Original Article

Associations between Serum 25-hydroxyvitamin D and Lipids, Lipoprotein Cholesterols, and Homocysteine

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Abstract

Background: Serum 25(OH) vitamin D levels are inversely associated with cardiovascular disease (CVD) mortality, mediated in part by independent positive relationships with high-density lipoprotein cholesterol (HDLC) and inverse relationships with low-density lipoprotein cholesterol (LDLC), triglyceride, and homocysteine. **Aims:** In this study, we assessed relationships between fasting serum vitamin D and lipids, lipoprotein cholesterols, and homocysteine. **Materials and Methods:** We studied 1534 patients sequentially referred to our center from 2007 to 2016. Fasting serum total 25(OH) vitamin D, plasma cholesterol, triglyceride, HDLC, LDLC, and homocysteine were measured. Stepwise regression models were used with total cholesterol, triglyceride, HDLC, LDLC, and homocysteine as dependent variables and explanatory variables age, race, gender, body mass index (BMI), and serum vitamin D levels. Relationships between quintiles of serum vitamin D and triglycerides, HDLC, LDLC, and homocysteine were assessed after covariance adjusting for age, race, gender, and BMI. **Results:** Fasting serum vitamin D was positively correlated with age, HDLC, and White race, and was inversely correlated with BMI, total and LDL cholesterol, triglyceride, and fasting serum homocysteine ($P \leq 0.0001$ for all). Serum vitamin D was a significant independent inverse explanatory variable for total cholesterol, triglyceride, and LDL cholesterol, and accounted for the largest amount of variance in serum total cholesterol (partial $R^2 = 3.6\%$), triglyceride (partial $R^2 = 3.1\%$), and LDLC (partial $R^2 = 2.9\%$) ($P < 0.0001$ for all). Serum vitamin D was a significant positive explanatory variable for HDLC (partial $R^2 = 1.4\%$, $P < 0.0001$), and a significant inverse explanatory variable for homocysteine (partial R^2 = 6.0–12.6%). **Conclusions:** In hyperlipidemic patients, serum vitamin D was a significant independent inverse determinant of total cholesterol, LDLC, triglyceride, and homocysteine, and a significant independent positive determinant of HDLC. Thus, serum vitamin D might be protective against CVD.

Keywords: Cardiovascular disease, cholesterol, estimated glomerular filtration rate, high density lipoprotein cholesterol, homocysteine, low density lipoprotein cholesterol, myocardial infarction, triglycerides, Vitamin D

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Introduction

Serum vitamin D levels are associated with adiposity, insulin resistance, and metabolic syndrome,[1,2] and it is not surprising that low levels of serum vitamin D are significant independent predictors of cardiovascular disease (CVD).[3,4] High levels of vitamin D and high

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density lipoprotein cholesterol (HDLC) are associated with a lower risk of CVD.[5] There are independent and significant positive correlations between serum vitamin D and apolipoprotein A1 and HDLC in adult men and women.[6] Vitamin D deficiency has been reported to be an independent predictor of elevated triglycerides in Spanish school children.[7] Vitamin D supplementation in Argentine school children increased HDLC and decreased triglyceride.[8] In women with polycystic ovary syndrome, vitamin D therapy decreased total cholesterol, triglyceride, and very low density lipoprotein cholesterol (LDLC), however, did not affect HDLC or apolipoprotein A1.[9]

In some studies, vitamin D supplementation has been reported to have no significant effect on CVD mortality[10] or on the incidence of myocardial infarction (MI) or stroke.[11] Wang *et al*. reported a nonsignificant reduction in CVD with moderate-to-high doses of vitamin D (risk ratio: 0.90; 95% confidence interval (CI): 0.77–1.05).[12] Vitamin D supplementation may protect against cardiac failure in older people, however, in a meta-analysis, it did not appear to protect against MI or stroke.[13]

In 1534 patients referred to our Cholesterol Center from 2007 to 2016, we assessed pretreatment entry relationships between fasting serum vitamin D and lipids, lipoprotein cholesterols, and homocysteine to better understand the independent relationships of serum vitamin D to CVD risk factors.

Materials and Methods

The study was carried out following protocol #12-03 approved by the Jewish Hospital Institutional Review Board.

Participants

We evaluated routine lipid, lipoprotein, homocysteine, and serum vitamin D determinations drawn at study entry after an overnight fast as part of our standard clinical initial evaluation of referred patients, following a protocol approved by the Institutional Review Board of the Jewish Hospital of Cincinnati. We studied 1534 patients in the consecutive order of their referral from 2007 to 2016.

Laboratory determinations

At the initial visit, after an overnight fast, blood was drawn for serum total 25(OH) vitamin D levels quantitated by two-dimensional high performance liquid chromatography (HPLC) with tandem mass spectrometry detection after protein precipitation.[14] The laboratory lower normal limit for total 25(OH) vitamin D was 32 ng/ml.[14] Additional measures included plasma cholesterol, triglyceride, HDLC, LDLC, and in some patients, fasting serum homocysteine and estimated glomerular filtration rate (eGFR).

Statistical analysis

Because the data was not normally distributed, Spearman correlation coefficients were calculated between serum vitamin D and other variables. Stepwise regression models were used with total cholesterol, triglyceride, HDLC, LDLC, and homocysteine as dependent variables, and explanatory variables such as age, race, gender, body mass index (BMI), and serum vitamin D levels.

To assess whether the association between serum vitamin D and homocysteine (or lipids) might be nonlinear, a spline second-degree function of vitamin D with a single knot at the cohort median of serum vitamin D (27.6 ng/ml) [Figure 1] was included in the linear regression model[15] to test the hypothesis that the association between serum vitamin D and homocysteine (or lipids) existed up to the median of serum vitamin D, but not above it.

We categorized serum vitamin D in quintiles, and assessed least square mean triglyceride, HDLC, LDLC, and homocysteine by D quintiles, after covariance adjusting for age, race, gender, and BMI [Figures 2-5].

Results

As displayed in Table 1, fasting serum vitamin D was positively correlated with age, HDLC, and White race ($P < 0.0001$ for all). Serum vitamin D was inversely correlated with BMI, total cholesterol and LDLC, triglyceride, eGFR, and fasting serum homocysteine ($P \leq 0.0001$ for all) [Table 1].

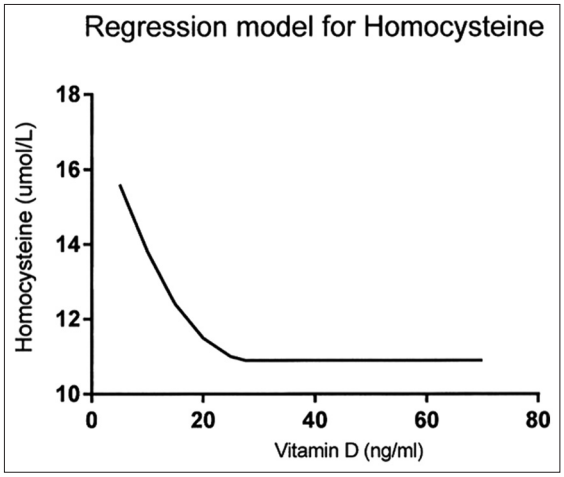

Figure 1: Curvilinear relationship between serum vitamin D and homocysteine. A splined function of vitamin D was used in regression models, knot = 27.6 ng/ml (cohort median for serum vitamin D), and degree = 2

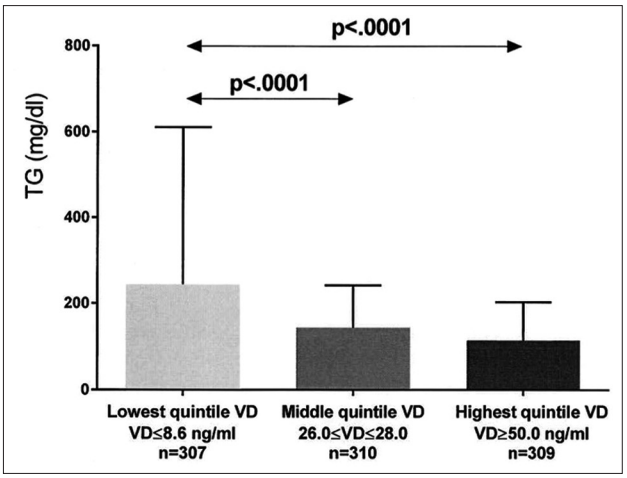

Figure 2: Unadjusted triglyceride (mean ± SD) in the lowest, middle, and highest vitamin D (VD) quintiles exhibited. Significant differences between groups are shown, with *P* values taken from comparisons of least squares means after adjustment for age, race, gender, and body mass index

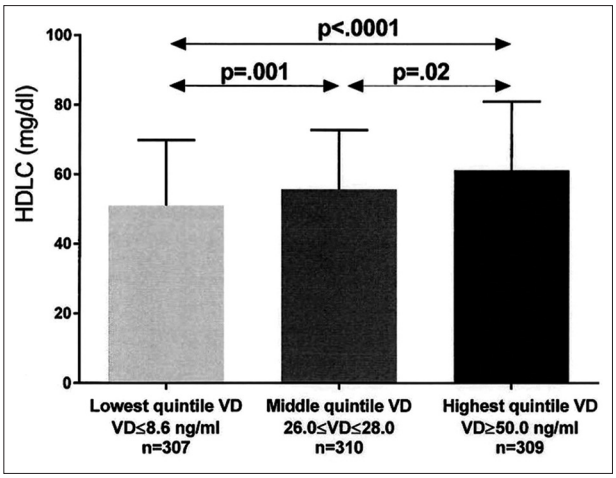

Figure 3: Unadjusted high density lipoprotein cholesterol (mean ± SD) in the lowest, middle, and highest vitamin D quintiles exhibited. Significant differences between groups are shown, with *P* values taken from comparisons of least squares means after adjustment for age, race, gender, and body mass index

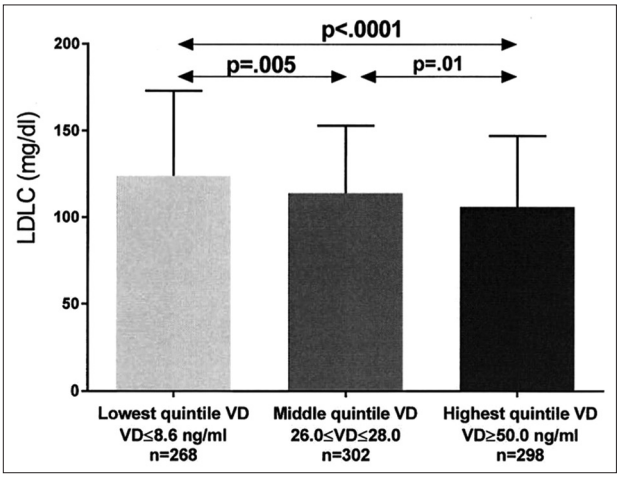

Figure 4: Unadjusted low density lipoprotein cholesterol (mean ± SD) in the lowest, middle, and highest vitamin D quintiles exhibited. Significant differences between groups are shown, with *P* values taken from comparisons of least squares means after adjustment for age, race, gender, and body mass index

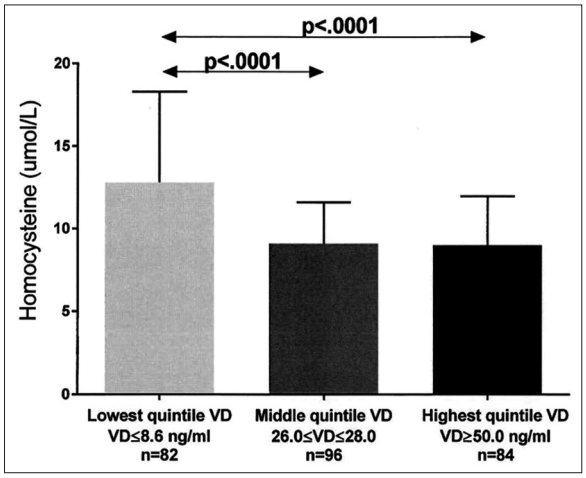

Figure 5: Unadjusted serum homocysteine (mean ± SD) in the lowest, middle, and highest vitamin D quintiles exhibited. Significant differences between groups are shown, with *P* values taken from comparisons of least squares means after adjustment for age, race, gender, and body mass index

Homocysteine was positively correlated with age, BMI, total cholesterol, LDLC, and was inversely correlated with HDLC, eGFR, female gender, and White race [Table 1].

Vitamin D, as a significant negative independent determinant, accounted for the largest amount of variance in serum total cholesterol (partial $R^2 = 3.6\%$), triglyceride (partial $R^2 = 3.1\%$), and LDLC (partial $R^2 = 2.9\%$) [Table 2]. Serum vitamin D was also a significant positive independent predictor for HDLC (partial $R^2 = 1.4\%$) [Table 2].

Vitamin D was a significant negative independent determinant of triglyceride, with a linear term and a splined quadratic term [Table 2, panel 2]. The spline quadratic term of vitamin D (with a positive coefficient) was significant for triglyceride in addition to the linear term [Table 2, panel 2]. The spline quadratic term for vitamin D accounted for an extra decrement in triglyceride up to the cohort mean of vitamin D, 26.7 ng/ml with no further decrements above the median.

Vitamin D was a significant negative independent determinant of LDLC [Table 2, panel 4] as a linear term, whereas the spline function for vitamin D provided a reduced decrement in LDLC up to the cohort vitamin D median, 26.7 ng/ml.

Table 1: Vitamin D, lipids, lipoprotein cholesterols, glomerular filtration rate (eGFR), [1]MTHFR genotype, [1]and homocysteine[1] in 1534 patients at study entry

Variables	*n*	Mean±SD	5th percentile	10th	50th	90th	95th	Spearman correlation with vitamin D *n*=1534	Spearman correlation with Homocysteine, *n*=813
Serum (ng/ml)	1534	31.9±22.7	5.9	7.1	27.6	95.7	73.7		*r*=−0.23, *P*<0.0001
Age (year)	1534	53.3±15.4	25.4	32.7	54.6	71.9	77.0	*r*=0.12, *P*<0.0001	*r*=0.36, *P*<0.0001
BMI (kg/m^2)	1534	30.7±7.6	20.7	22.2	29.5	40.8	45.0	*r*=−0.27, *P*<0.0001	*r*=0.068, *P*=0.054
TC (mg/dl)	1534	213±61	131	146	205	294	323	*r*=−0.16, *P*<0.0001	*r*=0.090, *P*=0.01
TG (mg/dl)	1534	201±285	52	61	128	335	558	*r*=−0.25, *P*<0.0001	*r*=0.16, *P*<0.0001
HDLC (mg/dl)	1534	53±19	29	33	51	78	86	*r*=0.19, *P*<0.0001	*r*=−0.13, *P*=0.0002
LDLC (mg/dl)	1408	124±49	58	68	117	190	217	*r*=−0.15, *P*<0.0001 *n*=1408	*r*=0.089, *P*=0.02 *n*=722
eGFR[1] (mL/min/1.73)	688	85.1±20.0	51	57	87	109	115	*r*=−0.15, *P*=0.0001 *n*=688	*r*=−0.43, *P*<0.0001 *n*=193
Race	1534	W 1293 (84%); B & O 241 (16%). Coded B & O=1, W=2						*r*=0.29, *P*<0.0001	*r*=−0.088, *P*=0.01 *n*=813
Gender	1534	M 570 (37%); F 964 (63%). Coded M=1, F=2						*r*=0.030, *P*=0.23	*r*=−0.23, *P*<0.0001 *n*=813
MTHFR[1]	572	CC 305 (53%); TC 102 (18%); TT 165 (29%). Coded CC=0, TC=1, TT=2						*r*=0.020, *P*=0.63 *n*=5 72	*r*=0.024, *P*=0.56 *n*=567
Homocysteine[1] (umol/L)	813	10.3±5.6	5.4	6.0	9.3	15.4	18.2	*r*=−0.23, *P*<0.0001 *n*=813	

[1]Obtained in subsets of the full cohort at entry, VD = Vitamin D, eGFR = Calculated glomerular filtration rate, MTHFR = Methlylenetetrahydrofolate reductase genotype, CC = Wild type normal, TC = Heterozygous, TT = Homozygous homocysteine

Table 2: Significant predictors for lipids, lipoprotein cholesterols, and homocysteine with explanatory variables race, gender, age, body mass index, serum vitamin D level (VD), and spline function[1] of VD

Dependent variable	Explanatory variables	Parameter±SE	*P*	Partial R^2 (%)
TC (1534 observations used) Model R^2=7.0%	Vitamin D (ng/ml)	−0.67±0.07	<0.0001	3.6
	Age (years)	0.40±0.10	<0.0001	1.3
	BMI (kg/m^2)	−0.74±0.21	0.0003	0.9
	Race (B & O=1, W=2)	15.9±4.3	0.0002	0.8
	Gender (Male=1, Female=2)	8.2±3.1	0.009	0.4
TG (1534 observations used) Model R^2=8.1%	Vitamin D	−1.68±0.40	<0.0001	3.1
	Gender (Male=1, Female=2)	−87.7±14.5	<0.0001	2.4
	Race (B and O=1, W=2)	130.9±20.7	<0.0001	1.8
	VD spline function, quadratic term	0.19±0.05	0.0005	0.7
HDLC (1534 observations used) Model R^2=23.5%	Gender (Male=1, Female=2)	12.7±0.9	<0.0001	11.9
	BMI (kg/m^2)	−0.63±0.06	<0.0001	8.3
	Vitamin D (ng/ml)	0.12±0.02	<0.0001	1.4
	Race (B & O=1, W=2)	−6.15±1.18	<0.0001	1.3
	Age (years)	0.089±0.027	0.001	0.5
LDLC (1408 observations used) Model R^2=5.4%	Vitamin D (ng/ml)	−0.59±0.07	<0.0001	2.9
	VD spline function, quadratic term	−0.036±0.009	0.0002	1.0
	Age (years)	0.28±0.08	0.0005	0.9
	Gender (Male=1, Female=2)	5.37±2.66	0.04	0.3
	BMI (kg/m^2)	−0.34±0.17	0.05	0.3
Homocysteine (813 observations used) Model R^2=18.1%	Age (year)	0.094±0.010	<0.0001	8.3
	VD spline function, quadratic term	0.0081±0.0010	<0.0001	6.0
	Gender (Male=1, Female=2)	−1.84±0.30	<0.0001	3.8

[1]Spline function of Vitamin D, degree=2, with knot at 27.6, the median of Vitamin D for the 1534 subjects

The curvilinear relationship between homocysteine and vitamin D is displayed in three regression models, which included the splined quadratic function of vitamin D [Table 2, panel 5 and Table 3, panels 1 and 2]

Table 3: Homocysteine 193 observations: Regression model of homocysteine, stepwise selection of explanatory variables: Age, gender, race, BMI, eGFR, TG, HDL, Vitamin D (VD), and spline function of VD

Dependent variable	Significant explanatory variables	Coefficient±SE	*P*	Partial R^2 (%)
Homocysteine 146 observations used Model R^2=23.3%	VD spline function, quadratic term	0.011±0.002	<0.0001	11.8
	eGFR	−0.071±0.018	<0.0001	7.8
	Gender (Male=1, Female=2)	−1.77±0.68	0.01	3.7
Homocysteine 193 observations used Model R^2=28.9%	eGFR	−0.080±0.014	<0.0001	11.7
	VD spline function, quadratic term	0.0092±0.0016	<0.0001	12.6
	Gender (Male=1, Female=2)	−1.90±0.54	0.0005	4.7

Regression model of homocysteine, stepwise selection on explanatory variables: Age, gender, race, BMI, eGFR, TG, HDL, Vitamin D (VD), and spline function of VD. [1]Spline function of VD, degree=2, with knot at 27.6, the median of VD for the 1534 subjects

[Figure 1]. The spline vitamin D quadratic term was a significant predictor of homocysteine; homocysteine decreased when vitamin D increased from low levels up to the cohort median (27.6 ng/ml), however, it did not decrease further as vitamin D increased above 27.6 ng/ml [Figure 1].

As displayed in Table 3, beyond the spline function of vitamin D, other significant explanatory variables for homocysteine included eGFR (inverse) and gender (female lower). Removing the MTHFR genotype, a nonsignificant explanatory variable, allowed an increase in the size of the model [Table 3, panel 2], and the resultant model was similar. Of the 3 regression models for homocysteine [bottom panel Table 2 and 3], the spline function of vitamin D was an important explanatory variable, partial R^2 = 6.0–12.6%.

After covariance adjusting for age, race, gender, and BMI, least square (LS) mean triglyceride was highest in patients with the lowest quintile serum vitamin D versus patients in both the middle and top quintiles [Figure 2]. Covariance adjusted HDLC was lowest in patients with lowest quintile serum vitamin D versus those with middle and top quintile vitamin D, and was lower in patients with middle quintile vitamin D than those with top quintile vitamin D [Figure 3]. Covariance adjusted LDLC was highest in patients with the lowest quintile serum vitamin D, lower in patients with middle quintile vitamin D, and was lowest in patients with highest quintile vitamin D [Figure 4]. Covariance adjusted fasting serum homocysteine was highest in patients with the lowest quintile serum vitamin D, and lower in patients with both middle and top quintile serum vitamin D levels [Figure 5].

Discussion

Our data is congruent with previous studies that reported that higher serum vitamin D levels are associated with lower CVD risk lipid profiles.[16,17] In our current study of patients referred for the diagnosis and treatment of hyperlipidemia and CVD, serum vitamin D was a significant independent inverse determinant of total cholesterol, LDLC, triglyceride, and homocysteine, and a significant independent positive determinant of HDLC. Within this frame of reference, if vitamin D supplementation lowered total cholesterol, LDLC, and triglyceride, as well as serum homocysteine, and elevated HDLC, it should be antiatherogenic.

Significant independent positive correlations between serum vitamin D and apolipoprotein A1 and HDLC have been reported in adult men and women.[6] In a database of 20360 participants,[18] serum vitamin D was positively associated with HDLC and inversely associated with triglyceride and LDLC. Low serum vitamin D was associated with high LDLC and triglyceride in diabetic and nondiabetic patients with stable CVD.[19] Low vitamin D levels were associated independently with reduced left ventricular ejection fraction.[19] In early childhood, serum vitamin D was inversely associated with nonHDLC, total cholesterol, and triglyceride.[20] Vitamin D deficiency was an independent predictor of elevated triglycerides in Spanish school children.[7]

Several mechanisms have been identified that might partially explain the effects of vitamin D on lipids and lipoproteins. *In vitro*, vitamin D metabolites can upregulate lipoprotein lipase,[21] increasing HDLC and lowering triglyceride. Vitamin D has anti-inflammatory effects, and might, speculatively, reduce insulin resistance by reducing low-grade chronic inflammation,[22,23] thus lowering triglycerides and increasing HDLC.

In placebo-controlled vitamin D intervention studies in adults, effects on LDLC, HDLC, and triglyceride have been varied and inconclusive.[24,25] In the Women's Health Initiative, where 1 g calcium-400 IU vitamin D were given in a double-blind randomized trial, LDLC reduced significantly (P = 0.03), and higher serum concentrations of vitamin D were associated with higher HDLC levels (P = 0.003), along with lower LDLC (P = 0.02) and triglyceride levels (P < 0.001).[26] In a placebo-controlled trial of vitamin D in patients with type 2 diabetes,[27] vitamin D reduced serum total cholesterol, but not

triglyceride or HDLC. Although most vitamin D supplementation trials have not demonstrated reduction in CVD, they have used relatively low doses of vitamin D supplementation, emphasizing the important of future trials with higher levels of vitamin D intervention, with focus on cardiovascular events.[25]

Inverse associations between serum vitamin D and homocysteine have been reported in Chinese[28] and North American (NHANES)[15] studies, but not in a Canadian[29] study. An curvilinear inverse association between serum vitamin D and homocysteine has been reported by Amer *et al*. in individuals with serum vitamin D below the group median (≤21 ng/ml),[15] however, in those with vitamin D levels >21 ng/ml, homocysteine did not fall as serum vitamin D increased. In our study, congruent with the report by Amer *et al*.,[15] vitamin D supplementation should lower homocysteine in subjects with serum vitamin D below the cohort median (27.6 ng/ml-our study), but may not be of benefit for those with serum vitamin D above 27.6 ng/ml. Overall, in the current report, with the spline term[15] in the model, vitamin D independently accounted for 6.0–12.6% of the variance of homocysteine, and, as such, vitamin D supplementation for subjects with serum vitamin D below the median should, speculatively, reduce the homocysteine-mediated risk of ischemic stroke.[30,31]

Risk factors for CVD including blood pressure,[32] smoking,[23] obesity,[33,34] physical inactivity,[35] and advanced age[36] are associated with lower serum vitamin D, making it difficult to ascribe an independent role of vitamin D in the development of CVD. However, there is an inverse association between vitamin D and all-cause mortality,[37] and Shotker *et al*. reported consistent inverse associations between vitamin D quintiles and both cardiovascular and all-cause mortality.[38] We speculate that the relationships between serum vitamin D and both lipoprotein cholesterols and homocysteine underlie the reported[38] inverse association of vitamin D with cardiovascular mortality. Placebo-controlled clinical trials of vitamin D supplementation in hyperlipidemic and hyperhomocysteinemic cohorts will be required to cross the bridge from correlation to causation.

Conclusion

In hyperlipidemic patients, serum vitamin D is a significant independent inverse determinant of total cholesterol, LDLC, triglyceride, and homocysteine, and a significant independent positive determinant of HDLC. We speculate that through these relationships, serum vitamin D might be protective against CVD.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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