However, genetically determined 25(OH)D was not associated with total cholesterol, non-HDL cholesterol or triglycerides.

Table 2 The associations of SNPs and allele score with serum 25(OH)D, total-, HDL-, and non-HDL-cholesterol as well as triglycerides levels in the HUNT2 study (n = 56,435)

SNP	Season-standardized 25(OH)D (nmol/L) (n = 5724)				Total cholesterol		HDL cholesterol		Non-HDL cholesterol		Triglycerides	
	Coef. (95% CI)[a]	*P* value	F statistic	R^2	Coef.[†] (95% CI)	*P* value	Coef.[†] (95% CI)	*P* value	Coef.[†] (95% CI)	*P* value	Coef.[†] (95% CI)	*P* value
rs2282679	4.01 (3.32 to 4.70)	9.0×10^{-30}	130	0.022	0.06 (−0.22 to 0.35)	0.66	0.43 (0.07 to 0.80)	0.02	−0.07 (−0.46 to 0.32)	0.73	−0.04 (−0.76 to 0.67)	0.91
rs10741657	2.46 (1.82 to 3.09)	3.8×10^{-14}	58	0.010	0.14 (−0.11 to 0.39)	0.28	0.37 (0.04 to 0.70)	0.03	0.08 (−0.27 to 0.43)	0.66	−0.96 (−1.61 to −0.31)	0.004
rs12785878	1.95 (1.29 to 2.60)	5.3×10^{-09}	34	0.006	0.00 (−0.26 to 0.26)	0.98	−0.02 (−0.36 to 0.32)	0.90	0.04 (−0.32 to 0.40)	0.83	−0.00 (−0.67 to 0.67)	1.00
Allele score[#]	2.66 (2.30 to 3.01)	1.1×10^{-48}	219	0.037	0.05 (−0.09 to 0.20)	0.46	0.26 (0.07 to 0.44)	0.007	−0.01 (−0.20 to 0.19)	0.95	−0.21 (−0.57 to 0.16)	0.27

25(OH)D 25-hydroxyvitamin D, *CI* confidence interval, *Coef* coefficient, *HDL* high-density lipoproteins, *HUNT2* The Nord-Trøndelag Health Study Survey 2, *SNP* single-nucleotide polymorphism

[a]Coefficient is difference in season-standardized 25(OH)D (nmol/L) per effect allele or allele score. Coef., F-statistic and R^2 value were derived from linear regression of season-standardized 25(OH)D level on SNPs or allele score. The *t*-distributions were used to calculate *P* values

[†]Coefficient is percentage difference in lipid level (mmol/L) per effect allele or allele score and was derived from linear regression of natural log-transformed lipid level on SNPs or allele score

[#]The weighted 25(OH)D allele score was calculated by multiplying the number of the effect allele for each SNP by its weight and summing across three SNPs (rs2282679, rs10741657 and rs12785878). Weight was calculated as dividing the effect of the individual SNP on 25(OH)D by the sum of effects of these three SNPs derived from a large consortium study of about 35,000 individuals by Vimaleswaran et al. [23]

Table 3 Observational and Mendelian randomization (MR) estimates of the associations between a 25 nmol/L increase in 25(OH)D and total-, HDL-, and non-HDL-cholesterol as well as triglycerides levels in the HUNT2 study (n = 56,435)

Outcome	Observational estimates (n = 5684)		MR estimates (IVW method)				MR-Egger method	
	Coefficient (95% CI)[a]	*P* value	MR estimate[†] (95% CI)	*P* value	I^2 (95% CI)	*P* value of Q statistic	Intercept (95% CI)	*P* value
Total cholesterol	−4.60 (−5.33 to −3.86)	< 0.001	0.60 (−0.73 to 1.94)	0.38	0.00 (0.00 to 0.64)	0.75	0.000 (−0.005 to 0.006)	0.91
HDL cholesterol	1.83 (0.85 to 2.81)	< 0.001	2.52 (0.79 to 4.25)	0.004	0.08 (0.00 to 0.90)	0.34	−0.002 (−0.011 to 0.006)	0.59
Non-HDL cholesterol	−6.46 (−7.44 to −5.47)	< 0.001	0.04 (−1.79 to 1.88)	0.96	0.00 (0.00 to 0.42)	0.84	0.002 (−0.005 to 0.009)	0.60
Triglycerides	−16.00 (−17.80 to −14.12)	< 0.001	−2.74 (−6.16 to 0.67)	0.12	0.66 (0.00 to 0.90)	0.05	−0.007 (−0.036 to 0.022)	0.65

25(OH)D 25-hydroxyvitamin D, *CI* confidence interval, *HDL* high-density lipoproteins, *IVW* inverse-variance weighted, *MR* Mendelian randomization

[a]Coefficient is percentage difference in lipid level (mmol/L) per 25 nmol/L increase in 25(OH)D, derived from linear regression of natural log-transformed lipid level on 25(OH)D, adjusted for age, sex, pack-years of smoking, alcohol consumption, body mass index, physical activity, education and socio-economic status (missing values of covariates were included)

[†]MR estimate is percentage difference in lipid level (mmol/L) per genetically determined 25 nmol/L increase in 25(OH)D

Comparison with previous studies

Most observational studies have observed that higher serum 25(OH)D level was associated with favorable lipid profiles in both adults [6, 7] and children [8]. Our results in the observational analyses were consistent with the previous findings, i.e. serum 25(OH)D level was positively associated with HDL cholesterol level but inversely associated with total cholesterol, non-HDL cholesterol and triglycerides. However, we only found a causal association between 25(OH)D and HDL cholesterol in the MR analysis. A lack of causal association with the lipids other than HDL cholesterol in our study was less likely due to low statistical power, since a much smaller sample size was needed for MR analysis of other lipids than for HDL cholesterol (Supplementary Table 5). Nevertheless, a recent meta-analysis of randomized controlled trials (RCTs) showed a beneficial effect of vitamin D supplementation only on LDL cholesterol [34]. We were not able to evaluate the relation between 25(OH)D and LDL cholesterol in the current MR study, as only non-fasting blood samples were collected in the HUNT population and it would not provide an accurate estimate for LDL cholesterol. The meta-analysis of RCTs showed no short-term effect of vitamin D supplementation on HDL cholesterol (16 weeks to 1 year), whereas the genetic variants in our MR study reflected a life-time exposure to vitamin D.

There has been a limited number of MR studies on evaluating the potential causation between vitamin D and lipids. One Danish study used genotypes for filaggrin gene mutations as instruments and found suggestive causal associations of serum 25(OH)D levels with fasting HDL cholesterol and triglycerides levels, but the associations disappeared after Bonferroni adjustment of the significance level [14]. The F statistic value of the genetic variants in this cited study was 15.1 that is much smaller than that in the present study. Another Danish study used one of the four SNPs suggested from the GWAS [16] and three other SNPs, and did not report any association with non-fasting lipid levels [15]. However, this Danish study found that genetically elevated remnant cholesterol (defined as non-fasting total cholesterol minus HDL-cholesterol and LDL-cholesterol) was associated with lower circulating 25(OH)D and genetically reduced HDL was associated with higher 25(OH)D levels [15].

Implications of the current study

Higher circulating HDL cholesterol levels have been associated with a reduced risk of cardiovascular diseases in observational studies [9, 10], but MR studies using HDL cholesterol-related genetic variants have not provided evidence for a causal inference [35–39]. Intervention studies by raising HDL cholesterol have not demonstrated beneficial effects on cardiovascular diseases [40]. Nevertheless, the latest research suggests that HDL cholesterol may reduce the risk of small artery disease, abdominal aortic aneurysm and chronic kidney disease [41–43]. HDL particles are complex and heterogeneous in lipid and protein composition, size and function. Future research to explore new genetic pathways and metabolism networks of HDL is warranted to clarify its role as a possible mediator in the relationships of vitamin D with cardiovascular disorders and other health outcomes [40, 44–46].

Strengths and limitations

Our study is among the few using several vitamin D SNPs derived from the GWAS conducted by Wang et al. [16] to assess the possibility of causal associations between vitamin D and lipid levels, and it is based on data from a large population cohort. Compared with observational studies, MR studies have

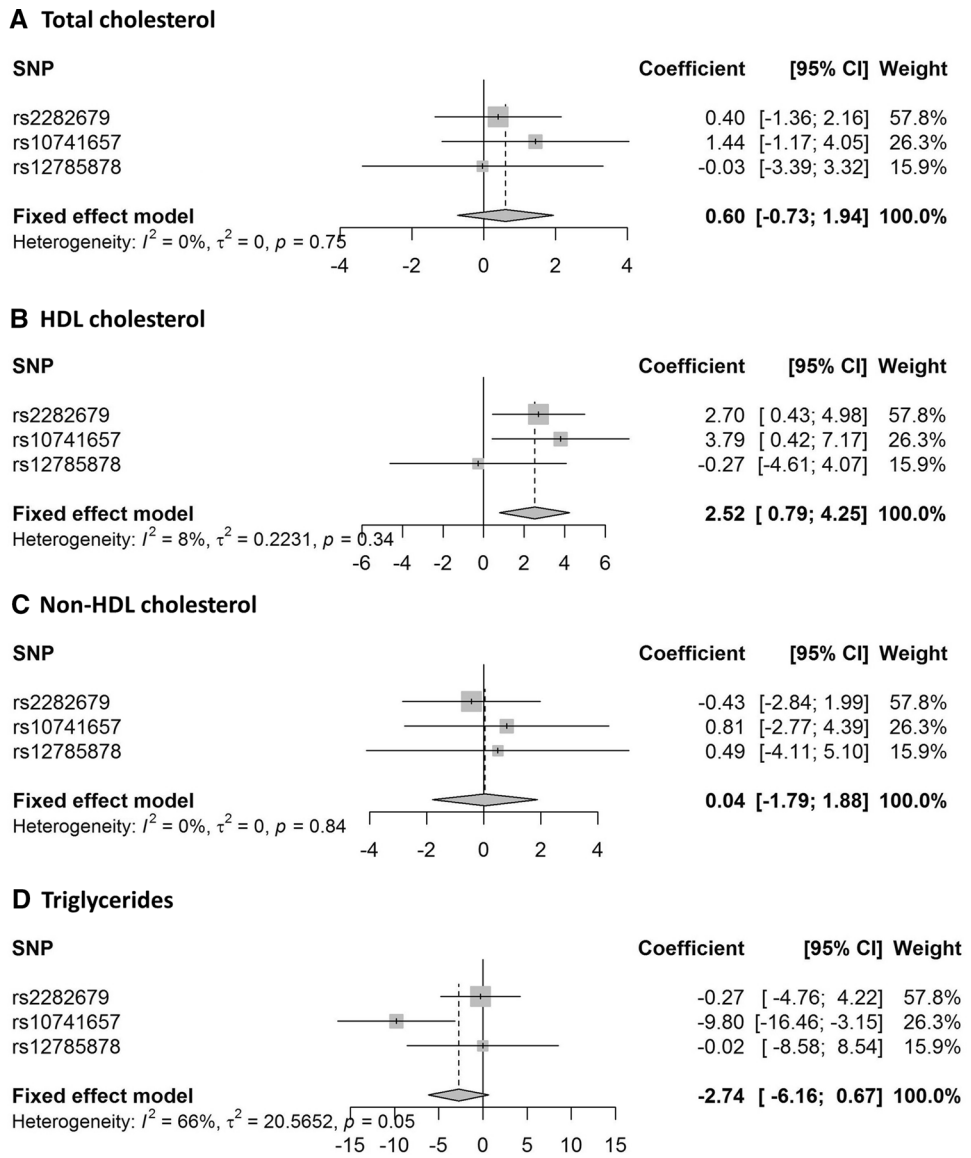

Fig. 1 Mendelian randomization estimate for each lipid calculated using inverse-variance weighted (IVW) method to summarize the effect from each individual single-nucleotide polymorphism (SNP) in a fixed effect model. Coefficient represents percentage difference in each lipid per genetically determined 25 nmol/L increase in serum 25(OH)D. 95% CI 95% confidence interval of the coefficient

an advantage of avoiding confounding and reverse causation if the three assumptions are met [12, 13]. An F-statistic value of 219 and an R^2 value of 3.7% from the regression of serum 25(OH)D on the allele score indicated sufficient strength of the three SNPs as instruments for the 25(OH)D levels. The investigation into the other two assumptions also provided some assurance in the current MR analysis. We performed a one-sample MR study in which the exposure data of serum 25(OH)D levels were available in a subcohort [12]. One-sample MR with subcohort compared with one-sample MR of full cohort often shows negligible effect on reduction in statistical power [28]. Thus, it can be regarded as a cost-efficient strategy for an MR study. The size of the subcohort required to reach a maximal power to detect causal effect depends on the strength of the instrumental variable [28]. The subcohort in the current study was a 10% random sample. As both the F statistic and the R^2 values suggested sufficient strength of the instrument, the size of this subcohort was likely to be adequate. To calculate the causal estimates in this one-sample analysis, we applied the IVW method that was initially developed for a two-sample MR. However, the IVW method can also be used to calculate the MR estimate in a one-sample MR [47], and it gave similar results as those from the 2SLS method. In addition, an MR study performed in a homogeneous population offers benefit to address population stratification [27]. This issue is minor in the HUNT population among which 97% were ethnic Norwegians [18].

Our study had several potential limitations. Among the 65,227 participants, we included 56,435 individuals (87%) with complete data on the vitamin D SNPs and lipid levels in the analysis. Compared to the excluded individuals, the included individuals were relatively younger and had a

healthier lifestyle and better socio-economic status (Table 1 and Supplementary Table 6). This would not have a major influence on the generalizability of our finding of a causal association. There were three vitamin D SNPs available in our study. They explained 3.7% of the variability of serum 25(OH) D levels, which was larger than the 1.9% explained by the four SNPs including rs6013897 in the large consortium study by Vimaleswaran et al. [23]. Furthermore, rs6013897, which was missing in our study, showed the weakest effect on 25(OH) D levels [16, 23]. We found no violation of the instrumental variable assumption on the pleiotropic effects of the SNPs according to MR-Egger tests, but with three SNPs the power to evaluate heterogeneity and horizontal pleiotropy was low [31]. A most recent GWAS has identified two additional novel SNPs (rs8018720 in *SEC23A* and rs10745742 in *AMDHD1*) that are not available in the HUNT data [48]. To increase the power of detecting causal effects and horizontal pleiotropy, future MR studies applying the six vitamin D SNPs are called for. Non-fasting blood samples were used for the lipid measurements. We adjusted for the duration between blood draw and the latest meal in the sensitivity analyses, and there were no substantial changes in the findings. In addition, non-fasting lipids have been suggested as a new standard for lipid measurement in the joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory [25, 26]. Furthermore, we applied a Bonferroni adjusted $p = 0.0125$ as the statistical significance level, which may increase possibilities of false negative findings when the outcomes are closely related. However, the conclusions of the study would be unaltered even if the unadjusted $p = 0.05$ was used. At last, it is worthy of mentioning that the relationship between vitamin D and lipids is complex. A previous Danish study showed that genetically reduced HDL was associated with higher 25(OH)D levels [15] and therefore, there is a possibility of bidirectional association. Unfortunately we were not able to perform a bidirectional MR analysis as serum 25(OH) D levels were only available in the subcohort but not the whole cohort.

Conclusions

In summary, the present MR study suggested a causal and positive association between serum 25(OH)D and HDL cholesterol based on data from a population-based cohort. The potential roles of vitamin D and HDL cholesterol in the prevention of cardiovascular diseases and other health outcomes warrant further investigation.

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Compliance with ethical standards

Conflict of interest XMM reports grant from "Nasjonalforeningen for folkehelsen" and YQS reports grant from The Norwegian Cancer Society during the conduct of the study. All other authors declare that they have no conflict of interest.

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