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ARTICLE

Exploring the association between serum 25-hydroxyvitamin D and serum lipids—more than confounding?

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Abstract

Background/objectives In observational, but not interventional, studies there are strong associations between serum 25-hydroxyvitamin D (25(OH)D) and serum lipids. The purpose of the present study was to examine potential causes of this association.

Subjects/methods A total of 17,411 subjects participating in the seventh survey of the Tromsø Study were included in the cross-sectional study; 5384 subjects who participated in both the sixth and seventh survey were included in the longitudinal study; 2365 subjects who participated in both the fourth and seventh survey were included in the genetic study; and 479 subjects with impaired glucose tolerance were included in the vitamin D binding protein (DBP) analyses.

Results For serum 25(OH)D, there were strong and positive associations with LDL-, HDL-, and total-cholesterol, and a negative association with triglycerides that remained after adjustment for gender, age, BMI, diet, supplements, and lifestyle factors. These associations were seen in winter as well as summer. Except for serum cholesterol, change of season for blood sampling did not affect lipid levels. However, when analyzing separately, subjects with low or no intake of vitamin D supplements, fish oil and fat fish, only the association between 25(OH)D and HDL-cholesterol remained significant. Serum DBP or single-nucleotide polymorphisms related to 25(OH)D had no relation to lipid levels.

Conclusions The associations between 25(OH)D and lipids (except for HDL-cholesterol) can be explained by known confounding factors. However, for HDL-cholesterol, the cause of the association with 25(OH)D still remains unknown.

Introduction

The main source of vitamin D is production in the skin upon UV-exposure. Some is also obtained from supplements and vitamin D-rich food like fatty fish [1]. In observational studies, a low level of serum 25-hydroxyvitamin D (25(OH)D), which is considered to reflect the vitamin D status, is associated with cardio-vascular diseases (CVD) and increased mortality [2–6]. Vitamin D deficiency is also associated with high serum levels of LDL-cholesterol and triglycerides [7], which may explain the association with CVD and mortality. However, intervention studies with vitamin D supplementation have not yielded convincing results [8–10]. The serum 25(OH)D level may therefore be a marker of good health, and not reflect causal relationships.

This is reasonable, since people in good health are more likely to stay out-doors than people who are ill, and also consume healthier and vitamin D-rich foods. Sunlight also causes release of NO and compounds with immunosuppressive effects which may be beneficial [11, 12]. Fatty fish is a source of vitamin D, but also provide fish oils, which may have cardio-protective effects [13]. Accordingly, the association between serum 25(OH)D, lipids, and cardio-vascular health could be explained by co-variation with other truly health promoting factors. However, this has so far not been properly tested, which for serum lipids was the purpose of the present study.

Since 1974, repeated health surveys have been conducted in Tromsø, Northern Norway [14]. In the seventh survey performed in 2015/2016, more than 21,000 subjects

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participated and serum 25(OH)D and lipids were measured. The subjects also filled in questionnaires about lifestyle factors, which gave us the opportunity to evaluate the cross-sectional association between serum 25(OH)D and lipids in relation to lifestyle factors, season, and sun exposure. With data from previous surveys and studies, we could also examine the longitudinal relationships and genetic factors, as well as the influence of the vitamin D binding protein (DBP) [15].

Materials and methods

The Tromsø Study, conducted in the municipality of Tromsø, Northern Norway at 69° North, was started in 1974 [14]. In the fourth survey of the Tromsø Study, performed in 1994/1995, all individuals aged 25 years or older (37,558) were invited to participate and 27,158 persons attended the first visit. All men aged 55–74 years, all women aged 50–74 years, and a sample of 5–10% of the remaining age groups between 25 and 84 years were invited to undergo a more extensive clinical examination (second visit), and 7965 persons, or 78% of those invited, attended. All participants who attended this second visit were genotyped for single-nucleotide polymorphisms (SNPs) related to serum 25(OH)D levels [16]. The sixth survey of the Tromsø Study was preformed in 2007/2008 and the following groups were invited: those who participated in the second phase of the fourth survey (1994/1995), a random 10% sample of subjects 30–39 years old, all subjects 40–42 and 60–87 years old, and a random 40% sample of subjects 43–59 years old. In total, 19,762 subjects were invited to the sixth Tromsø Study, and 12,984 subjects attended [14]. The seventh survey was performed in 2015/2016. All citizens aged 40 years and above (32,591) living in the municipality of Tromsø were invited to participate and 21,083 attended.

All subjects filled in questionnaires on use of lipid-lowering medication, intakes of fatty fish, cod liver oil, Omega 3 capsules (fish oil and seal oil), supplements containing vitamin D, sunny holidays last 2 months, use of solarium, physical activity, smoking, and alcohol habits. A physical activity score was created based on reported frequency, intensity, and duration. Non-fasting blood samples were drawn and analyzed consecutively for serum LDL-cholesterol, HDL-cholesterol, total-cholesterol, and triglycerides using an enzymatic colorimetric method with reagents from Roche (Roche Diagnostics). Serum 25(OH)D was measured in the sixth survey with ECLIA (Roche) using an automated clinical chemistry analyzer (Modular E170, Roche Diagnostics). This assay overestimates serum 25(OH)D levels in smokers [17] and where serum 25(OH)D levels from the sixth survey are used, smokers are excluded. All other serum 25(OH)D measurements were performed with an LC-MSMS method that discriminates between 25(OH)D_3 and 25(OH)D_2 [18]. Unless otherwise specified, the sum of 25(OH)D_3 and 25(OH)D_2 is used and designated 25(OH)D.

For the evaluation of DBP effects and potential importance of using measured free 25(OH)D, baseline data from an RCT in subjects with impaired glucose tolerance with development of type 2 diabetes as end point, was used [18]. Serum DBP was measured with competitive RIA using a polyclonal antibody [15]. Direct measurement and calculation of serum free 25(OH)D were performed as previously described [19–21].

For evaluation of genetic effects on the 25(OH)D and lipid association genotyping of the rs10741657 SNP in the 25-hydroxylase gene (*CYP2R1*) and the rs2282679 SNP in the DBP gene (*DBP or GC*) was performed in blood samples collected in the fourth survey of the Tromsø Study in 1994/1995 [16].

Statistical analyses

Normal distribution of dependent variables was determined using skewness, curtosis, and visual inspection of histograms and found normal except for serum triglycerides that attained normal distribution after log transformation and used as such in the analyses. The relations between the lipids and serum 25(OH)D were evaluated with linear regression models with covariates as appears in the tables. The effect of season was adjusted for with dummy variables for month of blood sampling. Correlations were evaluated with Pearson's correlation coefficient r. In the longitudinal analyses, May, June, July, and August were pooled together as "summer months" and November, December, January, and February were pooled together as "winter months." The effects of the selected SNPs on serum 25(OH)D and lipid levels were evaluated with linear regression across the genotypes. $P < 0.05$ was considered as statistically significant. The data are presented as mean (SD).

Ethics

The study was approved by the Regional Committee for Medical Research Ethics (2007/81 and 2017/806) and the Norwegian Medicines Agency (EUDRACTNR. 2007-002167-27). All subjects gave written, informed consent.

Results

Cross-sectional relations between serum 25(OH)D and lipids

Among the 21,083 subjects who attended the seventh survey of the Tromsø Study in 2015/2016, 20,898 had

Table 1 Serum 25(OH)D and lipid levels in relation to gender, age, BMI, and lifestyle factors, seventh survey in the Tromsø Study

	N	Serum 25(OH)D (nmol/L)	Serum LDL-cholesterol (mmol/L)	Serum HDL-cholesterol (mmol/L)	Serum total-cholesterol (mmol/L)	Serum triglycerides (mmol/L)
Females	9345	65.2 (22.2)	3.64 (0.96)	1.74 (0.48)	5.63 (1.04)	1.31 (0.73)
Males	8066	60.1 (21.2)*	3.80 (0.91)*	1.40 (0.40)*	5.57 (0.99)*	1.73 (1.09)*
Age (years)						
<50	6143	56.3 (20.2)	3.45 (0.93)	1.49 (0.43)	5.25 (0.98)	1.49 (1.04)
50–70	9260	65.2 (21.9)	3.87 (0.92)	1.61 (0.49)	5.79 (0.98)	1.54 (0.91)
>70	2008	72.4 (21.4)*	3.80 (0.92)	1.69 (0.52)*	5.77 (1.02)	1.40 (0.70)
BMI (kg/m^2)[a]						
<24.1	4340	67.0 (22.8)	3.40 (0.90)	1.86 (0.50)	5.42 (1.00)	1.08 (0.55)
24.1–26.5	4342	64.6 (21.8)	3.70 (0.92)	1.63 (0.46)	5.59 (1.00)	1.37 (0.76)
26.6–29.5	4340	61.8 (21.1)	3.87 (0.94)	1.48 (0.41)	5.72 (1.01)	1.66 (0.97)
>29.5	4341	58.1 (20.9)*	3.88 (0.93)*	1.35 (0.38)*	5.67 (1.03)*	1.91 (1.15)*
How often do you eat fat fish[a]						
0–1 times per month	2161	59.7 (23.5)	3.70 (0.91)	1.50 (0.46)	5.55 (0.98)	1.63 (0.99)
2–3 times per month	5652	62.0 (22.0)	3.75 (0.93)	1.54 (0.47)	5.62 (1.01)	1.56 (1.01)
1–3 times per week	8685	64.0 (21.3)	3.69 (0.95)	1.61 (0.48)	5.59 (1.03)	1.45 (0.88)
4–6 times per week	563	65.3 (22.0)	3.69 (0.95)	1.68 (0.52)	5.64 (0.99)	1.42 (0.94)
Once a day or more	186	67.3 (21.5)*	3.60 (0.92)	1.74 (0.56)	5.59 (0.96)	1.36 (0.74)
Do you use cod liver oil[a]						
No	9262	60.2 (22.0)	3.72 (0.95)	1.56 (0.47)	5.60 (1.02)	1.55 (0.96)
Sometimes	3125	58.7 (19.9)	3.67 (0.95)	1.55 (0.46)	5.53 (1.01)	1.54 (1.06)
Daily during winter season	1753	65.6 (19.9)	3.73 (0.95)	1.62 (0.48)	5.63 (1.04)	1.43 (0.85)
Daily	2358	74.7 (20.3)*	3.72 (0.91)	1.67 (0.51)*	5.65 (0.99)	1.37 (0.79)*
Do you use Omega 3 capsules (fish oil, seal oil)[a]						
No	9944	59.5 (21.4)	3.70 (0.94)	1.56 (0.47)	5.57 (1.01)	1.52 (0.93)
Sometimes	2696	61.0 (20.7)	3.69 (0.96)	1.55 (0.47)	5.57 (1.03)	1.57 (1.14)
Daily during winter season	864	66.8 (21.1)	3.73 (0.92)	1.60 (0.48)	5.62 (0.99)	1.48 (0.88)
Daily	3051	73.0 (21.2)*	3.75 (0.93)*	1.67 (0.51)*	5.69 (1.01)	1.42 (0.81)
Do you use vitamin D supplements[a]						
No	10398	59.4 (20.9)	3.72 (0.95)	1.56 (0.47)	5.60 (1.02)	1.53 (0.95)
Sometimes	2647	61.3 (20.8)	3.70 (0.94)	1.57 (0.47)	5.57 (1.00)	1.52 (1.04)
Daily during winter season	972	68.1 (21.0)	3.68 (0.94)	1.61 (0.48)	5.59 (1.01)	1.45 (0.84)
Daily	2598	75.6 (22.2)*	3.69 (0.94)	1.68 (0.51)**	5.64 (1.03)	1.42 (0.86)
Have you been on sunny vacation last two months[a]						
No	13917	60.3 (21.0)	3.71 (0.94)	1.58 (0.48)	5.59 (1.01)	1.51 (0.94)
Yes	3178	74.3 (21.9)*	3.71 (0.96)	1.61 (0.49)**	5.63 (1.04)	1.49 (0.93)
Do you use a solarium[a]						
Yes, weekly	44	69.6 (24.0)	3.93 (0.94)	1.55 (0.62)	5.86 (1.03)	1.70 (0.90)
Yes, sometimes	3450	66.7 (21.2)	3.65 (0.95)	1.61 (0.48)	5.55 (1.00)	1.44 (0.90)
Never	13631	61.9 (21.9)*	3.73 (0.94)	1.58 (0.48)	5.61 (1.02)	1.52 (0.95)
Physical activity score[a]						
Quartile 1 (lowest activity)	4149	58.4 (21.7)	3.81 (0.94)	1.46 (0.44)	5.64 (1.01)	1.69 (1.00)
Quartile 2	3791	61.9 (21.4)	3.74 (0.96)	1.56 (0.47)	5.62 (1.03)	1.55 (0.93)
Quartile 3	4009	64.3 (21.5)	3.71 (0.94)	1.61 (0.49)	5.61 (1.01)	1.46 (0.89)
Quartile 4	4859	66.4 (22.0)*	3.61 (0.93)*	1.69 (0.49)*	5.53 (1.01)	1.34 (0.84)*

Table 1 (continued)

	N	Serum 25(OH)D (nmol/L)	Serum LDL-cholesterol (mmol/L)	Serum HDL-cholesterol (mmol/L)	Serum total-cholesterol (mmol/L)	Serum triglycerides (mmol/L)
How often do you usually drink alcohol[a]						
Never	1249	61.0 (24.3)	3.72 (0.93)	1.50 (0.46)	5.55 (1.02)	1.56 (0.87)
Monthly or less	4129	60.7 (21.5)	3.69 (0.95)	1.49 (0.44)	5.51 (1.03)	1.55 (1.01)
2–4 times a month	6646	62.8 (21.2)	3.73 (0.94)	1.55 (0.46)	5.60 (1.01)	1.53 (0.93)
2–3 times a week	4285	64.8 (22.0)	3.71 (0.94)	1.68 (0.50)	5.60 (1.00)	1.44 (0.91)
4 or more times a week	1039	66.5 (23.8)*	3.72 (0.93)	1.79 (0.55)*	5.77 (0.99)*	1.41 (0.88)*
Do you smoke daily[a]						
Yes	2315	59.7 (22.7)	3.86 (0.99)	1.49 (0.46)	5.74 (1.04)	1.66 (1.09)
No	14768	63.4 (21.7)*	3.69 (0.93)*	1.60 (0.48)*	5.58 (1.01)*	1.48 (0.91)*

*$P < 0.01$, **$P < 0.05$, comparison between two groups or trend across groups, linear regression with adjustment for gender, age, BMI, and season

[a]Missing data in a few subject

successful serum lipids and 25(OH)D measurements, and of these 17,411 were non-statin users; 9345 females and 8066 males. The results are presented for these non-statin users, except when specified otherwise (Table 1).

For both serum 25(OH)D and serum lipids, there were strong associations with gender, age, and BMI. There was a particularly strong co-variation between serum 25(OH)D and HDL-cholesterol (Table 1). With increasing serum 25 (OH)D level, there was an increase in serum LDL-cholesterol, HDL-cholesterol, and total-cholesterol and a decrease in serum triglycerides (Table 2). Similar results were found with serum 25(OH)D as a continuous variable in different linear regression models (Supplemental Table 1).

To minimize the effect of food and supplements containing both vitamin D and fish oil that could possibly cause 25(OH)D and lipids to covariate [22], a subgroup analysis was made in subjects who reported the use of cod liver oil, Omega 3 tablet, and vitamin D supplements as "never" or "sometimes" and who reported intake of fatty fish as 0–1 times per month. In this subgroup, only the relation between 25(OH)D and HDL-cholesterol remained significant after adjusting for the other covariates (Table 2).

To minimize potential effects of season, the analyses were done stratified for the summer and winter months with exclusion of subjects recently been on a sunny holiday and subjects using solarium. This did not affect the relation between 25(OH)D and HDL-cholesterol, LDL-cholesterol, and total-cholesterol, whereas the relation with triglycerides became non-significant (data not shown).

Longitudinal relations between serum 25(OH)D and lipids

Among the 12,984 subjects who attended the sixth survey in the Tromsø Study 2007/2008, 10,055 were non-smokers and had successful serum 25(OH)D measurements (4780 men and 5275 women, mean age 58.0 (12.8) years, mean serum 25(OH)D 55.0 (17.8) nmol/L).

Since the serum 25(OH)D levels were substantially lower in the sixth than the seventh survey (probably due to differences between the assays employed), the serum 25 (OH)D levels from both survey were transformed to Z-scores to evaluate correlations between change in serum 25 (OH)D level and change in lipids. There was a significant positive correlation between delta serum 25(OH)D Z-score (value in the seventh survey minus the value in the sixth) with delta HDL-cholesterol ($n = 5384$, $r = 0.09$, $P < 0.001$) and a significant negative correlation with delta triglycerides ($n = 5384$, $r = -0.08$, $P < 0.001$), whereas no significant correlations were found with LDL- or total-cholesterol.

Using untransformed values, the lack of effect of season on the relation between 25(OH)D and lipids was further demonstrated by comparing those who changed season of blood sampling between the two surveys. Thus, for those who were examined during the winter months in the sixth survey in 2007/2008 and during the summer months in the seventh survey in 2015/2016 ($n = 782$), there was an increase in mean serum 25(OH)D of 17.9 (19.8) nmol/L; whereas those who changed from summer to winter ($n = 320$) had an increase of only 4.9 (22.6) nmol/L ($P < 0.01$). On the other hand, change in season of examination had no effect on change in serum lipid levels except for serum cholesterol that showed an increase if examined during the winter compared to summer (Table 3). Similarly, subjects who did not use cod liver oil in 2007/2008 but daily in 2015/2016 ($n = 284$) had an increase in mean serum 25 (OH)D of 17.8 (21.1) nmol/L, whereas those who stopped use of cod liver oil ($n = 269$) had a mean increase of 8.8 (23.5) nmol/L ($P < 0.001$). However, no significant

Table 2 Serum lipid levels in relation to serum 25(OH)D levels in all subjects and in those with low intake of fish oils and no use of vitamin D supplements, seventh survey in the Tromsø Study

	N	Serum LDL-cholesterol (mmol/L)	Serum HDL-cholesterol (mmol/L)	Serum total-cholesterol (mmol/L)	Serum triglycerides (mmol/L)
All subjects					
Serum 25(OH)D (nmol/L)					
0–24	383	3.63 (0.98)	1.39 (0.39)	5.40 (1.03)	1.71 (1.05)
25–49	4738	3.66 (0.94)	1.46 (0.44)	5.47 (1.01)	1.65 (1.09)
50–74	7617	3.72 (0.93)	1.58 (0.46)	5.60 (1.01)	1.48 (0.89)
75–99	3736	3.77 (0.94)	1.70 (0.50)	5.75 (1.01)	1.40 (0.82)
100–	937	3.72 (0.98)*	1.83 (0.56)*	5.79 (1.04)*	1.30 (0.76)*
Subjects with low intake of fish oils[a]					
Serum 25(OH)D (nmol/L)					
0–24	68	3.78 (1.02)	1.29 (0.34)	5.54 (1.10)	2.08 (1.41)
25–49	549	3.61 (0.89)	1.37 (0.39)	5.37 (0.95)	1.74 (1.11)
50–74	427	3.72 (0.91)	1.47 (0.43)	5.57 (0.97)	1.67 (1.06)
75–99	142	3.66 (0.88)	1.56 (0.48)	5.58 (0.97)	1.58 (0.87)
100–	19	4.23 (1.14)	1.77 (0.41)**	6.19 (1.14)	1.28 (0.60)

*$P < 0.01$, linear regression with gender, age, BMI, intake of fat fish, use of cod liver oil, Omega 3 capsules (fish oil, seal oil), use of vitamin D supplements, physical activity, sunny vacation last two months, use of solarium, smoking and frequency of alcohol intake and month of blood sampling as covariates

**$P < 0.05$, linear regression with gender, age, BMI, physical activity, sunny vacation last two months, use of solarium, smoking, frequency of alcohol intake, and month of blood sampling as covariates

[a]Eating fat fish 0–1 per month; use of cod liver oil, Omega 3 capsules (fish oil and seal oil), use of vitamin D supplements never or only sometimes

differences were seen for the changes in the serum lipids (Table 3). On the other hand, improvement in lipid profile was seen in those who started using statins after 2007/2008 and a worsening in lipid status in those who stopped statins after 2007/2008. However, change in statin status did not effect change in mean serum 25(OH)D between the two surveys (Table 3).

Genetic effects on serum 25(OH)D and lipids

In total, 2365 subjects attended both the fourth and the seventh survey of the Tromsø Study and had successful genotyping in 1994/1995 and serum 25(OH)D and lipid measurements in 2015/2016. As expected, the rs10741657 SNP in the 25-hydroxylase gene (*CYP2R1*) and rs2282679 SNP in the DBP gene (*DBP or GC*) were significantly related to the serum 25(OH)D level; however, there were no significant relation with the serum lipids (Table 4).

Relation between serum DBP, serum 25(OH)D, and serum lipids

In total, 511 subjects were included in the prevention of diabetes study [18], of whom 479 at baseline had successful measurements of vitamin D metabolites, DBP, and lipids. As expected, serum 25(OH)D correlated positively with DBP, measured and calculated free fraction of serum 25 (OH)D, and also with the serum lipids as in the Tromsø study (Supplemental Table 2). The calculated serum free 25 (OH)D had almost identical relations to serum lipids as total 25(OH)D, whereas the associations were weaker for the measured serum free 25(OH)D. Serum DBP had a weak, but significant correlation with HDL-cholesterol ($P < 0.02$); however, after adjustment for age, gender, and BMI, this was no longer significant. Similarly, in a linear regression model, serum 25(OH)D was a significant predictor of HDL-cholesterol after adjustment for gender, age, and BMI, a relation that was not affected by including DBP in the analysis (data not shown).

Discussion

To our knowledge, this is the largest observational study exploring the relations between serum 25(OH)D and lipids. We have confirmed that high serum 25(OH)D levels are associated with high serum LDL-, HDL-, and total-cholesterol levels, and with low serum triglyceride levels [7]. The association with HDL-cholesterol was particularly strong, and remained significant after extensive adjustments for relevant confounders and in all subgroups analyzed.

Obviously, a substantial part of the cross-sectional associations between serum 25(OH)D and lipids could be caused by co-variation with gender, age, BMI, and lifestyle factors like intake of fat fish, use of cod liver oil, vitamin supplements, sunny vacations, use of solarium, physical

Table 3 Change in serum 25(OH)D and lipids from the sixth to the seventh survey in the Tromsø Study in those who had a change in season of blood sampling, cod liver oil status, or statin status

Change from 2007/2008 to 2015/2016	Season of blood sampling[a]		Cod liver oil status[a]		Statin status	
	Winter 2007/2008, but summer 2015/2016 ($n = 782$)	Summer 2007/2008, but winter 2015/2016 ($n = 320$)	Never cod liver oil 2007/2008, but daily cod liver oil 2015/2016 ($n = 284$)	Daily cod liver oil 2007/2008, but never cod liver oil 2015/2016 ($n = 269$)	Not using statins 2007/2008, but using statins 2015/2016 ($n = 747$)	Using statins 2007/2008, but not using statins 2015/2016 ($n = 118$)
Delta serum 25(OH)D (nmol/L)	17.9 (19.8)	4.9 (22.6)*	17.8 (21.1)	8.8 (23.5) *	12.5 (23.4)	13.5 (22.2)
Delta serum LDL-cholesterol (mmol/L)	0.27 (0.65)	0.34 (0.62)	0.18 (0.66)	0.17 (0.63)	−1.62 (0.97)	1.26 (1.18)*
Delta serum HDL-cholesterol (mmol/L)	0.10 (0.29)	0.11 (0.28)	0.14 (0.32)	0.10 (0.29)	0.09 (0.27)	0.08 (0.30)
Delta serum total-cholesterol (mmol/L)	0.10 (0.77)	0.32 (0.69)*	0.10 (0.77)	0.05 (0.75)	−1.88 (1.06)	1.19 (1.26)*
Delta serum triglycerides (mmol/L)	−0.02 (0.77)	0.02 (0.68)	0.00 (0.70)	0.05 (0.78)	−0.33 (0.86)	0.04 (0.78)*

*$P < 0.001$, versus corresponding group, Student's t test

[a]Statin users excluded

activity, smoking, and alcohol habits. Such co-variation was particularly seen for 25(OH)D and HDL-cholesterol that increased or decreased in striking parallel with changes in these lifestyle factors. However, after adjustment for BMI inclusion of the lifestyle factors had only marginal effect on the relation between the lipids and 25(OH)D that remained highly statistically significant.

Because of the high number of subjects included, we had the opportunity to do subgroup analyses that could eliminate, or at least reduce the influence of the two main sources of vitamin D, production in the skin upon UVB exposure and intake of supplements and vitamin D containing food. We therefore examined serum 25(OH)D and lipids both during the summer and winter months and found basically the same relations. Similarly, change in season of blood sampling from the sixth (2007/2008) to the seventh (2015/2016) survey in the Tromsø Study had the expected effect on the serum 25(OH)D levels, whereas the lipids levels, except for serum total-cholesterol, were not affected. For total-cholesterol, the higher serum levels during the winter is well known [23], and as the seasonal change was opposite that for serum 25(OH)D, this further strengthens the argument that co-variation with UVB exposure cannot explain the 25(OH)D-lipid association [24].

Similarly, we attempted to minimize effects of diet by only including subjects with very low intake of fish and no intake of cod liver oil or vitamin D containing fish oil. With this approach, all relations between serum 25(OH)D and the lipids became non-significant, except for the association with HDL-cholesterol that was hardly affected at all.

In RCTs, supplementation with vitamin D has generally had little or no effect on the serum lipid levels [7]. However, the association between 25(OH)D and lipids could be bi-directional, so that changes in lipids could result in 25 (OH)D changes. However, that was not seen in our study; starting statin medication gave the expected changes in the serum lipids but no effect on 25(OH)D. This is slightly contrary to that reported by Mazedi et al. who in their review on statins and serum 25(OH)D in RCTs found a significant increase in serum 25(OH)D of ~7 nmol/L by statins, whereas in observational studies (like ours) there was a decrease in serum 25(OH)D [25].

Another way of demonstrating a causal effect, as well as the direction of the effect, is to use Mendelian randomization analysis. As an example, Vimaleswaran et al. showed that SNPs related to serum 25(OH)D had no relation to BMI, whereas SNPs related to BMI were also related to serum 25(OH)D [26]. Accordingly, the association between BMI and 25(OH)D is driven by BMI. This approach has also been used for lipids and serum 25(OH)D by Ooi et al., where SNPs related to lipids and 25(OH)D were analyzed in 85,868 Danish subjects [27]. Their main finding was that genetically low serum 25(OH)D was not related to lipid

Table 4 Serum 25(OH)D and lipid levels in the seventh survey of the Tromsø Study in relation to genotypes, subjects who participated in both the fourth and seventh survey of the Tromsø Study

	N	Serum 25(OH)D (nmol/L)	Serum LDL-cholesterol (mmol/L)	Serum HDL-cholesterol (mmol/L)	Serum total-cholesterol (mmol/L)	Serum triglycerides (mmol/L)
SNPs related to serum 25(OH)D						
Rs10741657 (*CYP2R1* gene)						
Major homozygote	817	68.3 (20.7)	3.88 (0.94)	1.71 (0.52)	5.88 (1.03)	1.44 (0.70)
Heterozygote	1111	71.1 (21.8)	3.79 (0.94)	1.69 (0.53)	5.76 (1.01)	1.44 (0.81)
Minor homozygote	438	74.2 (23.1)*	3.79 (0.93)	1.70 (0.53)	5.77 (1.05)	1.38 (0.69)
Rs2282679 (*DBP* or *GC* gene)						
Major homozygote	1349	74.0 (22.8)	3.81 (0.93)	1.71 (0.53)	5.79 (1.03)	1.41 (0.76)
Heterozygote	873	67.4 (19.8)	3.86 (0.94)	1.68 (0.51)	5.83 (1.01)	1.47 (0.77)
Minor homozygote	143	60.3 (16.4)*	3.76 (0.97)	1.70 (0.54)	5.72 (1.05)	1.33 (0.62)

*$P < 0.01$, linear trend adjustment for gender and age

levels as in our study, whereas genetically elevated remnant cholesterol was associated with low serum 25(OH)D levels. However, genetically reduced HDL-cholesterol was not associated with low serum 25(OH)D. This could potentially fit with some of the reported RCT results with increase in 25(OH)D by statin use, indicating a causal relation with the line of direction from cholesterol to 25(OH)D, but not the other way around. A physiological explanation for this could be the shared biosynthetic pathway for cholesterol and vitamin D [28]; however, it should be recalled that the Mendelian randomization approach gives no final proof as the results may reflect pleiotropic effects as well as linkage with other genes properly having the observed effect.

Apart from effects through shared biosynthetic pathways, vitamin D could influence serum lipids by a number of mechanisms. Thus, vitamin D increases intestinal calcium absorption and thereby amount of calcium-fatty acid soaps excreted in the feces [29]. Increased calcium levels may reduce hepatic triglyceride formation and secretion [30], and in isolated adipocytes $1,25(OH)_2D$ inhibits lipolysis [31]. Vitamin D may also have an effect on both insulin secretion and insulin sensitivity [32], and vitamin D deficiency may therefore lead to an increase in triglyceride level and a decrease in HDL-C level [33].

It should finally be mentioned that measurement of serum 25(OH)D includes the free, unbound fraction as well as that bound to albumin and DBP. The fraction bound to DBP is by far the largest, and the serum level of DBP therefore has a large effect on the serum 25(OH)D measured [15]. A connection between DBP and lipids could therefore potentially explain the lipid and 25(OH)D co-variation; however, that was not found. Nor did using calculated or directly measured free 25(OH)D change the 25(OH)D and lipid associations.

Our study has several limitations. As an observational study, no causal relationships can be established, and the study therefore only had an exploratory function. We used self-reported lifestyle data, and our genetic analysis probably lacked power to detect minor associations. On the other hand, our study also has some strength; we had a large number of subjects both in the cross-sectional and longitudinal analyses, enabling us to do targeted subgroup analyses.

Conclusions

The associations between 25(OH)D and lipids (except for HDL-cholesterol) appear to be explained by known confounding factors. However, for HDL-cholesterol, we were not able to indicate a plausible cause of the association with 25(OH)D, which can hardly be explained by co-variation with sun exposure or be the result of nutrients or supplements with dual effects on their serum levels.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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