

# Does Vitamin D Modulate Asymmetric Dimethylarginine and C-Reactive Protein Concentrations?

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## ABSTRACT

**BACKGROUND:** Vitamin D deficiency is associated with significant increases in the incidence of cardiovascular risk factors and mortality. However, the mechanisms underlying this association remain unclear. The current study evaluated the possible relationships among vitamin D status, endothelial dysfunction, and inflammation.

**METHODS:** Plasma concentrations of 25-hydroxyvitamin D<sub>3</sub> were determined by radioimmunoassay in a normal population cohort (n = 253) aged 51 to 77 years (mean 63.4 ± 6 years). Asymmetric dimethylarginine, a marker/mediator of endothelial dysfunction, was assayed by high-performance liquid chromatography. High-sensitivity C-reactive protein levels were used as a marker of inflammatory activation.

**RESULTS:** On univariate analyses, low 25-hydroxyvitamin D<sub>3</sub> levels were inversely correlated with asymmetric dimethylarginine concentrations, high-sensitivity C-reactive protein levels, and body mass index. Seasonal fluctuations in 25-hydroxyvitamin D<sub>3</sub> levels were associated with reciprocal asymmetric dimethylarginine concentration fluctuations. Hypertension and treatment with an angiotensin-converting enzyme inhibitor/angiotensin receptor blocker also were associated with low 25-hydroxyvitamin D<sub>3</sub> levels. On multiple linear analysis, both asymmetric dimethylarginine ( $\beta = -0.19$ ,  $P = .003$ ) and high-sensitivity C-reactive protein ( $\beta = -0.14$ ,  $P = .03$ ) concentrations were inversely correlated with plasma 25-hydroxyvitamin D<sub>3</sub> concentrations; other significant correlates were male gender ( $\beta = 0.19$ ,  $P = .003$ ), calcium levels ( $\beta = 0.14$ ,  $P = .03$ ), and use of angiotensin-converting enzyme inhibitor ( $\beta = -0.17$ ,  $P = .007$ ).

**CONCLUSION:** Low 25-hydroxyvitamin D<sub>3</sub> levels are associated with markers of endothelial dysfunction and inflammatory activation, representing potential mechanisms for incremental coronary risk.

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**KEYWORDS:** Asymmetric dimethylarginine; Cardiovascular risk; Endothelial dysfunction; High-sensitivity C-reactive protein; Vitamin D

The “classic” effects of vitamin D are pivotal to bone development, growth, mineralization, and maintenance of skeletal integrity.<sup>1</sup> These effects also are reflected in its use

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for the treatment of metabolic bone disease. However, in the past 10 years, an array of evidence suggests that vitamin D status also is important in the cause of various chronic diseases. Vitamin D receptors have widespread tissue distribution, including endothelial cells, vascular smooth muscle cells, cardiomyocytes,<sup>1,2</sup> and most cells of the immune system.<sup>3</sup> Associated with this widespread distribution of receptors is the emergence of “new” physiologic effects, including regulation of cell proliferation/differentiation, modulation of the immune system, influence on pancreatic  $\beta$ -cell function, and regulation of cardiac contractility and hypertrophy.<sup>4</sup>

In regard to the cardiovascular system, administration of vitamin D (calcitriol) has been reported to improve cardiac

performance in patients receiving hemodialysis<sup>5</sup> and to suppress myocardial hypertrophy.<sup>6</sup> Notably, supplementation of the active vitamin D<sub>3</sub> derivative in patients receiving hemodialysis was a correlate of reduced cardiovascular mortality.<sup>7</sup> Similar suggestions have been made regarding low vitamin D status without supplementation: vitamin D deficiency has been raised as a potential explanation for higher cardiovascular morbidity and mortality in populations with reduced sun exposure for geographic/seasonal reasons.<sup>8-11</sup> However, the mechanisms underlying the putative cardioprotective effects of vitamin D are unclear. Two potential explanations relate to preservation of endothelial function,<sup>12-14</sup> or suppression of inflammation, which itself might affect endothelial function.<sup>15</sup>

Asymmetric dimethylarginine, a physiologically occurring competitive antagonist of endothelial nitric oxide synthase, is both a strong marker and a mediator of many aspects of endothelial dysfunction.<sup>16-18</sup> Asymmetric dimethylarginine seems not only to inhibit endothelial nitric oxide synthase-mediated bioconversion of arginine to release nitric oxide but also to regulate endothelial nitric oxide synthase activity under certain conditions.<sup>18</sup> No previous studies have evaluated a putative relationship between any form of vitamin D and asymmetric dimethylarginine.

The emergence of asymmetric dimethylarginine as an independent cardiovascular risk factor has paralleled that of high-sensitivity C-reactive protein.<sup>19,20</sup> Furthermore, because acute inflammation also can affect asymmetric dimethylarginine kinetics,<sup>21</sup> there is a potential interaction between implications of inflammatory activation (high-sensitivity C-reactive protein) and effects of vitamin D on asymmetric dimethylarginine kinetics. Thus, investigation of the possible impact of vitamin D status on cardiovascular outcomes, evaluation of markers of endothelial function and of inflammation is warranted.

The current study therefore tested the primary hypothesis that plasma 25-hydroxyvitamin D concentrations (25-hydroxyvitamin D<sub>3</sub>), a measure of vitamin D status, are correlated with asymmetric dimethylarginine concentrations in a randomly selected cohort of aging “normal” individuals. We also sought to determine whether 25-hydroxyvitamin D<sub>3</sub> concentrations are correlated with those of high-sensitivity C-reactive protein.

## MATERIALS AND METHODS

### Study Population

The study cohort (n = 253) represented a subset of the North Western Adelaide Health Study<sup>22</sup> aged 51 to 77 years. This

cohort of ambulant but aging individuals were initially evaluated to identify risk factors for aortic valve calcification.<sup>23</sup> Subject characteristics are summarized in Table 1. All but 1 of the subjects were white. All volunteers gave informed consent before the study. The study was approved by the Ethics of Human Research Committee of The Queen Elizabeth Hospital.

### Study Variables

All patients' cardiovascular risk factors were delineated at interview. Hypertension was defined on the basis of treatment with antihypertensive drugs or blood pressure greater than 140/80 mm Hg. Hypercholesterolemia was defined by current treatment with cholesterol-lowering drugs or a total cholesterol greater than 5.5 mmol/L. Diabetes mellitus was defined as current treatment for diabetes or a fasting blood glucose greater than 7.8 mmol/L. Known coronary artery disease was defined on the basis of patient history of coronary revascularization, history of myocardial infarction, or known significant coronary disease from previous angiogram if available. Routine transthoracic echocardiography was performed in all subjects. Left ventricular diameters and wall thicknesses were measured from 2-dimensionally guided M-mode echocardiography. Left ventricular mass index was calculated by the method described by Devereux and

### CLINICAL SIGNIFICANCE

- Vitamin D has been increasingly shown to have cardioprotective effects, but the mechanism(s) underlying this are uncertain.
- In an aging population (n = 253), plasma concentrations of vitamin D (25-hydroxyvitamin D<sub>3</sub>) varied inversely with those of asymmetric dimethylarginine (a marker/mediator of endothelial dysfunction) and C-reactive protein.
- These correlations might explain the strong associations between vitamin D deficiency and coronary risk.

and

**Table 1** Patient Characteristics

Parameter	N	%
Age (mean ± SD) (y)	63.4 ± 6.0	
BMI (kg/m <sup>2</sup> )	28.2 ± 5.0	
Gender (% male)	110/253	43.5
History of hypercholesterolemia	148/252 <sup>a</sup>	58.7
Statin therapy	81/251 <sup>a</sup>	32.1
Previous angina/MI	34/252 <sup>a</sup>	13.5
ACEI/ARB therapy	83/251 <sup>a</sup>	33.1
Hypertension	105/251 <sup>a</sup>	41.8
Family history	131/252 <sup>a</sup>	52.2
Smoking	36/252 <sup>a</sup>	14.3
Diabetes mellitus	28/251 <sup>a</sup>	11.2
Subjects with ≥ 3 cardiovascular risk factors	80/253	31.7
History of CVA	9/251 <sup>a</sup>	3.6
Calcium supplementation	42/251 <sup>a</sup>	16.7
Vitamin D supplementation	4/250 <sup>a</sup>	1.6

BMI = body mass index; MI = myocardial infarction; ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; CVA = cerebrovascular accident; SD = standard deviation.

<sup>a</sup>Indicates the number of subjects for whom the data are available.

colleagues<sup>24</sup> and indexed by height in meters raised to the 2.7 power.

Augmentation index is a measure of apparent arterial stiffness modulated by vascular endothelial function<sup>25</sup> and was measured by radial pulse-wave analysis in all patients.

Biochemical measurements were performed. In all patients, blood was collected into heparinized tubes and centrifuged at 4°C at 2700g for 20 minutes, and plasma was stored at -80°C until assay. Concentrations of asymmetric dimethylarginine in plasma were measured by high-performance liquid chromatography using the derivatization reagent AccQ-Fluor (Waters, Milford, Mass) after solid-phase extraction as previously described.<sup>26</sup> The recovery rate for asymmetric dimethylarginine was 92% ± 2%, and the detection limit of the assay was 0.1 μM. Lipid profile, high-sensitivity C-reactive protein, serum creatinine, calcium, phosphate, and 25-hydroxyvitamin D<sub>3</sub> (normal reference range 60-160 nmol/L) were assayed by commercially available radioimmunoassay after extraction (Immunodiagnostic Systems, Boldon, UK). This assay has 100% specificity for 25-hydroxyvitamin D<sub>3</sub>, with a coefficient of variation less than 8% within run and coefficient of variation less than 10% between runs. Creatinine clearance was calculated according to the Cockcroft-Gault equation and indexed for body surface area using the Dubois and Dubois formula.

## Statistical Analyses

All data are expressed as mean ± standard deviation unless otherwise stated. Normal distribution was tested for all continuous variables, and skewed data were normalized by log or square root transformation. Comparisons between groups for nonparametric data were made using the Mann-Whitney test. Correlations between transformed, continuous nonparametric data were made using linear regression. Backward multiple linear regression analyses were performed to assess independent predictors of 25-hydroxyvitamin D<sub>3</sub> levels. Parameters examined as putative correlates of 25-hydroxyvitamin D<sub>3</sub> levels were age, gender, plasma calcium, plasma phosphorus, diabetes mellitus, hypertension, use of angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, calculated creatinine clearance, body mass index, high-sensitivity C-reactive protein, augmentation index, and asymmetric dimethylarginine concentrations. These variables were included because of statistical significance on univariate analyses or as suspected clinical correlates. All analyses were performed using SPSS 13 software (SPSS Inc, Chicago, Ill), and a *P* value of less than .05 was considered to be statistically significant.

## RESULTS

### Patient Characteristics

Baseline patient characteristics are shown in Table 1. There was a high proportion of obese subjects, multiple coronary risk factors were frequently present, and there was extensive therapy with statins and angiotensin-converting enzyme inhibitor/angiotensin receptor blockers. No patient had clinically

**Table 2** Baseline Subject Biochemistry Data

	Mean	SD	Minimum	Maximum
Vitamin D (nmol/L)	72.2	24.3	22	159
LDL (mmol/L)	2.9	0.9	0.5	5.5
HDL (mmol/L)	1.3	0.3	0.6	2.9
Calcium (mmol/L)	2.24	0.15	1.61	2.75
CaxPO <sub>4</sub>	2.3	0.5	1.1	3.7
CrCl (indexed for BSA) (mL/min/1.73 m <sup>2</sup> )	90.8	30.3	22.9	176.8
hs-CRP (mg/L)	3.6	4.0	0.2	29.0
AIx (%)	27.6	8.5	1.0	50.7
ADMA (μM)	0.52	0.08	0.34	0.91

LDL = low-density lipoprotein; HDL = high-density lipoprotein; CaxPO<sub>4</sub> = calcium-phosphate product; CrCl = creatinine clearance; BSA = body surface area; hs-CRP = high-sensitivity C-reactive protein; AIx = augmentation index; ADMA = asymmetric dimethylarginine; SD = standard deviation.

significantly significant hepatic dysfunction. No patient had undergone coronary revascularization within the preceding 6 months. The prevalence of clinically overt ischemic heart disease was somewhat greater than for a comparable overall South Australian population (D. Banham, personal communication, 2009).

### Biochemistry

Biochemical findings are summarized in Table 2. According to the current literature,<sup>27-29</sup> vitamin D deficiency is generally considered to correspond to 25-hydroxyvitamin D<sub>3</sub> levels of 50 nmol/L or less. On the basis of these criteria, 46 subjects (18.5%) had vitamin D deficiency. Mean plasma asymmetric dimethylarginine concentrations were within the previously described normal range for the methodology used.<sup>26,30</sup> In 39.2% of subjects, high-sensitivity C-reactive protein was greater than 3 mg/L.

Plasma cholesterol concentrations were elevated beyond normal (>5.5 mmol/L) in 26.4% of subjects at entry. In general, renal function was well preserved. There was no patient receiving dialysis, with only 2 subjects with creatinine clearance less than 30 mL/min/1.73 m<sup>2</sup>.

### Univariate and Multivariate Results

Univariate analyses between 25-hydroxyvitamin D<sub>3</sub> levels versus continuous and categorical variables are documented in Table 3. Age, creatinine clearance, augmentation index, left ventricular mass index, and calcium-phosphate product were not significant correlates of 25-hydroxyvitamin D<sub>3</sub> levels; there was a positive trend with calcium and phosphate levels. 25-hydroxyvitamin D<sub>3</sub> levels were inversely correlated with asymmetric dimethylarginine concentrations, high-sensitivity C-reactive protein levels (Figure 1), and body mass index. The presence of hypertension and treatment with an angiotensin-converting enzyme inhibitor/angiotensin receptor blocker also were associated with low 25-hydroxyvitamin D<sub>3</sub> levels. There was no significant cor-

**Table 3** Univariate Correlates of Normalized 25-Hydroxyvitamin D<sub>3</sub> Levels

A) Continuous Univariate Correlates

	$\beta$ Coefficients	P Value
Age (y)	-0.06	.35
CrCl (mL/min/1.73 m <sup>2</sup> )	0.07	.29
BMI (kg/m <sup>2</sup> )	-0.15	.02
Ca <sup>2+</sup> (mmol/L)	0.09	.18
PO <sub>4</sub> <sup>2-</sup> (mmol/L)	-0.07	.27
CaxPO <sub>4</sub>	-0.03	.60
AIx (%)	-0.07	.31
LV mass index (g/m <sup>2.7</sup> )	0.02	.80
ADMA concentration ( $\mu$ M)	-0.21	.001
hs-CRP (mmol/L)	-0.17	.009

B) Categorical Univariate Correlates: Differences in Median Levels Were Performed Using the Mann-Whitney U Test

	Median Yes vs No	P Value
Hypertension	65.0 vs 74.1	.008
ACEI/ARB	65.0 vs 73.8	.008
Gender (male vs female)	74.5 vs 65.5	.002
Hypercholesterolemia	68.6 vs 71.0	.7
DM	63.7 vs 70.8	.26
Previous ischemia/angina	68.5 vs 69.7	.5

CrCl = creatinine clearance; BMI = body mass index; CaxPO<sub>4</sub> = calcium-phosphate product; AIx = augmentation index; LV = left ventricular; ADMA = asymmetric dimethylarginine; hs-CRP = high-sensitivity C-reactive protein; ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; DM = diabetes mellitus.

relation between plasma asymmetric dimethylarginine concentrations and high-sensitivity C-reactive protein levels.

In view of the known correlation between 25-hydroxyvitamin D<sub>3</sub> levels and sunlight exposure,<sup>8-11</sup> we evaluated seasonal fluctuations in 25-hydroxyvitamin D<sub>3</sub> levels in comparison with those of asymmetric dimethylarginine and high-sensitivity C-reactive protein. There was significant seasonal variability ( $P = .005$ , Kruskal-Wallis test) in 25-hydroxyvitamin D<sub>3</sub> and asymmetric dimethylarginine concentrations with reciprocal variation between these (Figure 2). High-sensitivity C-reactive protein levels showed no significant seasonal variability (data not shown).

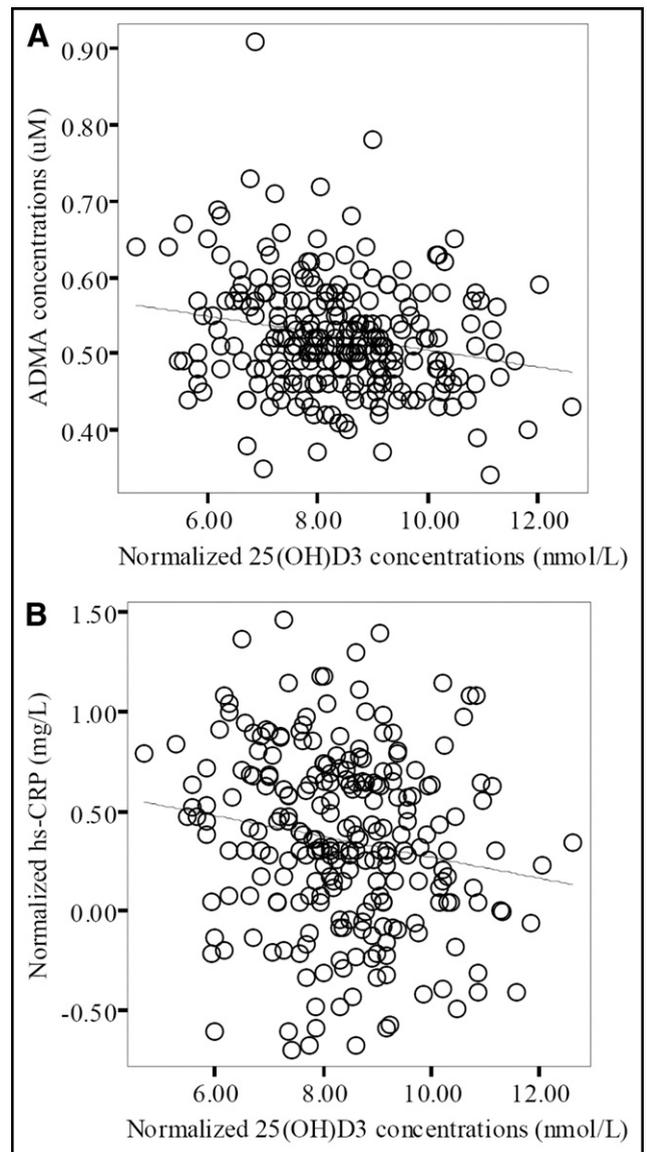
On backward multiple linear analysis (Table 4), direct correlates of high 25-hydroxyvitamin D<sub>3</sub> levels that remained were male gender ( $P = .003$ ), calcium levels ( $P = .03$ ), absence of angiotensin-converting enzyme inhibitor/angiotensin receptor blocker ( $P = .007$ ), low high-sensitivity C-reactive protein ( $P = .03$ ), and low asymmetric dimethylarginine concentrations ( $P = .003$ ).

**DISCUSSION**

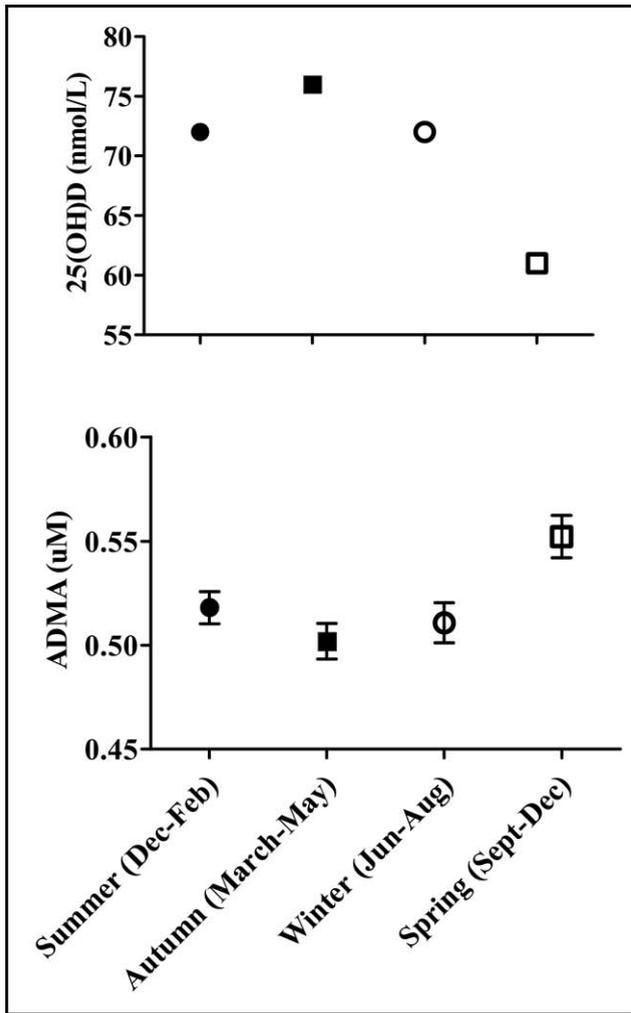
The results of this study, conducted in an aging predominantly white population, suggest for the first time that low 25-hydroxyvitamin D<sub>3</sub> concentrations are associated with

increases in both asymmetric dimethylarginine concentrations and high-sensitivity C-reactive protein levels in this random, cross-sectional Western population. Both high-sensitivity C-reactive protein and asymmetric dimethylarginine have been implicated as markers of cardiovascular risk.<sup>20,31</sup>

There is increasing evidence that low 25-hydroxyvitamin D<sub>3</sub> concentrations are associated with increased cardiovascular risk.<sup>27,32-34</sup> In turn, this newly established nexus between low levels of 25-hydroxyvitamin D<sub>3</sub> and cardiovascular events raises the issue of underlying mechanism(s). For example, vitamin D levels have been correlated both with various cardiac risk factors and with cardiac events.



**Figure 1** Correlation of 25-hydroxyvitamin D<sub>3</sub> levels with (A) plasma asymmetric dimethylarginine concentrations (there was an inverse correlation with 25-hydroxyvitamin D<sub>3</sub> levels:  $\beta = -0.21$ ,  $P = .001$ ) and (B) high-sensitivity C-reactive protein levels (there also was an inverse correlation with 25-hydroxyvitamin D<sub>3</sub> levels:  $\beta = -0.17$ ,  $P = .009$ ). ADMA = asymmetric dimethylarginine; hs-CRP = high-sensitivity C-reactive protein.



**Figure 2** Seasonal variability of (A) 25-hydroxyvitamin D<sub>3</sub> and (B) asymmetric dimethylarginine plasma concentrations. Median values are provided for 25-hydroxyvitamin D<sub>3</sub> concentrations; mean ± standard error of the mean for asymmetric dimethylarginine concentrations. ADMA = asymmetric dimethylarginine.

Martins et al<sup>32</sup> demonstrated inverse associations between vitamin D (25-hydroxyvitamin D<sub>3</sub>) levels and incidence of obesity, hypertension, and diabetes. The Framingham Offspring Study<sup>33</sup> found that vitamin D (25-hydroxyvitamin D<sub>3</sub>) deficiency is associated with incremental risk of cardiovascular events irrespective of preexisting cardiovascular risk factors. Furthermore, low vitamin D status (25-hydroxyvitamin D<sub>3</sub>) is associated with increased risk of myocardial infarction independently of conventional cardiovascular risk factors<sup>34</sup> and all-cause and cardiovascular mortality.<sup>27</sup> With the emergence of these epidemiologic findings, the potential importance of vitamin D status to cardiovascular disease/outcomes is apparent; mechanistic insights of these findings remain largely elusive.

Elevation of asymmetric dimethylarginine concentrations alone offers a potential mechanism for increased cardiovascular event rates. We recently reviewed the clinical significance of small increases in plasma asymmetric dimethylarginine concentrations.<sup>30</sup>

For example, Schnabel et al,<sup>31</sup> examining a population with known coronary artery disease, found that an increase in asymmetric dimethylarginine concentrations of 0.21 μmol/L was associated with an approximately 2.5-fold increase in myocardial infarction and cardiovascular mortality rates. In a population of asymptomatic middle-aged men, Valkonen et al<sup>35</sup> found that after logistic regression modeling there was a 27-fold increase in coronary risk associated with a 0.1 μmol/L increase in asymmetric dimethylarginine.

A number of previous experimental findings raise the possibility that vitamin D may increase activity and expression of endothelial nitric oxide synthase (or nitric oxide synthase III), the enzyme critical to the generation and bioavailability of nitric oxide. For example, treatment of endothelial cells with calcitriol significantly reversed advanced glycation end product-induced down-regulation of endothelial nitric oxide synthase mRNA and activity;<sup>36</sup> aortic endothelial nitric oxide synthase expression and urinary nitrate/nitrite excretion were reduced in vitamin D receptor knockout mice.<sup>37</sup> Clinically, however, evidence of a relationship between low vitamin D levels and endothelial dysfunction is currently limited: In a small study of patients with end-stage renal disease receiving hemodialysis, serum concentrations of 25-hydroxyvitamin D<sub>3</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub> correlated directly with measures of intact vascular/endothelial function, including flow-mediated dilatation.<sup>38</sup> Furthermore, a single, high dose of vitamin D<sub>2</sub> improved flow-mediated dilatation in a small number of patients with type 2 diabetes.<sup>15</sup> Thus, there is intriguing evidence that vitamin D might beneficially modulate vascular endothelial function. To date, the only mechanism proposed for this is augmentation of endothelial nitric oxide synthase expression and activity.

The mechanism of the nexus between low 25-hydroxyvitamin D<sub>3</sub> concentrations and increased asymmetric dimethylarginine concentrations was not explored in the current study and does not necessarily reflect inhibition of endothelial nitric oxide synthase alone. One potential additional mechanism might involve activation of the renin-angiotensin system. Li et al<sup>39</sup> demonstrated that 1,25(OH)<sub>2</sub>D<sub>3</sub> suppresses renin biosynthesis. In vitamin D receptor null mice, renin expression and

**Table 4** Variables Independently Associated with High 25-Hydroxyvitamin D<sub>3</sub> Levels after Backward Multiple Linear Regression Analysis

	β Coefficient	P Value
Male gender	0.19	.003
Calcium levels (mmol/L)	0.14	.03
Presence of ACEI/ARB	-0.17	.007
hs-CRP (mg/L)	-0.14	.03
ADMA concentrations (μM)	-0.19	.003

ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; hs-CRP = high-sensitivity C-reactive protein; ADMA = asymmetric dimethylarginine.

plasma angiotensin II levels are elevated,<sup>39</sup> corresponding to an increase in water and sodium retention and development of hypertension in these mice. This also potentially explains the previously described link<sup>40</sup> between low 25-hydroxyvitamin D<sub>3</sub> status and development of hypertension; however, in the currently evaluated population, most cases of hypertension have been treated by angiotensin-converting enzyme inhibitor or angiotensin receptor blocker.

Given the demonstrated inverse association between 25-hydroxyvitamin D<sub>3</sub> and asymmetric dimethylarginine concentrations, we examined potential seasonal variability. In fact, 25-hydroxyvitamin D<sub>3</sub> concentrations demonstrated marked seasonal fluctuation, with highest concentrations reported in autumn: asymmetric dimethylarginine concentrations varied in a reciprocal manner as shown in Figure 2, these variations being substantially greater than normal week-to-week fluctuations in asymmetric dimethylarginine concentrations.<sup>30</sup> Thus, these changes in asymmetric dimethylarginine concentrations might contribute to seasonal fluctuations in cardiovascular event rates,<sup>8-11</sup> and these findings remain consistent with the suggested regulatory function of 25-hydroxyvitamin D<sub>3</sub> over asymmetric dimethylarginine kinetics.

The other major finding in this study was the correlation between low levels of 25-hydroxyvitamin D<sub>3</sub> and elevated high-sensitivity C-reactive protein, suggesting that 25-hydroxyvitamin D<sub>3</sub> has protective effects on inflammation, which has been demonstrated to be a fundamental precursor of atheromatous plaque rupture.<sup>41</sup> Similar relationships have been reported by others.<sup>27,42</sup> Dobnig et al<sup>27</sup> also found that low 25-hydroxyvitamin D<sub>3</sub> levels were associated with significant elevation of markers of cell adhesion, namely, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1. In a study of patients with heart failure, Forman et al<sup>40</sup> found that although vitamin D<sub>3</sub> supplementation did not lower C-reactive protein specifically, it did lead to improvements in other inflammatory markers, such as interleukin-10 and tumor necrosis factor- $\alpha$ . In cardiac transplant recipients<sup>41</sup> and patients with moderate- to high-risk cardiac disease in general,<sup>42</sup> elevated C-reactive protein levels independently predicted low calcitriol levels, which in turn were predictors of 1-year mortality. In addition, we also demonstrated that the association between 25-hydroxyvitamin D<sub>3</sub> and high-sensitivity C-reactive protein was independent of the elevation of asymmetric dimethylarginine concentrations, suggesting that different mechanisms of association might apply. Although the mechanisms whereby 25-hydroxyvitamin D<sub>3</sub> status affects high-sensitivity C-reactive protein levels are uncertain, experimental studies have shown that calcitriol can suppress nuclear factor kappaB<sup>36</sup> and release of tumor necrosis factor- $\alpha$ , all active participants of inflammation.

## LIMITATIONS

A limitation of the current study is its cross-sectional nature, and caution must be exercised in interpreting the associations delineated. For example, the association between high

25-hydroxyvitamin D<sub>3</sub> levels and absence of angiotensin-converting enzyme inhibitor/angiotensin receptor blocker therapy is counterintuitive and likely to be confounded by the concordance (96.4%) between such therapy and presence of hypertension  $\pm$  diabetes. We did not specifically take into account subjects' mobility status, sun exposure, and geographic location, and the season during which the participants attended their study visits. However, these volunteers were virtually all white, non-institutionalized, and sufficiently mobile to attend the assessment clinic. Furthermore, because we took volunteers specifically around the Western Adelaide Health Area, interpatient variability in regard to sun and environmental exposures is likely to be small. There was no seasonal variability in proportional recruitment.

## CONCLUSIONS

In this cross-sectional population study, aging subjects with low 25-hydroxyvitamin D<sub>3</sub> status had higher asymmetric dimethylarginine and high-sensitivity C-reactive protein concentrations. These associations were robust and present after adjustment for both statistical and clinical confounders. These findings therefore suggest that endothelial and inflammatory activation could explain the recently established nexus among low 25-hydroxyvitamin D<sub>3</sub> status and cardiovascular risk. It is therefore appropriate to test the hypothesis that vitamin D supplementation normalizes asymmetric dimethylarginine and high-sensitivity C-reactive protein concentrations.

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