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Deficient serum 25-hydroxy vitamin D is associated with an atherogenic lipid profile: The Very Large Database of Lipids (VLDL-3) Study

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Abstract

Background—Cross-sectional studies have found an association between deficiencies in serum vitamin D, as measured by 25-hydroxyvitamin D (25(OH)D), and an atherogenic lipid profile. These studies have focused on a limited panel of lipid values including low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG).

Objective—Our study examines the relationship between serum 25(OH)D and an extended lipid panel (Vertical Auto Profile) while controlling for age, sex, glycemic status, and kidney function.

Methods—We used the Very Large Database of Lipids (VLDL), which includes U.S. adults clinically referred for analysis of their lipid profile from 2009 to 2011. Our study focused on 20,360 subjects who had data for lipids, 25(OH)D, age, sex, hemoglobin A1c, insulin, creatinine, and blood urea nitrogen. Subjects were split into groups based on serum 25(OH)D: deficient (<20 ng/ml), intermediate (20-30 ng/ml), and optimal (30 ng/ml). The deficient group was compared to the optimal group using multivariable linear regression.

Results—In multivariable-adjusted linear regression, deficient serum 25(OH)D was associated with significantly lower serum HDL-C (–5.1%) and higher total cholesterol (+9.4%), non-HDL-C (+15.4%), directly measured LDL-C (+13.5%), intermediate-density lipoprotein cholesterol (+23.7%), very low-density lipoprotein cholesterol (+19.0%), remnant lipoprotein cholesterol (+18.4%), and TG (+26.4%) when compared to the optimal group.

Conclusion—Deficient serum 25(OH)D is associated with significantly lower HDL-C and higher directly-measured LDL-C, IDL-C, VLDL-C, RLP-C, and TG. Future trials examining

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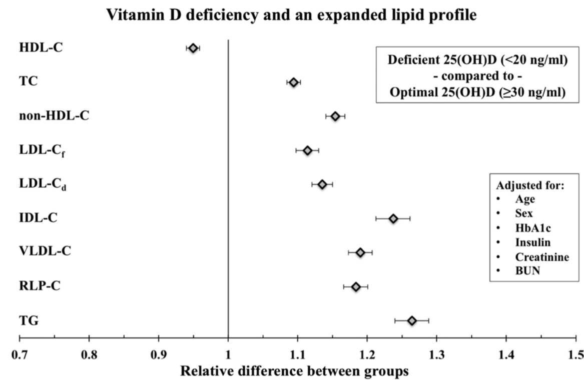
vitamin D supplementation and cardiovascular disease risk should consider using changes in an extended lipid panel as an additional outcome measurement.

Graphical Abstract



Keywords

Vitamin D; 25(OH)D; lipid; lipoprotein; glycemic status; kidney function

Introduction

Atherosclerotic cardiovascular disease (CVD) is the leading cause of death and disability-adjusted life years lost worldwide.[1] Elevated serum concentrations of low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) and low concentrations of high-density lipoprotein cholesterol (HDL-C) are known to be major risk factors for developing CVD.[2-5] A growing body of cross-sectional evidence indicates that blood levels of vitamin D, a fat-soluble vitamin, are inversely associated with an atherogenic lipid profile.[6-9] These studies have found that individuals with low serum 25-hydroxyvitamin D (25(OH)D) (defined as either <20 ng/mL,[6] <30 ng/mL,[7] or in the lowest quartile[8]) have higher LDL-C, higher TG, and lower HDL-C compared to those with higher levels of 25(OH)D (defined as 30 ng/mL[6,7] or higher quartiles[8]). Serum 25(OH)D is considered the best indicator for vitamin D status.[10] Lower serum levels of 25(OH)D are also independently associated with CVD events and mortality, even after adjusting for traditional risk factors including hyperlipidemia, diabetes, hypertension, smoking, body mass index (BMI), and prior history of myocardial infarction.[11-20] The impact of vitamin D supplementation on CVD risk reduction remains inconclusive and is a subject of much investigation and debate.[21]

Past studies examining the association between 25(OH)D and atherogenic lipid profiles used Friedewald-estimated LDL-C (LDL-C_f), which is less accurate than directly measured LDL-C (LDL-C_d), especially in the setting of low LDL-C and high TG.[22] LDL also consists of different densities, with small, dense LDL suggested as a more significant CVD risk factor than large, buoyant LDL particles.[23] The overall LDL particle density can be determined using the Logarithmic LDL Density Ratio (LLDR), which is the ratio of dense-to-buoyant LDL subclasses (defined as $\ln[(LDL_3\text{-}C + LDL_4\text{-}C)/(LDL_1\text{-}C + LDL_2\text{-}C)]$).[24] Higher values of LLDR indicate denser LDL, which is potentially more atherogenic. No studies have

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examined associations between 25(OH)D and LDL-C_d or LDL density. Similarly, no studies have evaluated the relationship between 25(OH)D and remnant lipoprotein cholesterol (RLP-C). RLP-C are triglyceride-rich lipoproteins consisting of intermediate-density lipoproteins (IDL) and dense forms of very-low density lipoproteins (VLDL). RLP-C has been independently associated with the development of CVD.[25-30]

Our study set out to examine the association of vitamin D deficiency, as defined by serum 25(OH)D <20 ng/mL,[31] with an extended lipid panel (Vertical Auto Profile) including HDL-C, total cholesterol (TC), non-HDL-C, LDL-C_f, LDL-C_d, IDL-C, VLDL-C, RLP-C, TG, and LLDR in a large cohort representative of the general U.S. population. The inability of randomized controlled trials and cross-sectional studies to thus far agree on the associations between 25(OH)D and CVD risk may be due to confounders, like glycemic status and kidney function, that were not accounted for in prior cross-sectional studies. Current literature suggests there is an inverse association between 25(OH)D and incidence of type II diabetes,[32] insulin resistance,[33,34] and glycosylated hemoglobin.[35-37] Given the link between diabetes and CVD,[38] our study sought to control for glycemic status in our analysis. Previous research has also shown an association between 25(OH)D and kidney function,[39-41] necessitating controlling for kidney function in our study given the link between declining kidney function and increasing risk for CVD.[42,43] Our study also adjusted for age and sex in addition to glycemic status and kidney function. By utilizing this database with directly measured lipid values and adjusting for clinical variables, we can further elucidate the relationship between 25(OH)D and lipids with greater power than prior studies. We hypothesized that 25(OH)D deficiency would be associated with a more atherogenic lipid profile.

Methods

Study Population

Data in the Very Large Database of Lipids (VLDL) database were collected from 1,340,614 adults (18 years of age) in the United States who were clinically referred for Vertical Auto Profile (VAP; Atherotech, Inc., Birmingham, Alabama) ultracentrifugation testing for lipid profiles from 2009 to 2011. The distribution of lipid values in this dataset match the distribution in the National Health and Nutrition Examination Survey 2007 to 2008.[44] For the primary analysis, we used a cohort of 20,360 individuals from the VLDL dataset who had measurements of 25(OH)D, lipid fractions, age, sex, hemoglobin A1c (HbA1c), insulin, creatinine, and blood urea nitrogen (BUN). All laboratory measures were performed at Atherotech Diagnostics Laboratory in Birmingham, AL.

Lipid Measurements

Direct measurements of LDL-C, IDL-C, VLDL-C, and HDL-C were conducted using inverted rate zonal, single vertical spin, and density gradient ultracentrifugation by the VAP technique. A high level of accuracy in VAP testing was confirmed through split sample comparisons conducted yearly (2007-2012) with beta quantification at Washington University's Core Laboratory for Clinical Studies reference laboratory for lipoprotein analysis, St. Louis, MO. Triglycerides were measured with the Abbott ARCHITECT C-8000

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system (Abbott Park, IL). Friedewald-estimated LDL-C was determined as described previously in individuals with TG <400 mg/dL.[45] LLDR was calculated as described previously as ln[(LDL_3-C + LDL_4-C)/(LDL_1-C + LDL_2-C)].[24] RLP-C was calculated as described previously as VLDL3-C + IDL-C.[30,46] To convert lipoprotein values from mg/dL to mmol/L, multiply by 0.0259. To convert TG values from mg/dL to mmol/L, multiply by 0.0113.

Vitamin D Measurements

Total 25(OH)D assay in serum was performed using LIAISON ® 25 OH Vitamin D Reagent Integral: REF 310600 kit and Liaison® chemistry analyzer both made by DiaSorin. The method is based upon chemiluminescent immunoassay (CLIA) principle. To convert 25(OH)D from ng/ml to nmol/L, multiply by 2.496.

The within-run, between-run, and total reproducibility (% Coefficient of Variation, %CV) of the assay was assessed using two levels of DiaSorin control material (low and high controls). The work was performed over the course of a 9-day testing period. A total of 45 data points were obtained for both controls. On at least one testing day, the assay was repeated a minimum of 10 times within a single run using each level of control material to measure within-run reproducibility. Data from within-run and between-run were combined to get the total reproducibility. Within-run, between-run, and total reproducibility (%CV) for low control was 7.2%, 6.0%, and 8.3%, respectively, and the corresponding %CVs for high control was 5.7%, 5.2%, and 7.0%.

The accuracy of the assay was evaluated by comparing our laboratory results with those obtained by another Liaison analyzer in the DiaSorin manufacturer's laboratory using split specimens (N=42). An excellent correlation coefficient of 0.983 with a slope of 0.89 and an intercept of 2.1 ng/mL was obtained. The bias between the two laboratories was 2.1%.

Hemoglobin A1c Measurements

HbA1c was measured using two different methodologies. In the initial stage of data collection an assay based on AxSym chemistry analyzer and an immunoassay reagent (catalog # 3L93-20) both made by Abbott Diagnostics was used. However, methodology was changed on 07/20/2010 to the one based on Tosoh Automated Glycohemoglobin Analyzer and G8 Variant Elution Buffer No. 1 (S) (Ref# 0021956), No. 2 (S) (Ref#0021957), and No. 3 (S) (Ref# 021958). The analyzer and the buffers were both manufactured by Tosoh Corporation. The Tosoh assay is traceable to the Diabetes Control and Complication Trial (DCCT) reference method. To convert HbA1c from % to mmol/mol, multiply by 10.93 and subtract 23.5.

Insulin Measurements

Insulin was measured using a chemiluminescent microparticle immunoassay (CMIA) principle. The ARCHITECT c System chemistry analyzer and Architect Insulin Reagent Kit Ref: 8K41 both made by Abbott Diagnostic Laboratory were used for testing insulin. To convert insulin from μIU/l to pmol/L, multiply by 6.945.

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Creatinine Measurements

Creatinine assay was performed using kinetic alkaline picrate methodology. The ARCHITECT c System chemistry analyzer and Creatinine Test Reagent kit REF: 3L81 were used to measure creatinine. To convert creatinine from mg/dL to μmol/L, multiply by 88.4.

BUN Measurements

The BUN test was performed using Abbott/ARCHITECT c System chemistry analyzer and Abbott's Urea Nitrogen Test Reagent Kit REF: 7D75. To convert BUN from mg/dL to mmol/L, multiply by 0.357.

Data Management and Review Board approval

Atherotech Diagnostics Lab recorded the initial patient data as part of routine patient clinical measurements, removed duplicates and de-identified patient data before providing the data to our investigators. The database is housed at Johns Hopkins Hospital in Baltimore, Maryland. All investigators had unrestricted access to the study data and authority over the manuscript. We take responsibility for the accuracy of the analysis. The Johns Hopkins University School of Medicine Institutional Review Board approved the study and granted it an exemption from informed consent.

Statistics

For initial comparisons, we examined the distribution of directly measured lipid factions across the following clinical cutoffs of 25(OH)D: deficient (<20 ng/ml), intermediate (20-30 ng/ml), and optimal (30 ng/ml) levels. The optimal level was chosen as 30 ng/ml based on previous studies[6,7] and the Endocrine Society's recommendations.[47] We conducted an unadjusted linear regression using the deficient and optimal 25(OH)D groups (n=13,831) as an independent variable (binary) and TC, non-HDL-C, LDL-C_f, LDL-C_d, IDL-C, VLDL-C, RLP-C, TG, as log-transformed, continuous dependent variables. This regression was repeated with adjustments using age (continuous) and sex (binary). The regression was repeated a third time adding the following log-transformed covariates to age and sex: HbA1c, insulin, creatinine, and BUN. All analysis was done comparing the deficient and optimal 25(OH)D groups (n=13,831) from the sample of 20,360 subjects. All regressions were conducted with 25(OH)D as a binary variable with deficient (<20 ng/ml) compared to optimal (30 ng/ml). Comparisons of lipid outcomes between the deficient and optimal groups are reported in the text as a percentage change and the absolute difference in geometric means. As the Institute of Medicine defines optimal 25(OH)D as 20 ng/ml [31] we also performed the above analysis as part of a sensitivity analysis comparing deficient 25(OH)D (<20ng/ml) to 25(OH)D 20 ng/ml. Sensitivity analyses were also performed stratified by HbA1c level, by creatinine level, and by sex.

Results

Our final study cohort consisted of 20,360 subjects whose baseline characteristics are listed as median and interquartile range in **Table 1**. This cohort has similar distributions of age, TC, HDL-C, LDL-C_f, LDL-C_d, and TG compared to all subjects in the VLDL database with a 25(OH)D measurement (n=70,207) and to all subjects included in the entire VLDL

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database (n=1,340,614). There was a greater proportion of women in both our study subset (57.7%) and the entire VLDL subset with 25(OH)D measurements (58.7%) compared to the VLDL database (47.7%). These sex differences may due to a higher proportion of women getting labs for 25(OH)D for osteoporosis or bone health screening.

Our analyses grouped subjects based on serum 25(OH)D levels. The clinical characteristics of subjects with optimal (30 ng/ml), intermediate (20-30 ng/ml) and deficient 25(OH)D (<20 ng/ml) can be seen as median and interquartile range in **Table 2**. The 25(OH)D deficient group was slightly younger, more likely to be female, and had higher HbA1c and serum insulin. While there were no differences seen in creatinine, BUN was higher in the 25(OH)D deficient group.

The median lipid values of each 25(OH)D group are also shown in **Table 2**. In unadjusted linear regression, compared to subjects with an optimal 25(OH)D (30 ng/ml), subjects who were deficient in 25(OH)D (<20 ng/ml) had lower serum levels of HDL-C (–10.4%, –5.5 mg/dL) and higher levels of all of the atherogenic lipids: TC (+8.4%, +15.2 mg/dL), non-HDL-C (+16.6%, +20.5 mg/dL), LDL-C$_f$ (+8.4%, +8.3 mg/dL), LDL-C$_d$ (+13.0%, +13.1 mg/dL), IDL-C (+27.3%, +2.6 mg/dL), VLDL-C (+26.9%, +5.7 mg/dL), RLP-C (+22.9%, +5.0 mg/dL), and TG (+42.2%, +45.7 mg/dL) (**Figure 1A**). In the multivariable-adjusted linear regression, deficient serum 25(OH)D was still associated with lower serum levels of HDL-C (–5.1%, –3.6 mg/dL) and higher levels of all of the atherogenic lipids: TC (+9.4%, +19.1 mg/dL), non-HDL-C (+15.4%, +20.3 mg/dL), LDL-C$_f$ (+11.4%, +19.3 mg/dL), LDL-C$_d$ (+13.5%, +18.3 mg/dL), IDL-C (+23.7%, +1.4 mg/dL), VLDL-C (+19.0%, +2.2 mg/dL), RLP-C (+18.4%, +2.5 mg/dL), and TG (+26.4%, +9.8 mg/dL) when compared to the optimal group. These differences were statistically significant for all lipid variables in unadjusted analyses and remained significant after adjusting for age, sex, HbA1c, insulin, creatinine, and BUN in multivariable regression analyses (p<0.001 for all variables in both analyses) (**Figure 1B**).

Median values for LDL and HDL subfractions across 25(OH)D groups are shown in **Table 2**. Subjects deficient in 25(OH)D did not have significantly different mean LLDR compared to subjects with optimal 25(OH)D in unadjusted regression (p=0.580). Using multivariable regression with age, sex, HbA1c, insulin, creatinine, and BUN yielded a –0.058 (95% CI –0.084, –0.032) decrease in LLDR in subjects with deficient 25(OH)D compared to the 25(OH)D optimal group. A higher LLDR is consistent with a denser LDL and is potentially more atherogenic.[24] While HDL subfractions HDL$_2$ and HDL$_3$ were both lower in the 25(OH)D deficient group compared to the 25(OH)D optimal group, the ratio of HDL$_2$ to HDL$_3$, a marker of HDL density, showed no significant association with 25(OH)D levels in multivariable regression analysis (p=0.730).

The magnitudes of the associations between deficient 25(OH)D and an atherogenic lipid profile were mildly attenuated in a sensitivity analysis comparing lipid profiles by 25(OH)D classified into binary categories of deficient (<20 ng/mL) and optimal as defined by the Institute of Medicine as levels 20 ng/mL.[31] However, there was no change in the statistical significance of any association. Presence of 25(OH)D deficiency (<20 ng/mL) was associated with an average increase in atherogenic lipid values ranging from 5.3-30.8%

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compared to the optimal 25(OH)D group (20 ng/mL) in the sensitivity analysis (n=20,360). This was mildly reduced compared to an 8.4-42.2% increase in atherogenic lipid values in the 25(OH)D deficient group when the optimal 25(OH)D group was defined as   30 ng/mL in the original analysis (n=13,831).

We also performed sensitivity analyses stratified by HbA1c and creatinine cutpoints and by sex to see if associations of vitamin D deficiency with lipids were similar among these subgroups (**Supplemental Table**). When stratified by HbA1c levels, associations for patients with HbA1c   6.5% were similar to the overall cohort, and for patients with HbA1c >6.5, the associations of low vitamin D with most lipid parameters were even stronger. When stratified by creatinine above and below 1.5 mg/dL, results were similar for both groups compared to the overall cohort. And when stratified by sex, associations for TG, VLDL-C, and RLP-C were slightly stronger among men.

Discussion

In one of the largest and most detailed studies investigating the association between serum 25(OH)D and lipids, we find that 25(OH)D deficiency is associated with an atherogenic lipid profile across all lipid outcomes examined. 25(OH)D deficient individuals have on average 8.4-42.2% more of the various atherogenic lipids and 10.4% less HDL-C compared with individuals with optimal serum 25(OH)D. After adjusting for age, sex, glycemic status, and kidney function, 25(OH)D deficient individuals still have an average of 9.4-26.4% more atherogenic lipids and 5.1% less HDL-C compared to the optimal 25(OH)D group.

These results are consistent with and extend prior work investigating the association between 25(OH)D and dyslipidemia. As seen in our study, patients deficient in 25(OH)D have previously been shown to have higher levels of LDL-C_f and TG along with lower levels of HDL-C.[6,8,48] In comparison to the largest of these earlier studies,[6] we find greater unadjusted geometric mean differences in lipids for patients with 25(OH)D deficiency compared to those with optimal 25(OH)D levels (+8.3 mg/dL, +45.7 mg/dL, and −5.5 mg/dL for LDL-C_f, TG, and HDL-C, respectively, compared to +5.2 mg/dL, +7.5 mg/dL, and −4.8 mg/dL in the prior study). The difference in TC is also greater in our cohort (+15.2 mg/dL compared to +1.9 mg/dL).

Extending prior investigations, our study is the first to show that 25(OH)D deficiency is associated with higher direct LDL-C in addition to LDL-C_f. LDL-C_f as estimated by the Friedewald equation (LDL-C_f = TC – HDL-C – TG/5 in mg/dL) is prone to underestimate LDL-C in certain patients, particularly those with low LDL-C and high TG.[22] In our study cohort, the median LDL-C_f is 106 mg/dL compared to a median LDL-C_d of 109 mg/dL; these values are similar to median LDL-C_f and LDL-C_d values in our entire VLDL cohort of over 1 million individuals. The difference in mean LDL-C_f values in 25(OH)D deficient compared to 25(OH)D optimal individuals (+8.3 mg/dL) is lower than the difference in mean LDL-C_d values (+13.1 mg/dL). This suggests that prior studies may have underestimated the impact of 25(OH)D levels on directly measured LDL-C and supports an inverse association between 25(OH)D and LDL-C that may be somewhat stronger than previously recognized.

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Prior studies have not been able to directly measure RLP-C, but rather have relied on TG levels as a marker. Our study is novel in showing that serum levels of remnant lipoprotein particles measured as RLP-C are higher in patients with 25(OH)D deficiency. These particles contain TG and apolipoprotein B and are formed by enzymatic modification of TG within chylomicrons and VLDLs. They have been implicated in the development of atherosclerosis by a number of molecular mechanisms and have been identified as a risk factor for development of CVD. [25-29] One prior study using Mendelian randomization has shown observational and genetic associations of elevated levels of TG-estimated remnant cholesterol with lower 25(OH)D,[49] but our study is the first to show an inverse association between 25(OH)D and directly measured RLP-C.

Several studies have shown a strong association between small, dense LDL and CVD events.[50-52] While LDL density has previously been categorized as pattern A, A/B, or B, with pattern B conferring the greatest CVD risk, LDL density can also be estimated using the continuous LLDR.[24] In our study, we find that LLDR differs between subjects with deficient 25(OH)D and those in the optimal 25(OH)D range only after adjusting for age, sex, HbA1c, insulin, creatinine, and BUN. The observed effect is small in magnitude, with a reduction in LLDR of only 0.058 in the 25(OH)D deficient group compared to the 25(OH)D optimal group. As increases in LLDR are considered more atherogenic, this change did not agree with the associations between 25(O)D deficiency and the other lipid variables. However, the small magnitude of difference and lack of significance in unadjusted analysis suggests that changes in serum 25(OH)D may not have any major effect on LDL density.

The pathophysiology for deficient 25(OH)D causing an atherogenic lipid profile is unclear. Inadequate dietary intake of vitamin D may reduce calcium absorption in the gastrointestinal system. Prior studies have shown that deficient dietary intake of calcium is associated with higher body weight and a more atherogenic lipid metabolism,[53,54] potentially due to increased lipid metabolism in the body or reduced intake of cholesterol in the gut. However, the observed association between 25(OH)D and dyslipidemia may be confounded by shared metabolic risk factors rather than a causal mechanism. Serum 25(OH)D has been shown to be decreased in obese patients, where sequestration of 25(OH)D in body fat reduces detectable 25(OH)D in the serum.[55] This phenomenon results in an inverse association between BMI and 25(OH)D. Given that BMI is also associated with dyslipidemia, the presence of obesity may explain the observed association of 25(OH)D with dyslipidemia. Unfortunately, we did not have BMI available in our dataset to adjust for this potentially confounding variable. There also appears to be an association between lipid-lowering medication usage and increases in 25(OH)D,[56,57] suggesting that the observed association between low 25(OH)D levels and dyslipidemia may be due to patients with lipid derangements not being on statin therapy.

Although vitamin D supplementation is inexpensive, widely available, and effective in raising serum 25(OH)D, clinical trials have yet to find any link between vitamin D supplementation and changes in lipid profile or reduced CVD mortality.[15,18,58-61] Mendelian randomization studies have used single nucleotide polymorphisms (SNPs) leading to variations in 25(OH)D to examine vitamin D's role in the development of an atherogenic lipid profile and CVD.[49,62,63] These studies have shown evidence that SNPs

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causing a genetically increased RLP-C[49] and BMI[62] are associated with reduced 25(OH)D. This supports the notion that 25(OH)D may be a marker for overall health rather than an independent risk factor for CVD. As there is not currently any definitive evidence that vitamin D has a causal role in atherosclerotic CVD,[21] the associations seen in observational studies may be due confounding factors that impact both serum 25(OH)D and serum lipids.

Limitations

The study has several limitations. As we do not have access to patient medical records, medication usage, including whether patients were taking vitamin D supplements or not, is unknown. Individuals with favorable health-seeking behavior might be more likely to take vitamin D supplements. Although conversely, older, frailer individuals who are at risk for osteoporosis or osteopenia may also be taking vitamin D supplements. The use of 25(OH)D in our analysis, which integrates sunlight, diet, and vitamin D supplementation as an overall marker for vitamin D status, still allows us to compare associations with an atherogenic lipid profile. If someone takes vitamin D supplementation but still has low 25(OH)D, our data suggest they may still have a risk for dyslipidemia. Additionally, as vitamin D supplements contribute to 25(OH)D in a causal manner, it would be inappropriate to adjust for them in an analysis.

Given the minimal demographic and clinical data available in our laboratory dataset, there are many other variables that we were unable to adjust for in our multivariable regression analyses. Such variables include diet, BMI, physical activity, statin usage, or family history, among other potentially important variables that may have impacted our results. For example, since sunlight is a large contributor to 25(OH)D levels, individuals with higher vitamin D levels might be following a healthier lifestyle with increased outdoor physical activity compared to those with lower levels. We tried to account for this possibility by including variables that may be associated with the degree of a healthy lifestyle, like HbA1c, which is associated with the dietary component of a healthy lifestyle.[64,65] We are unable to adjust for the potential confounding influence of physical activity. Although 25(OH)D has been shown to be negatively correlated with BMI, past studies of the association between 25(OH)D and variables from a limited lipid panel (i.e. HDL-C or TGs) still found a significant association after adjusting for physical activity, waist circumference or BMI.[8,48,66,67] While these uncontrolled variables in our study reduce the ability to make causal conclusions, it does not diminish the result in this study that an atherogenic lipid profile as measured by an extended lipid panel (VAP) is associated with deficient 25(OH)D levels independently of HbA1c, insulin, creatinine, and BUN.

Of note, the mean HbA1c was relatively low in our sample (5.8%). However, the individuals included in the VLDL dataset are from referrals for VAP testing of lipids, making it likely that the majority of subjects used in this analysis are from primary care referrals where the prevalence of diabetes is lower than in specialty populations. While we do not know the reason for the laboratory tests that were ordered, it is possible a good portion were for health screening rather than disease monitoring. This may be the simplest explanation for why the mean HbA1c is relatively low, not because this dataset excludes diabetic patients but perhaps because patients with known diabetes were more likely to receive Point of Care

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testing for A1c. Additionally, the mean HbA1c in this study (5.8%) is comparable to that of National Health and Nutrition Examination Survey 2007 to 2008 (5.6%).[68] We did perform a sensitivity analysis stratified by HbA1c level above and below the 6.5% cutpoint, and results were similar to the overall cohort, and even slightly stronger among diabetic patients.

Individuals with advanced chronic kidney disease have reduced ability to convert 25(OH)D into the active form, 1,25 dihydroxy-vitamin D (calcitriol), but the prevalence of chronic kidney disease was also low in our sample (n=483, 3% with a creatinine 1.5). We did perform a sensitivity analysis stratified by creatinine above and below the 1.5 cutpoint and the magnitudes of associations were similar to the overall cohort.

Like any cross-sectional, observational study, our results are limited to the associations between variables and do not prove a causal role of 25(OH)D deficiency on the development of an atherogenic lipid profile. Nevertheless, the sample size, precision, and consistency of our results even after adjusting for potential confounding variables lends support to the hypothesis that 25(OH)D deficiency plays a role in promoting an atherosclerotic lipid profile. Additionally, the observation of a dose effect in the sensitivity analysis, where smaller changes in 25(OH)D between groups had smaller magnitudes of atherogenic lipid changes, also supports a role of 25(OH)D in promoting an atherogenic lipid profile.

Future Directions

The limitations discussed above do not diminish the role of our study in generating hypotheses for future investigations. Prior observational studies have found links between deficient 25(OH)D and CVD risk, but the mechanism remains unclear.[11-20] Our results indicate that the mechanism by which deficient 25(OH)D increases CVD risk may be mediated through changes in lipids. Our study and others suggest it is likely that only deficient individuals (rather than a broad general population) would benefit from vitamin D supplements. However, whether the treatment of vitamin D deficiency through supplementation or modest sunlight exposure can improve dyslipidemia and reduce CVD outcomes is currently unknown.

Randomized controlled trials of vitamin D supplementation that are currently in progress or in the future should consider using an extended lipid panel for comparison with outcomes. In our study LDL-C, HDL-C, and TC had a smaller magnitude of association with 25(OH)D level compared to IDL-C, VLDL-C, RLP-C, and TGs, indicating that future trials may benefit from utilizing an extended lipid panel as an outcome measurement. Additionally, the major limitation of our study and other observational studies is the potential for confounding variables we were unable to adjust for. Most notably, future trials should, in addition to being randomized, measure body anthropometrics (BMI, waist circumference, etc.), take a detailed dietary history, and assess the amount of outdoor activity for additional adjustments in analysis.

Conclusions

Despite inconclusive evidence from clinical trials, there remains strong evidence in the literature that 25(OH)D is inversely associated with LDL-C_f and TG and directly associated

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with HDL-C. Our study not only supports these findings but also shows for the first time, to the best of our knowledge, that this inverse association holds true for directly measured LDL-C, IDL-C, VLDL-C, and RLP-C. Investigators conducting future clinical trials examining the impact of vitamin D supplementation on hyperlipidemia and CVD risk should consider studying an extended lipid panel, which includes directly measured LDL-C, IDL-C, VLDL-C, RLP-C, and LLDR in addition to LDL-C_f, HDL-C, and TG. In doing so, such studies may be able to show reductions in CVD risk factors that might not otherwise be recognized, revealing potential benefits that may not be apparent in mortality and morbidity outcomes alone.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We examine the association between vitamin D (25(OH)D) and an extended lipid panel.
- We compare deficient 25(OH)D (<20 ng/mL) to optimal 25(OH)D (30 ng/mL).
- We adjust for age, sex, HbA1c, insulin, creatinine, and blood urea nitrogen.
- Deficient 25(OH)D is associated with 5% lower high-density lipoprotein cholesterol.
- Deficient 25(OH)D is associated with 9-26% higher atherogenic lipids.

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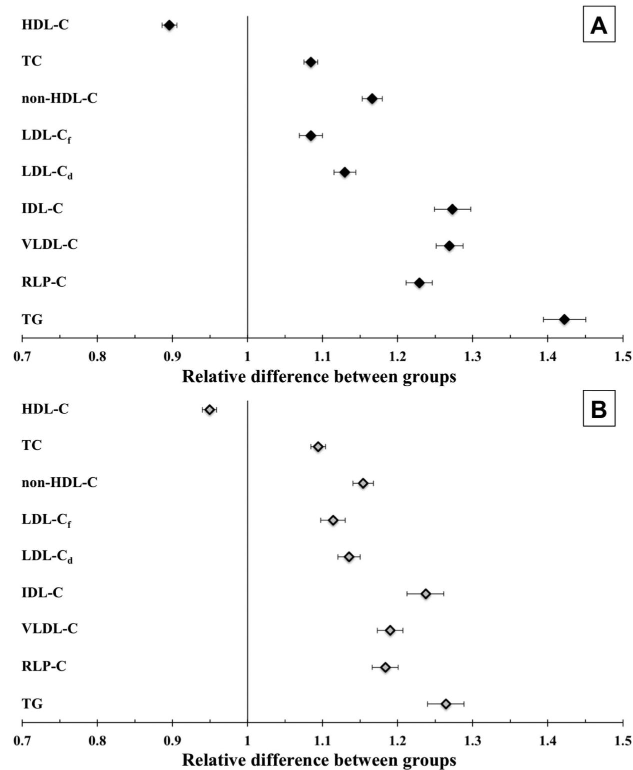

Figure 1.

The relative difference in extended lipid profile of subjects with deficient 25(OH)D (<20 ng/ml) compared to optimal 25(OH)D (30 ng/ml) without adjustment (A) and with adjustment for age, sex, hemoglobin A1c, insulin, creatinine, and blood urea nitrogen (B). In unadjusted analysis (A), the 25(OH)D deficient group had 10% lower HDL-C and 8-42% higher atherogenic lipid values than the group with optimal 25(OH)D. With adjustment (B), the 25(OH)D deficient group had 5% lower HDL-C and 9-26% higher atherogenic lipid values than the group with optimal 25(OH)D. Abbreviations: HDL-C = high density lipoprotein cholesterol, TC = total cholesterol, $LDL-C_f$ = Friedewald-estimated low-density lipoprotein cholesterol, $LDL-C_d$ = directly-measured low-density lipoprotein cholesterol, IDL-C = intermediate-density lipoprotein cholesterol, VLDL = very low-density lipoprotein cholesterol, RLP = remnant lipoprotein cholesterol, TG = triglycerides. Error bars indicate 95% confidence intervals. For all variables p<0.001.

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Table 1

Characteristics of study and VLDL subjects

	Study subset n=20,360	**All 25(OH)D n=70,207**	**VLDL n=1,340,614**
Age (years)	58 (48 to 68)	59 (49 to 69)	59 (49 to 70)
Sex (% female)	55.5	56.3	47.7
HDL-C (mg/dL)	50 (42 to 62)	51 (42 to 63)	52 (42 to 63)
TC (mg/dL)	188 (160 to 219)	188 (160 to 219)	188 (159 to 220)
LDL-C_f (mg/dL)	106 (81 to 133)	106 (82 to 133)	106 (82 to 134)
LDL-C_d (mg/dL)	109 (85 to 136)	109 (86 to 136)	108 (85 to 135)
Triglycerides (mg/dL)	120 (84 to 176)	118 (83 to 172)	115 (82 to 166)

Except for sex, values are median (interquartile range). Abbreviations: HDL-C = high density lipoprotein cholesterol, TC = total cholesterol, LDL-C_f = Friedewald-estimated low-density lipoprotein cholesterol, LDL-C_d = directly-measured low-density lipoprotein.

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Table 2

Distribution of adjusting and outcome variables by clinical categories of 25(OH)D

	Deficient 25(OH)D <20 ng/ml (n=3962)	Intermediate 25(OH)D 20-30 ng/ml (n=6529)	Optimal 25(OH)D 30 ng/ml (n=9869)
Age (years)	58 (48 to 68)	59 (49 to 69)	59 (49 to 70)
Sex (% female)	58.9	53.3	55.6
HbA1c (%)	5.8 (5.4 to 6.3)	5.7 (5.4 to 6.0)	5.6 (5.3 to 5.9)
Insulin (μIU/L)	12.7 (7.7 to 22.6)	10.3 (6.6 to 17.8)	8.5 (5.3 to 14.1)
Creatinine (mg/dL)	0.8 (0.8 to 1.0)	0.9 (0.8 to 1.0)	0.9 (0.8 to 1.0)
BUN (mg/dL)	14.7 (11.8 to 18.5)	15.5 (12.6 to 19.0)	16 (13.1 to 19.6)
HDL-C (mg/dL)	48 (39 to 58)	49 (41 to 60)	53 (43 to 65)
TC (mg/dL)	196 (167 to 228)	192 (164 to 223)	182 (101 to 154)
non-HDL-C (mg/dL)	147 (118 to 179)	139 (113 to 169)	125 (101 to 154)
LDL-C_f (mg/dL)	111.4 (85 to 139.2)	109 (84.6 to 136.4)	101.6 (78.4 to 127.2)
LDL-C_d (mg/dL)	117 (92 to 144)	113 (90 to 140)	103 (82 to 129)
IDL-C (mg/dL)	12 (9 to 17)	11 (8 to 16)	10 (7 to 14)
VLDL-C (mg/dL)	24 (18 to 33)	23 (18 to 31)	20 (16 to 26)
RLP (mg/dL)	26 (20 to 35)	24 (19 to 32)	21 (17 to 28)
Triglycerides (mg/dL)	137 (94 to 207)	128 (90 to 185)	105 (76 to 149)
LDL_1-C (mg/dL)	13.4 (8.9 to 18.4)	12.6 (8.4 to 17.7)	10.6 (6.8 to 15.6)
LDL_2-C (mg/dL)	11.2 (4.8 to 21.9)	11.4 (5 to 21.9)	11.9 (5.4 to 21.6)
LDL_3-C (mg/dL)	46.9 (32.9 to 62.1)	45.5 (32.9 to 60.4)	41.3 (29.3 to 55.0)
LDL_4-C (mg/dL)	17.8 (10.7 to 28.8)	17.4 (10.7 to 27.2)	15.1 (10.2 to 22.7)
LLDR	0.99 (0.52 to 1.41)	1.00 (0.52 to 1.41)	0.95 (0.47 to 1.39)
HDL_2-C (mg/dL)	10 (8 to 14)	11 (8 to 15)	12 (9 to 17)
HDL_3-C (mg/dL)	37 (31 to 44)	38 (32 to 45)	41 (34 to 49)

Except for sex, values are median (interquartile range). Abbreviations: HbA1c = hemoglobin A1c, BUN = blood urea nitrogen, HDL-C = high density lipoprotein cholesterol, TC = total cholesterol, LDL-C_f = Friedewald-estimated low-density lipoprotein cholesterol, LDL-C_d = directly-measured low-density lipoprotein cholesterol, IDL-C = intermediate-density lipoprotein cholesterol, VLDL = very low-density lipoprotein cholesterol, RLP = remnant lipoprotein cholesterol, LLDR = logarithmic LDL density ratio.