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Vitamin D status is a determinant of atorvastatin effect on carotid intima medial thickening progression rate in children with lupus: an Atherosclerosis Prevention in Pediatric Lupus Erythematosus (APPLE) substudy

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ABSTRACT

Objective: Epidemiological associations suggest that vitamin D status may play a role in inflammation and progression of atherosclerosis. Using frozen serum, carotid intima medial thickness (CIMT) measurements and other existing data from the Atherosclerosis Prevention in Pediatric Lupus Erythematosus (APPLE) trial, we assessed interactions between serum 25-hydroxyvitamin D (25(OH)D), atorvastatin randomisation and CIMT progression rate.

Methods: Participants in the 3-year APPLE trial were randomised to placebo or atorvastatin and CIMT progression rate was measured. Baseline frozen serum was used to measure 25(OH)D concentrations. Mixed effect longitudinal models for CIMT progression at 3 years were used to evaluate interaction between vitamin D deficiency (serum 25(OH)D <20 ng/mL) at baseline and atorvastatin or placebo treatment, adjusting for key systemic lupus erythematosus disease variables and cardiovascular risk factors.

Results: 201/221 APPLE participants had available samples and were included in this analysis; 61/201 (30%) had vitamin D deficiency at baseline. In adjusted longitudinal modelling, there was significant interaction between baseline vitamin D deficiency and atorvastatin randomisation in 3-year progression of mean-max CIMT. In four out of six carotid segments, there was a greater decrease in mean-max CIMT progression rate in subjects who were treated with atorvastatin compared with placebo if they had baseline serum 25(OH)D levels ≥20 ng/mL.

Conclusions: Subjects with serum 25(OH)D ≥20 ng/mL had less mean-max CIMT progression following 3 years of atorvastatin treatment. Results from secondary analyses must be interpreted cautiously, but findings suggest that underlying vitamin D deficiency may be involved in response to atorvastatin in atherosclerosis prevention.

Trial registration number: NCT00065806.

KEY MESSAGES

► Vitamin D deficiency at baseline was associated with increased baseline hsCRP levels in children and adolescents with SLE.
► No change in longitudinal disease activity measures was seen based on baseline vitamin D status.
► Findings suggest that underlying vitamin D deficiency may be involved in response to atorvastatin in atherosclerosis prevention in children and adolescents with SLE.

INTRODUCTION

Over the last three decades, systemic lupus erythematosus (SLE)-related mortality has decreased in all areas except cardiovascular disease (CVD).[1] Women with SLE who are less than 40 years of age are at a 50-fold increased risk of myocardial infarction compared with control populations.[2] This increase in risk cannot be attributed solely to traditional cardiovascular risk factors, and immune and vascular pathology in SLE are postulated to contribute to the increased CVD risk.[3 4]

Vitamin D deficiency has emerged as a potential risk factor for CVD.[5] In epidemiological studies of the general population, lower vitamin D levels have been associated with CVD, hypertension, diabetes, high-density lipoprotein cholesterol and low-density lipoprotein (LDL) cholesterol, and surrogate measurements of cardiovascular risk such as coronary artery calcification and carotid intima medial thickness (CIMT).[5] One prospective study found that a serum 25(OH)D <15 ng/mL had a multivariable-adjusted

HR of 1.62 (95% CI 1.11 to 2.36, p=0.01) for incident CVD events.[6]

Vitamin D status is mainly determined by the level of circulating 25-hydroxyvitamin D (25(OH)D), which is converted into an active secosteroid hormone, 1,25-dihydroxyvitamin D (1,25(OH)$_2$D) by the kidney and cells of the immune system. The activated hormone then regulates transcription of several inflammatory cytokines.[7] Studies have shown that 25(OH)D deficiency (defined as a serum level <20 ng/mL) is common in SLE and has been associated with increased photosensitivity, fatigue, renal disease, SLE disease activity and proteinuria.[8–12] In vitro, 1,25(OH)$_2$D blocks dendritic cell differentiation, lowers interleukin 12 secretion, and modulates T lymphocyte proliferation and function.[13–16] Differentiation of dendritic cells and type I interferon are important in the pathogenesis of SLE.[17]

The Atherosclerosis Prevention in Pediatric Lupus Erythematosus (APPLE) trial was originally designed to prospectively assess the effect of atorvastatin on progression of CIMT in 221 children and young adults (aged 10–21 years) with SLE.[18] Subjects were randomised to 36 months of atorvastatin (10–20 mg/day based on weight) versus placebo treatment. Primary results showed overall, no significant difference in mean-mean CIMT progression between treatment and placebo groups.[18] Atorvastatin is a hydroxymethyl glutaryl coenzyme A (HMG-coA) reductase inhibitor, which decreases the synthesis of cholesterol and is used in adults with hyperlipidaemia to reduce CVD progression. Cholesterol is one of the precursors of vitamin D. 1,25(OH)$_2$D activates an enzyme of the cytochrome P450 system which metabolises atorvastatin. One study showed differential hypolipaemic response to atorvastatin based on serum 25(OH)D levels.[19] Another study found that in dyslipidaemic subjects, the addition of vitamin D to atorvastatin synergistically lowered total cholesterol and LDL cholesterol levels.[20] Two large studies in SLE (APPLE and Lupus Atherosclerosis Prevention Study (LAPS)) showed no significant change in CIMT between subjects taking atorvastatin and those taking placebo,[18 21] but given the high proportion of patients with SLE with vitamin D deficiency, it is possible that these results may be confounded by vitamin D deficiency. There are no studies that have evaluated the relationship between vitamin D status, inflammation and subclinical vascular disease in paediatric subjects with lupus.

The objective of this subanalysis was to use samples prospectively obtained during participation in the APPLE trial to evaluate the relationship between vitamin D status and atorvastatin treatment on CIMT progression.

METHODS
Subjects
Participants in the 3-year APPLE trial were randomised to placebo or atorvastatin in addition to routine care and CIMT progression was measured. The design and methods of the APPLE trial have been reported previously.[18] SLE was classified by American College of Rheumatology criteria from 21 North American centres. Subjects were excluded from the study if they had baseline fasting total cholesterol >350 mg/dL, familial hypercholesterolaemia, nephrotic syndrome, renal insufficiency, liver disease, or were pregnant or nursing. Subjects were randomised to daily atorvastatin (>50 kg: 10 mg/day, increasing to 20 mg/day at day 30; ≤50 kg: 10 mg/day). Hydroxychloroquine, low-dose aspirin, multivitamins containing folate and American Heart Association Therapeutic Lifestyle Changes diet were recommended.

Two baseline CIMT examinations were performed using an ultrasound protocol described previously.[18] CIMT measurements have been used in paediatric populations as a surrogate marker of cardiovascular risk in multiple diseases including chronic renal failure, chronic hypertension, obesity and familial hypercholesterolaemia.[22] Ultrasound scans were read by a single experienced reader at Ward A. Riley Ultrasound Center, Wake Forest University School of Medicine, Winston-Salem, North Carolina, USA using Image Pro software (Media Cybernetics, Bethesda, Maryland, USA). Standardised longitudinal B-mode images were collected for three arterial segments defined relative to the tip of the flow divider (TFD) as the common carotid artery (10–20 mm proximal to the TFD), the carotid bifurcation (from the TFD to 10 mm proximal to the TFD) and the proximal 10 mm of the internal carotid artery. Near and far walls were imaged simultaneously in the common carotid artery, but separately in the carotid bifurcation and internal carotid artery to improve the ability to align each wall horizontally in these segments. For each arterial segment, Meijer's Arc was used to collect images at 90°, 120°, 150° and 180° on the right side and at 270°, 240°, 210° and 180° on the left side. For a set of 68 studies reread to evaluate intrareader reliability, the intraclass correlation coefficient was 0.74 (95% CI 0.61 to 0.83) for mean-mean common and 0.71 (95% CI 0.56 to 0.81) for mean-max CIMT measurements. The combination of 3 arterial segments, 2 walls, and 2 sides of the neck provided a set of 12 CIMT measurement sites, each imaged from 4 angles. For each measurement site, a maximum CIMT value was defined as the largest of the four angle-specific maximum CIMT values. The 12 maximum CIMT values were then averaged to determine the mean-max CIMT over near and far walls of the right and left common carotid artery, carotid bifurcation and internal carotid artery. For each of the four measurement sites in the common carotid artery, a mean CIMT value defined as the average of the four angle-specific mean CIMT values was also calculated. The four mean CIMT values were then averaged to determine the mean-mean common CIMT. Overall mean-mean and other segment/wall-specific mean-max or mean-mean CIMT measures were computed accordingly.

Other assessments including fasting lipid levels, disease activity scores (Safety of Estrogens in Lupus Erythematosus,

National Assessment (SELENA), SLE Disease Activity Index (SLEDAI)), and a disease-related damage score (Systemic Lupus International Collaborating Clinics/ American College of Rheumatology Damage Index (SDI)) were obtained as previously described.[18] High sensitivity C reactive protein (hsCRP) was obtained along with lipid profiles after 12-h or 4-h fasts at randomisation and analysed at a central commercial laboratory (purified protein derivative Global Central Laboratories, Highland Heights, Kentucky, USA). Institutional Review Board (IRB) approval was obtained for the original APPLE trial and additionally for this secondary analysis.

Serum 25(OH)D determinations

Frozen serum collected at baseline and at 1-year follow-up was used to measure 25(OH)D levels after IRB approval was secured. Frozen serum samples stored in −80° freezers were shipped to the laboratory of Dr Vin Tangpricha at Emory University, Atlanta, Georgia, USA. Serum 25(OH)D was measured by chemiluminescent assay using the Immuno Diagnostic Systems immunoassay System (IDS-iSYS) automated system (Fountain Hills, Arizona, USA). Laboratory technicians were blinded to the study assignment of the samples. Intra-assay and interassay coefficients of variation for serum 25(OH)D were 1.8–4.0% and 10.1–13.0%, respectively. The laboratory participates in a vitamin D external quality control assessment schema (http://www.deqas.org) and the NIH standard quality control programme for vitamin D and tested proficient in the measurement of 25(OH)D during the study period.

Statistical analysis

All statistical analysis was performed using SAS V.9.2 statistical software (SAS, Cary, North Carolina, USA). All statistical tests were two-sided with p values less than 0.05 considered statistically significant for this analysis. Baseline characteristics were summarised using descriptive statistics with categorical data presented as percentages and continuous data presented as means, SDs and medians. The primary efficacy analysis for APPLE compared rates of mean-mean common CIMT progression between treatment groups based on a test of two-way interaction between treatment group and time in a longitudinal linear mixed effects model under data missing at random assumptions. From that model, it was assumed that the effect of treatment could be estimated as the difference in mean progression rates between participants assigned to atorvastatin versus placebo, with negative differences indicating progression for those on atorvastatin was slower than for those on placebo. Similar mixed-effects models were used for analysing continuous secondary longitudinal end points or changes from baseline over time for lipid data. Log transformation was used for hsCRP to achieve normality. Generalised estimating equations were used for binary longitudinal outcomes.

To examine heterogeneity of treatment effects by vitamin D status (25(OH)D levels <20 ng/mL), the efficacy model used in the primary APPLE analysis was extended to include an indicator variable for subgroup as well as two-way and three-way interactions between subgroup, treatment group and time. From these models, we provide estimated mean progression rates with 95% CIs for each combination of subgroup and treatment group. Finally, the three-way interaction between subgroup, treatment group and time provides a test of whether treatment effects in terms of progression rate differ significantly between subgroups. Initially, models were fit examining one subgroup variable at a time, then adjusted for lupus duration, sex, systolic blood pressure, pubertal status, LDL and natural log of hsCRP. These results should be interpreted cautiously as hypothesis generating and not hypothesis testing.

RESULTS
Baseline characteristics

A total of 201/221 (91%) of APPLE subjects had available baseline samples and were included in the analysis; 98 were randomised to atorvastatin and 103 to placebo. At 1-year follow-up, 79 subjects in each group had available serum for analysis. In the original APPLE trial, 81.6% of each arm completed the 3-year study, 70% of them still on study drug. Among the 201 subjects included in the current subanalysis, 180 remained on study drugs at the 1-year follow-up. As shown in table 1, subjects were 83% female, 51% Caucasian, 27% African American and had a mean age of 16 years at entry into the study.

Vitamin D status

Overall, 61/201 (30%) had vitamin D deficiency at baseline and 139 (69%) had vitamin D insufficiency (25(OH)D <30 ng/mL); 12 subjects (6%) had levels less than 10 ng/mL indicating severe deficiency. Mean baseline 25(OH)D levels were 25.9 ng/mL (SD 11.0). At 1 year follow-up, mean 25(OH)D levels were 27.7 ng/mL (SD 14.0), with no statistically significant difference between atorvastatin and placebo groups in mean vitamin D levels after 1 year (p=0.97). There was no statistically significant difference between baseline and 1 year follow-up levels within or between arms. Sixty-six per cent of subjects stayed in their original vitamin D status category at follow-up (sufficient, insufficient or deficient). Percentage taking corticosteroids, and mean prednisone dose adjusted for weight did not differ between deficient and insufficient/sufficient groups (0.19 mg/kg for both groups, p=0.80).

Vitamin D status and CIMT progression

In unadjusted longitudinal modelling, baseline vitamin D deficiency was associated with increased baseline mean-max CIMT (p=0.01). Other baseline associations between vitamin D deficiency and cardiovascular risk factors were detailed in a previous paper.[23] In adjusted

Table 1 Baseline characteristics of APPLE substudy subjects

Variable	All patients, n=201
Female	167 (83.1%)
Age, years (SD)	15.7 (2.7)
Latitude (SD)	39.3 (3.3)
Season	
1st quarter	36 (17.9%)
2nd quarter	54 (26.9%)
3rd quarter	52 (25.9%)
4th quarter	59 (29.4%)
Race	
White	102 (50.7%)
Black	54 (26.9%)
Asian	19 (9.5%)
Native American	3 (1.5%)
Native Hawaiian	5 (2.5%)
Hispanic or Latino	47 (23.4%)
History of smoking	6 (3.0%)
Postmenarchal	137/167 (82.0%)
Annual household income	
<$25 000	57/187 (30.5%)
$25 000–49 999	51/187 (27.3%)
$50 000–74 999	31/187 (16.6%)
$75 000–99 999	24/187 (12.8%)
$100 000–149 999	16/187 (8.6%)
>$150 000	8/187 (4.3%)
Body mass index percentile (SD)	72.1 (25.2)
Duration of lupus, months (SD)	30.4 (28.9)
SLEDAI (SD)	4.5 (4.0)
SDI=0	151 (75.1%)
Hypertension	65/195 (33.3%)
Glomerulonephritis	81/200 (40.5%)
Creatinine clearance (SD)	138.7 (31.8)
Timed urine protein, mg/24 h (SD)	214.6 (491.5)
Serologies	
Lupus anticoagulant	68/190 (35.8%)
Anticardiolipin antibody	86/196 (43.9%)
AntidsDNA antibody	163/201 (81.1%)
Corticosteroid usage	163/200 (81.5%)
Multivitamin usage	147 (73.1%)
Hydroxychloroquine usage	196 (97.5%)
Baseline hsCRP, mg/L (SD)	2.9 (8.4)
Homocysteine, μmol/L (SD)	7.4 (3.0)
Lipids, mg/dL (SD)	
Total cholesterol	154.7 (38.5)
HDL cholesterol	46.0 (13.0)
LDL cholesterol	86.0 (31.4)
Triglycerides	115.2 (68.6)
Lipoprotein A, mg/dL (SD)	22.6 (25.3)
Baseline mean-mean mm (SD) common CIMT mm (SD)	0.468 (0.042)
Baseline mean-max CIMT mm (SD)	0.583 (0.055)

APPLE, Atherosclerosis Prevention in Pediatric Lupus Erythematosus; CIMT, carotid intima media thickening; dsDNA, double-stranded DNA; HDL, high-density lipoprotein; hsCRP, high-sensitivity C reactive protein; LDL, low-density lipoprotein; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; SLICC, Systemic Lupus International Collaborating Clinics.

longitudinal modelling, there was a significant interaction effect between baseline vitamin D deficiency and atorvastatin treatment in 3-year progression of mean-max CIMT (see table 2 and figure 1). In four out of six carotid segments, there was a greater decrease in mean-max CIMT progression rate in subjects treated with atorvastatin compared with placebo if they had baseline 25(OH)D levels ≥20 ng/mL. In only one of six carotid segments, there was a greater decrease in mean-mean CIMT progression rate in those treated with atorvastatin with sufficient vitamin D levels. Of the subjects who changed in vitamin D status from deficient to either insufficient or sufficient at 1 year into the trial, there was a trend towards response to atorvastatin treatment in 3-year CIMT progression, but this did not reach statistical significance.

When we repeated this analysis with 30 ng/mL as our new 25(OH)D cut point, we found similar results with interaction in overall mean-max CIMT progression rate with 2/6 carotid mean-mean segments and 2/6 carotid mean-max segments showing evidence of interaction between vitamin D insufficiency and sufficiency with atorvastatin usage ($p < 0.05$). All interaction effects were in the same direction with greater decrease in CIMT progression rate in those treated with atorvastatin with higher serum 25(OH)D levels. Using race and ethnicity as an additional adjustment variable, we noted no changes in our conclusions, and the numbers of subjects in each subgroup were too small to evaluate changes in CIMT progression with any accuracy.

Vitamin D status and secondary outcomes
In the original APPLE trial, the atorvastatin group displayed reductions from baseline in total cholesterol, LDL and hsCRP, which were maintained over time. Changes from baseline in SLEDAI and SDI did not differ between groups. We found no evidence of interaction between vitamin D deficiency and response to atorvastatin in change in LDL, SLEDAI or SDI over 2 years. When looking at SLEDAI and SDI by vitamin D status alone, there was a trend towards higher SLEDAI area under the curve over 3 years (65.8, 95% CI −14.9 to 146.5) and higher proportion of subjects with damage index greater than 0 (3.4%, 95% CI −1.3% to 8.0%) in those with baseline vitamin D deficiency, but this was not statistically significant. Baseline vitamin D deficiency was not a predictor of change in hsCRP over 3 years and change in vitamin D over 1 year was not associated with change in hsCRP at 1 year.

DISCUSSION
For the first time in SLE, we find that vitamin D status may affect response to atorvastatin in CVD risk and CIMT progression over time. The associations we found between vitamin D deficiency and increased age, body mass index, winter season and minority status in the APPLE paediatric

Table 2 CIMT progression in participants treated with atorvastatin or placebo for 3 years by baseline serum 25(OH)D levels (mg/dL)*

| Segment	CIMT progression with atorvastatin (mm)	CIMT progression with placebo (mm)	Interaction effect	p Value
Mean-mean CIMT				
25(OH)D ≤20	0.0014 (−0.0005, 0.0032)	0.0031 (0.0013, 0.0049)	−0.0027 (−0.0077, 0.0022)	0.275
25(OH)D >20	0.0039 (0.0013, 0.0066)	0.0084 (0.0051, 0.0117)		
Mean-max CIMT				
25(OH)D ≤20	0.0024 (−0.0004, 0.0052)	0.0031 (0.0004, 0.0057)	−0.0101 (−0.0175, −0.0027)	**0.008**
25(OH)D >20	0.0021 (−0.0019, 0.0061)	0.0129 (0.0080, 0.0178)		
Mean-mean common CIMT				
25(OH)D ≤20	0.0006 (−0.0015, 0.0027)	0.0007 (−0.0013, 0.0027)	−0.0038 (−0.0095, 0.0018)	0.182
25(OH)D >20	0.0002 (−0.0029, 0.0032)	0.0041 (0.0004, 0.0078)		
Mean-max common CIMT				
25(OH)D ≤20	0.0000 (−0.0032, 0.0032)	−0.0028 (−0.0058, 0.0003)	−0.0115 (−0.0199, −0.0031)	**0.008**
25(OH)D >20	−0.0015 (−0.0061, 0.0031)	0.0073 (0.0017, 0.0129)		
Mean-mean internal CIMT				
25(OH)D ≤20	0.0047 (0.0013, 0.0082)	0.0066 (0.0033, 0.0099)	−0.0092 (−0.0183, −0.0000)	**0.049**
25(OH)D >20	0.0057 (0.0007, 0.0106)	0.0167 (0.0107, 0.0227)		
Mean-max internal CIMT				
25(OH)D ≤20	0.0073 (0.0024, 0.0122)	0.0118 (0.0071, 0.0165)	−0.0108 (−0.0239, 0.0023)	0.104
25(OH)D >20	0.0074 (0.0003, 0.0145)	0.0227 (0.0141, 0.0314)		
Mean-mean bifurcation CIMT				
25(OH)D ≤20	0.0007 (−0.0020, 0.0033)	0.0036 (0.0011, 0.0061)	−0.0005 (−0.0075, 0.0065)	0.886
25(OH)D >20	0.0045 (0.0008, 0.0083)	0.0080 (0.0034, 0.0126)		
Mean-max bifurcation CIMT				
25(OH)D ≤20	0.0034 (−0.0008, 0.0075)	0.0034 (−0.0006, 0.0073)	−0.0143 (−0.0254, −0.0032)	**0.012**
25(OH)D >20	−0.0007 (−0.0067, 0.0052)	0.0135 (0.0062, 0.0209)		
Mean-mean far wall CIMT				
25(OH)D ≤20	0.0026 (0.0004, 0.0048)	0.0049 (0.0028, 0.0071)	−0.0021 (−0.0080, 0.0038)	0.491
25(OH)D > 20	0.0051 (0.0019, 0.0083)	0.0095 (0.0056, 0.0134)		
Mean-max far wall CIMT				
25(OH)D ≤20	0.0033 (−0.0002, 0.0068)	0.0054 (0.0020, 0.0088)	−0.0077 (−0.0171, 0.0017)	0.107
25(OH)D > 20	0.0036 (−0.0014, 0.0087)	0.0135 (0.0073, 0.0197)		
Mean-mean near wall CIMT				
25(OH)D ≤20	−0.0001 (−0.0026, 0.0024)	0.0007 (−0.0017, 0.0031)	−0.0034 (−0.0101, 0.0032)	0.312
25(OH)D > 20	0.0026 (−0.0010, 0.0062)	0.0069 (0.0025, 0.0113)		
Mean-max near wall CIMT				
25(OH)D ≤20	0.0012 (−0.0024, 0.0047)	−0.0001 (−0.0035, 0.0033)	−0.0130 (−0.0224, −0.0036)	**0.007**
25(OH)D > 20	0.0003 (−0.0048, 0.0054)	0.0121 (0.0059, 0.0183)		

Bold represents p<0.05.
*Multivariable mixed effects longitudinal modelling adjusted for lupus duration, female gender, systolic blood pressure, pubertal level, LDL cholesterol and hsCRP.
CIMT, carotid intima medial thickness; hsCRP, high-sensitivity C reactive protein; LDL, low-density lipoprotein; 25(OH)D, 25-hydroxyvitamin D.

cohort are consistent with those seen in larger epidemiological studies of the general population.[24] A higher, although not statistically significant, proportion of subjects with vitamin D deficiency were also on multivitamins, but multivitamin use in this population was high overall, and it is possible that subjects with a history of vitamin D deficiency may have been encouraged by their providers to take multivitamins. In addition, in this cohort we found notable differences at baseline between vitamin D deficient subjects and those not deficient, notably in some CVD risk measures (mean-max CIMT, LDL cholesterol hsCRP), and in SLE disease related variables (duration of SLE, SDI and proteinuria).[25]

The relationship between vitamin D status and the inflammation marker hsCRP has been previously described in adults with SLE, specifically in the LAPS study, a randomised study of atorvastatin in adults with lupus.[26] In LAPS, baseline 25(OH)D levels ≥21 ng/mL were associated with lower baseline hsCRP levels. HsCRP is associated with higher SLE disease activity measured by physician's global assessment or SLEDAI, and has been associated with increased serositis and arthritis.[27–29] In newly diagnosed patients with SLE, hsCRP levels have correlated with disease activity.[30] Inflammation is important in the pathogenesis of atherosclerosis,[31] and is an independent predictor of future stroke and myocardial infarction in the general population.[32] However, the relationship between hsCRP and CVD events in SLE is less clear. For instance, cross-sectional studies of patients with SLE evaluating hsCRP and CIMT have shown
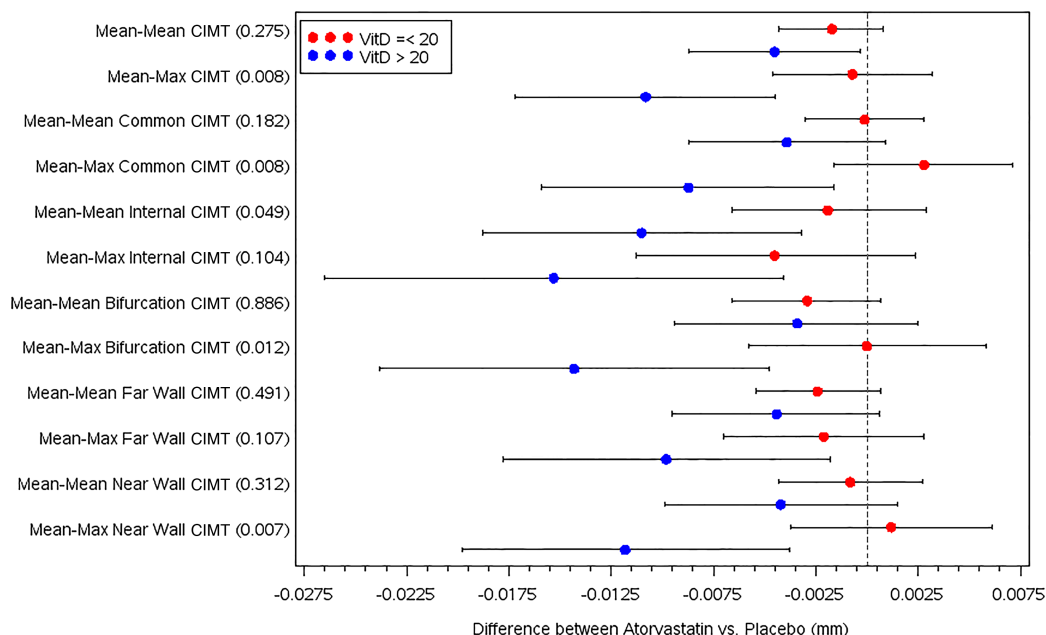
Figure 1 Forest plot of CIMT progression rate for atorvastatin treatment versus placebo for 3 years by baseline serum 25-hydroxyvitamin D status. Multivariable mixed effects longitudinal modelling adjusted for lupus duration, female gender, systolic blood pressure, pubertal level, LDL cholesterol and hsCRP. VitD, serum 25-hydroxyvitamin D status, ng/mL; CIMT, carotid intima medial thickness, in mm; hsCRP, high-sensitivity C reactive protein; LDL, low-density lipoprotein. p Values for the interaction effect are listed in parentheses on the y-axis.

inconsistent results. Secondary analysis of the APPLE cohort suggested that atorvastatin may reduce atherosclerosis prevention in pubertal patients with lupus with higher hsCRP.[33]

Studies suggest that vitamin D has potent effects on innate and acquired immunity, including modulation of T lymphocyte proliferation and function, and inhibition of Th1 cytokine expression while augmenting the anti-atherogenic Th2 cytokines.[34 35] In addition, vitamin D inhibits tumor necrosis factor (TNF)-α-induced adhesion molecule expression in endothelial cells.[36] Thus, through modulation of inflammatory cells and inflammatory cytokine secretion, low vitamin D may adversely affect the cardiovascular system in several chronic inflammatory conditions. Indeed, vitamin D receptors have broad tissue distribution that includes vascular smooth muscle, endothelium and cardiomyocytes. In vitro, activated $1,25(OH)_2D$ directly suppresses renin gene expression[37] and regulates the growth and proliferation of vascular smooth muscle cells and cardiomyocytes.[38] Vitamin D suppresses foam cell formation by reducing oxidised LDL-cholesterol uptake, suppresses CD36 expression, and improves insulin signalling.[39] Clinical studies have reported associations between lower vitamin D levels and hypertension,[23 40] low high-density lipoprotein cholesterol, coronary artery calcification,[41] increased CIMT[42 43] and prevalent CVD.[44 45] Thus, putative vascular effects of vitamin D include modulation of smooth muscle cell proliferation, inflammation, thrombosis, insulin sensitivity and blood pressure.

The findings in this study suggest that there may be interaction between vitamin D levels and response to atorvastatin in CIMT progression, especially in mean-max CIMT measurements. In general, we note that the lowest CIMT progression rates were seen in the atorvastatin-treated subgroup with serum 25(OH)D >20 ng/mL, although these numbers should be interpreted cautiously in segments where the p value of the interaction effect was greater than 0.05. In the original APPLE trial, mean-max CIMT progression was not found to be significantly different between atorvastatin and placebo groups (0.0037 mm/year in the atorvastatin group vs 0.0064 mm/year in the placebo group, p=0.083). Further analysis of the LAPS trial concluded that 25(OH)D levels were not associated with progression of coronary artery calcium or CIMT over 2 years, although the mean hsCRP decreased over the study period.[25] Difference in results between the LAPS trial secondary analyses and the present study could be due to the fact that in LAPS, no adjustments were made for atorvastatin or placebo usage, there was a shorter duration of follow-up, and only single measurements of the common carotid arteries were performed. Baseline CIMT was not controlled for in this analysis, which looked strictly at progression alone, nor was it controlled for in the original APPLE study. However, vitamin D levels and CIMT levels were not statistically different between atorvastatin and placebo groups at baseline.

Mean-mean and mean-max CIMT commonly have been used in trials of statins to moderate cardiovascular risk. In the original APPLE trial, due to slow recruitment, the primary outcome was changed during the trial to mean-mean common CIMT from mean-max

CIMT. The initial analysis showed a difference in mean-max CIMT progression over 3 years after controlling for covariates related to baseline CIMT.[18] Mean-max CIMT is thought to be more predictive of clinical cardiovascular events than mean-mean CIMT, and more strongly associated with the presence of symptomatic CVD in adults.[46] The mean-max IMT is a measure of plaque taken at the carotid bifurcation and proximal internal carotid artery (ICA), where complex oscillatory low shear stress promotes the primary deposition of LDL cholesterol in the wall. However, carotid plaque is a later effect of atherosclerosis and is not present in children and adolescents. Further research is needed to better define the biological relevance of different CIMT measures in patients with SLE compared with the general population.

In our study of APPLE trial data, the relationship between vitamin D status and mean-max CIMT progression persisted despite adjustment for multiple confounders. Evaluation of the interaction effect between atorvastatin and vitamin D levels is a strength of this analysis, especially after adjustment for factors such as duration of lupus, gender, baseline blood pressure, baseline pubertal status, baseline LDL cholesterol and baseline hsCRP.

The trend towards higher SLEDAI over 3 years, and higher proportion of participants with SDI>0 in subjects with baseline vitamin D deficiency found in this analysis was interesting, and matches with prior studies finding associations between vitamin D deficiency and increased disease activity,[8–12] although the difference did not reach statistical significance. This may be related to the fact that subjects with severe disease such as proteinuria were excluded from entry into the trial, and the cohort reported overall low SLEDAI and SDI scores.

CONCLUSIONS

APPLE participants with higher serum 25(OH)D (≥20 ng/mL) had less mean-max CIMT progression in multiple carotid segments following 3 years of atorvastatin treatment than participants receiving placebo. Results from secondary analyses must be interpreted cautiously, but these findings suggest that underlying vitamin D deficiency may negatively impact the efficacy of atorvastatin in atherosclerosis prevention. More studies are needed to determine if vitamin D replacement therapy can boost response to atorvastatin in prevention of CVD in SLE and the general population.

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