Letter to the Editor

Vitamin D Supplementation Is Associated With Stabilization of Cardiac Autonomic Tone in IgA Nephropathy

Michelle C. Mann, Brenda R. Hemmelgarn, Derek V. Exner, David A. Hanley, Tanvir C. Turin, David C. Wheeler, Darlene Y. Sola, Linda Ellis, Sofia B. Ahmed

To the Editor:

Vitamin D deficiency is a cardiovascular risk factor. Deficiency in both 25-hydroxy vitamin D, the barometer of vitamin D status, and the more biologically active 1,25-dihydroxy vitamin D is associated with increased risk of sudden cardiac death (SCD), particularly in the chronic kidney disease (CKD) population. Studies have suggested that vitamin D supplementation may alter SCD risk by influencing activity of the cardiac autonomic nervous system, although this phenomenon has not been studied in the CKD population.

Assessment of heart rate variability, including derivations of cardiac autonomic tone (CAT), provides predictive measures of cardiovascular risk, namely through quantification of the electric output of the cardiac autonomic nervous system. Patients with CKD show a marked withdrawal of parasympathetic tone, a strong indicator of loss of cardioprotective control and a risk factor for SCD. Alterations in CAT are attenuated by renin-angiotensin system (RAS)-interrupting medications, such as angiotensin-converting enzyme inhibitors. Given that vitamin D is a negative regulator of the RAS and supplementation with oral vitamin D is associated with improvements in CAT in healthy humans, we sought to determine whether oral vitamin D supplementation was associated with a similar improvement in CAT in humans with CKD due to IgA nephropathy.

This study was approved by the Conjoint Health Research Ethics Board at the University of Calgary, and all subjects provided written informed consent. Fifteen subjects (87% men, 41±4 years) with IgA nephropathy (estimated glomerular filtration rate: 101±7 mL/min per 1.73 m²; proteinuria: 1.03±0.3 g/d) were studied after an overnight fast in high-salt balance before and after 28 days of vitamin D₃ supplementation (10,000 IU/d). CAT, quantified by spectral analysis of heart rate variability (low-frequency power [LF], high-frequency power [HF], and overall cardiac autonomic activity [LF:HF]), was measured at baseline and in response to a graded angiotensin-II (Ang-II) challenge (3 ng/kg per min×30 min, 6 ng/kg per min; recovery). Before supplementation, individuals with levels of 1,25-dihydroxy vitamin D below the median value of 105 pmol/L demonstrated a decline in parasympathetic activity in response to increasing doses of Ang-II challenge between subjects. Changes in 25-hydroxy and 1,25-dihydroxy vitamin D levels on each study day were significant factors in association with differences in CAT responses to Ang-II challenge between subjects. Increases in 25-hydroxy and 1,25-dihydroxy vitamin D levels from pre–vitamin D₃ supplementation to post–vitamin D₃ supplementation study days were assessed independently and together as a multiplicative variable to determine the magnitude of the association between the individual vitamin D responses to supplementation and changes in Ang-II challenge responses within each subject. Greenhouse–Geisser corrections were made where appropriate. The following covariates were included: age, race, sex, baseline estimated glomerular filtration rate, and baseline heart rate. On the basis of the work of Kontopoulos et al, a sample size of 15 was calculated to achieve 90% power to detect a 1-SD increase in baseline measures of HF before and after vitamin D₃ supplementation. All statistical analyses were performed using SPSS (version 19; IBM), with 2-tailed significance levels of 0.05.

Levels of 25-hydroxy vitamin D significantly increased (63±7 versus 136±12 nmol/L; P<0.001) but not the levels of 1,25-dihydroxy vitamin D (107±9 versus 126±13 pmol/L; P=0.14) with vitamin D₃ supplementation. No changes in mineral metabolism parameters or circulating RAS components were observed with supplementation. No changes were observed in resting CAT with vitamin D₃ supplementation (LF, P=0.7; HF, P=0.4; LF:HF, P=0.2; all values pre–vitamin D₃ supplementation versus post–vitamin D₃ supplementation; Table). Compared with presupplementation, vitamin D₃ supplementation was associated with stabilization of the LF:HF response to Ang-II (Table). Before supplementation, LF:HF fluctuated in response to Ang-II and during recovery (Figure). In comparison, after vitamin D₃ supplementation, subjects displayed significantly decreased LF:HF in response to graded Ang-II infusion and during recovery (∆LF:HF: 3 ng/kg per min Ang-II, −0.21±0.14, P=0.04; 6 ng/kg per min Ang-II, −0.21±0.11, P=0.03; recovery, −0.14±0.07, P=0.035 versus presupplementation response; Figure), largely because of a decline in measures of LF, most notably during recovery (∆LF: 0.014 versus presupplementation response). Blood pressure and circulating RAS components responded to Ang-II as expected, although no differences in any parameters were observed before and after vitamin D₃ supplementation.

Secondary analyses with repeated measures ANOVA showed that intrindividually (independent) differences in 1,25-dihydroxy vitamin D levels appeared to account for the observed divergence in CAT responses over the 3 time points during and after Ang-II challenge (3 ng/kg per min, 6 ng/kg per min; recovery). Before supplementation, individuals with levels of 1,25-dihydroxy vitamin D below the median value of 105 pmol/L demonstrated a decline in parasympathetic tone throughout Ang-II challenge and recovery, whereas those with 1,25-dihydroxy vitamin D levels above 105 pmol/L showed a favorable and graded increase in cardioprotective parasympathetic activity in response to increasing doses of Ang-II throughout the study irrespective of 25-hydroxy vitamin D levels. After 4 weeks of oral vitamin D₃ supplementation, 1,25-dihydroxy vitamin D levels above the postsupplementation median value (120 pmol/L) remained strongly associated with the same significant trend of steadily increasing parasympathetic activity in response to Ang-II challenge and recovery (HF: 6.96; df=1; P=0.039).
In a post hoc analysis, baroreflex sensitivity was analyzed in a subgroup of subjects (n=5; 1 woman and 4 men) with adequate baroreflex sensitivity measurement as a secondary measure of parasympathetic activity. Although not statistically significant, there was a trend toward increased baroreflex sensitivity after vitamin D3 supplementation as throughout Ang-II challenge, baroreflex sensitivity followed the same trend as measured HF (Table).

To the best of our knowledge, this is the first study investigating the effect of vitamin D supplementation on CAT, a potentially modifiable marker of cardiovascular risk, in humans with CKD. Our key findings are as follows: (1) vitamin D supplementation was associated with favorable changes in LF:HF during and after a physiological stressor in subjects with IgA nephropathy and early CKD and (2) 1,25-dihydroxy, rather than 25-hydroxy vitamin D, levels were associated with a graded increase in cardioprotective parasympathetic activity in response to increasing doses of Ang-II. A decrease in parasympathetic activity contributes to SCD risk,7 and previous studies have suggested that 1,25-dihydroxy vitamin D may be the most robust marker of cardiovascular risk in the CKD population.1,4 Together with our present findings, these observations suggest that vitamin D supplementation, with the specific goal of increasing 1,25-dihydroxy vitamin D levels and improving overall cardiac autonomic activity, may be a potential preventive cardiovascular therapy in this high-risk population. Interestingly, we did not find that vitamin D, supplementation was associated with a change in resting (baseline) heart rate variability parameters. Our findings suggest that increasing vitamin D levels are associated with improved modulation of CAT in response to an Ang-II stressor, perhaps highlighting an important interaction between vitamin D and cardiovascular risk specific to populations with increased RAS activity, such as CKD.

Previous studies have suggested that vitamin D, and specifically 1,25-dihydroxy vitamin D, affects the cardiac autonomic system in several ways, including enhancement of electrophysiological β-adrenergic signaling between cardiac myocytes.5 1,25-dihydroxy vitamin D crosses the blood–brain barrier and therefore may have physiological implications at the molecular level within the nervous system.5 In support of our findings, a large cross-sectional study of patients with end-stage kidney disease on hemodialysis reported a positive association between 25-hydroxyvitamin D levels and all-cause and cardiovascular mortality, but that this relationship was no longer observed in patients treated with analogues of 1,25-dihydroxy vitamin D,4 suggesting that cardioprotection is mediated by the activated form of vitamin D. Similarly, Dobnig et al1 reported that serum levels of 1,25-dihydroxy vitamin D were independently associated with cardiovascular mortality in a large cohort of patients undergoing coronary angiogram. Our observations of an increase in cardioprotective parasympathetic activity (HF) in response to Ang-II challenge observed only in those subjects with higher levels of 1,25-dihydroxy vitamin D support the findings from these larger studies.

This study has limitations. In humans, vitamin D metabolism is related to that of other minerals and hormones that might influence cardiovascular risk, including parathyroid hormone.2 Inclusion of these other variables did not significantly change our results, perhaps because of the narrow and clinically insignificant ranges observed in the participants of

### Table. Cardiac Autonomic Tone at Baseline and in Response to Angiotensin-II, Pre–Vitamin D₃ supplementation vs Post–Vitamin D₃ Supplementation

<table>
<thead>
<tr>
<th>Cardiac Autonomic Parameter</th>
<th>Baseline 3 ng/kg per min</th>
<th>6 ng/kg per min</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF</td>
<td>63±5</td>
<td>69±5</td>
<td>63±3</td>
</tr>
<tr>
<td>Postsupplementation</td>
<td>66±4</td>
<td>65±3</td>
<td>65±4</td>
</tr>
<tr>
<td>HF</td>
<td>27±4</td>
<td>32±5</td>
<td>32±3*</td>
</tr>
<tr>
<td>Postsupplementation</td>
<td>32±4</td>
<td>35±3</td>
<td>37±3*</td>
</tr>
<tr>
<td>LF:HF</td>
<td>1.61±0.2</td>
<td>1.72±0.1*</td>
<td>1.51±0.1†</td>
</tr>
<tr>
<td>Postsupplementation</td>
<td>1.60±0.2</td>
<td>1.39±0.08‡</td>
<td>1.39±0.09*</td>
</tr>
<tr>
<td>BRS, ms/mm Hg§</td>
<td>4.53±1.9</td>
<td>6.87±2.3</td>
<td>6.44±1.3</td>
</tr>
<tr>
<td>Postsupplementation</td>
<td>10.5±1.9</td>
<td>8.14±2.6</td>
<td>7.08±2.1</td>
</tr>
</tbody>
</table>

All data are expressed as mean±SE. BRS indicates baroreflex sensitivity; HF, high frequency; LF, low frequency; and LF:HF, low- to high-frequency ratio.

*P<0.05 vs response at baseline.
†P<0.05 vs response at 3 ng/kg per min angiotensin-II dose.
‡P<0.05 postsupplementation vs presupplementation at the same angiotensin-II dose.
§Subgroup analysis, n=5.

In a post hoc analysis, baroreflex sensitivity was analyzed in a subgroup of subjects (n=5; 1 woman and 4 men) with adequate baroreflex sensitivity measurement as a secondary measure of parasympathetic activity. Although not statistically significant, there was a trend toward increased baroreflex sensitivity after vitamin D₃ supplementation as throughout Ang-II challenge, baroreflex sensitivity followed the same trend as measured HF (Table).

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### Figure. Cardiac autonomic response to angiotensin-II (Ang-II) challenge: pre–vitamin D₃ supplementation vs post–vitamin D₃ supplementation. Error bar: ±1SE. *P<0.05 post–vitamin D₃ supplementation vs pre–supplementation response at the same Ang-II dosage; error bar: ±1SE. LF:HF indicates low frequency to high frequency ratio.
our study. Although 1,25-dihydroxy vitamin D has a short $t_1/2$, all serum analyses were conducted at the same central laboratory, thus ensuring the highest accuracy and eliminating intra-assay variability.

We have shown that humans with CKD secondary to IgA nephropathy, but who are otherwise healthy, demonstrate more stable and favorable CAT responses to an acute physiological stressor after oral vitamin D supplementation, a phenomenon primarily observed in subjects with greater levels of 1,25-dihydroxy vitamin D. The potential for activated vitamin D as a therapy in mitigating SCD risk in the CKD population merits attention.

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Disclosures
None.

References
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