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Daily Supplementation with 4000 IU Vitamin D3 for Three Years Does Not Modify Cardiovascular Risk Markers in Patients with Advanced Heart Failure: The Effect of Vitamin D on Mortality in Heart Failure Trial

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Keywords
Vitamin D · Cardiovascular risk markers · Dyslipoproteinemia · Fetuin-A · Matrix-gla-protein

Abstract
Background/Aims: We aimed to investigate the effect of a moderately high vitamin D dose on lipid parameters and biochemical markers of vascular calcification (VC) in patients with established cardiovascular disease. ***Methods:*** We included in this pre-specified secondary analysis of a randomized controlled trial 161 patients with advanced heart failure and 25-hydroxyvitamin D (25OHD) concentrations <75 nmol/L (vitamin D group: $n = 80$; placebo group: $n = 81$), who received a daily vitamin D_3 supplement of 4,000 IU for 3 years. We assessed between-group differences of the lipid parameters total-cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, and triglycerides, and the VC markers fetuin-A and non-phosphorylated undercarboxylated matrix gla protein (MGP) at study termination, with adjustment for baseline values. ***Results:*** Lipid parameters, the percentage of patients with dyslipoproteinemia, and VC markers did not differ significantly between groups at study termination (p values: 0.395–0.939). Likewise, vitamin D achieved no significant treatment effect on these markers in subgroup analyses in patients with 25OHD concentrations <30 nmol/L, nonusers of lipid-lowering drugs, or diabetic patients (p values: 0.245–0.998). ***Conclu-***

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sion: Our data indicate that vitamin D does not improve the lipid profile and does not influence the calcification inhibitors fetuin-A and non-phosphorylated undercarboxylated MGP in patients with advanced heart failure.

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Introduction

Dyslipidemia is an important predictor of cardiovascular disease (CVD) [1] and lowering atherogenic lipoproteins is an established therapeutic strategy to prevent CVD events [2]. Observational studies indicate that a poor vitamin D status is associated with high triglycerides concentrations and an unfavorable low-density lipoprotein (LDL)-cholesterol or total-cholesterol to high-density lipoprotein (HDL)-cholesterol ratio [3]. Results of randomized controlled trials (RCTs) regarding vitamin D and lipid metabolism are, however, conflicting: A meta-analysis of published RCTs showed a significant increase in LDL-cholesterol but no effect on triglycerides by vitamin D supplementation [4], whereas another meta-analysis in patients with non-insulin-dependent diabetes mellitus showed a significant vitamin D-induced decrease in total-cholesterol and LDL-cholesterol and a non-significant decrease in triglycerides [5]. Notably, the vast majority of the RCTs included into the 2 meta-analyses were only short-term studies (duration: <6 months), whereas data regarding long-term effects of vitamin D supplementation on lipid parameters are scarce.

Similar to dyslipidemia, vascular calcification (VC) is a predictor of CVD [6]. VC is an actively regulated process where death and damage of vascular smooth muscle cells (VSMCs) occur, with the transformation of VSMCs into osteoblast-like cells and observed deficiencies in calcification inhibitors [7]. It has been assumed that vitamin D deficiency promotes VSMCs differentiation into osteoblast-like cells through the suppression of several inhibitors of VC such as matrix Gla protein (MGP) and fetuin-A [8]. Both substances are able to make up a complex with calcium and phosphate, thereby transporting and clearing the insoluble calcium-phosphate salt, and preventing its extra skeletal deposition [9]. MGP contains 5 glutamic acid-residues and gammacarboxylation of these glutamic acid-residues is responsible for its inhibitory effect on VC, whereas high concentrations of non-phosphorylated undercarboxylated, and thus inactive MGP (dp-ucMGP) is considered to be a risk factor/marker for VC, disease severity, and mortality in patients with chronic heart failure [10, 11]. In experimental animals, the deletion of the vitamin D receptor as well as diets low in vitamin D content stimulated osteoblast-like transformation of VSMCs and aortic calcification [12, 13]. In humans, data about the effects of vitamin D deficiency on biomarkers of VC are scant: Administration of the precursor of the active form of vitamin D, alfacalcidol, may increase fetuin-A concentrations in hemodialysis patients [14]. In postmenopausal women, beneficial vitamin D effects, which occurred in combination with vitamin K administration on the elastic properties of the vessel wall, were attributed to its effect on gammacarboxylated MGP concentrations [10]. However, the relationship of vitamin D status with dp-ucMGP concentrations is unclear at present.

The present study therefore aimed to investigate in a cohort of patients with established CVD and inadequate vitamin D status the effect of a daily vitamin D_3 dose of 4,000 IU for 3 years on cardiovascular risk markers such as lipid parameters and biochemical risk markers of VC.

Materials and Methods

Study Design and Patients

To answer our research questions, we did a pre-specified secondary analysis of the effect of vitamin D on mortality in heart failure (EVITA) trial. EVITA is a randomized, placebo-controlled, single-center study performed at the Heart and Diabetes Center North Rhine-Westphalia, Germany (geographic latitude: 52° N). Four hundred patients with advanced heart failure and 25-hydroxyvitamin D (25OHD) concentrations <75 nmol/L were recruited between November 2010 and July 2013. Patients were supplemented with either 4,000 IU vitamin D_3 daily (the upper tolerable intake level of the European Food Safety Authority and the North American Institute of Medicine for vitamin D) or placebo for 3 years. The main results of the EVITA trial have already been published elsewhere [15]. Of the 400 patients enrolled, 75 patients died, 113 dropped out, and 35 were lost to follow-up (Fig. 1). Sixteen patients provided insufficient sample volume for the present analyses at baseline or study termination (36-month visit). Thus, data analysis was performed in 161 patients, of whom 80 were assigned to the vitamin D group and 81 to the control group. In an additional number of 89 samples, the volume was insufficient to analyze dp-ucMGP concentrations, whereas all other outcome parameters were available in the above described sample of 161 patients. Therefore, dp-ucMGP measurements were restricted to a subset of 70 pairs at baseline and study termination (vitamin D group: $n = 38$; placebo group: $n = 32$). During the study, all patients remained on guideline-recommended medications [16].

Biochemical Analyses

Blood samples were drawn in the morning between 7 and 11 am after an overnight fast. 25OHD was analyzed on the day of blood drawing. The DiaSorin autoanalyzer (DiaSorin, Stillwater, MN, USA) was used to measure 25OHD. Aliquots of blood samples for the other measurements were stored at –80 °C until analysis. The following parameters were measured for this pre-specified

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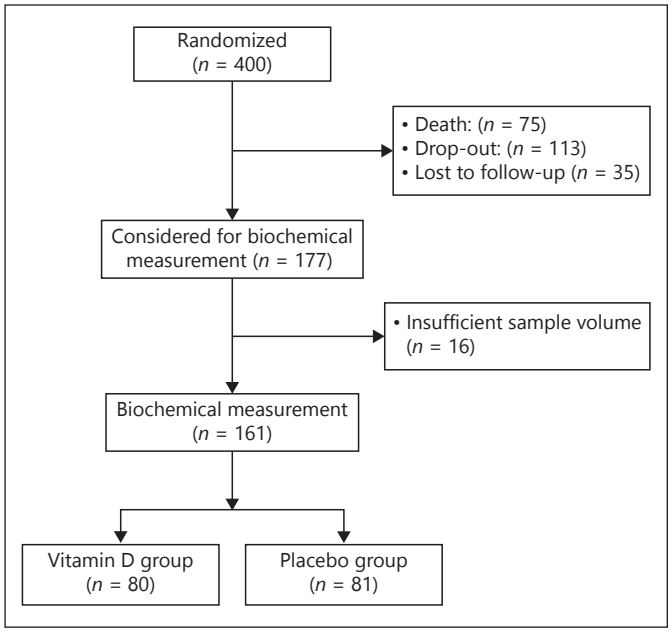

Fig. 1. Study flowchart.

data analysis at baseline and study termination: total-cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, fetuin-A, and dp-ucMGP. Lipid parameters were assessed using the Architect auto-analyzer (Abbott, Wiesbaden, Germany). Fetuin-A and dp-ucMGP were analyzed by ELISA test kit provided by IBL (Fetuin-A: IBL International GmbH, Hamburg, Germany) and IDS (dp-ucMGP: IDS Immunodiagnostic Systems GmbH, Frankfurt/M, Germany) respectively. We used the following cutoff values for classifying lipid parameters to be associated with a low atherosclerosis risk: total-cholesterol: <220 mg/dL, LDL-cholesterol: <150 mg/L, HDL-cholesterol: >55 mg/dL (males) and > 65 mg/dL (females), total-cholesterol/HDL-cholesterol ratio <4, LDL-cholesterol/HDL-cholesterol ratio <3.5, and triglycerides <150 mg/dL. Regarding fetuin-A and dp-ucMGP, the reference range according to the manufacturer was 0.35–0.95 g/L and <750 pmol/L respectively. For dp-ucMGP, the limit of quantitation was 300 pmol/L and values below this concentration were considered 290 pmol/L. Intra-assay and inter-assay coefficients of variation were according to the manufacturers for fetuin-A <6 and <7%, respectively, and for dp-ucMGP <6 and <8%, respectively.

Outcome Measures

In the present analysis of the EVITA trial, we assessed between-group differences of the lipid parameters total-cholesterol, HDL-cholesterol, LDL-cholesterol, total-cholesterol/HDL-cholesterol ratio, LDL-cholesterol/HDL-cholesterol ratio and triglycerides, and the VC parameters fetuin-A and dp-ucMPG at study termination, with adjustment for baseline values.

Statistics

Categorical variables are reported as a percentage of observations. Since most continuous parameters were not normally distributed, as checked by the Kolmogorov-Smirnov test, all continuous variables are presented as median with 25th and 75th percentiles, unless otherwise stated. Fisher's exact test and the Mann-Whitney U-test were used for group comparison at baseline, when appropriate. ANCOVA was used to test for differences in biochemical parameters between the vitamin D and placebo groups at the 36-month follow-up visit. Results were adjusted for baseline values. Skewed variables were normalized by log(e) transformation before use in ANCOVA, but all results are shown in the original units. Treatment effects are shown as mean and 95% CI of the mean. We also performed subgroup analyses in patients with circulating 25OHD concentrations below 30 nmol/L, non-users of lipid-lowering drugs, and diabetic patients. In addition, data were analyzed in male and female patients separately. p values <0.05 (2-sided) were considered statistically significant.

To account for multiple testing (i.e., 48 ANCOVA tests of between-group differences in biochemical outcome variables, 8 in the entire cohort and 40 in the subgroups of statin non-users, diabetic patients, patients with 25OHD concentrations below 30 nmol/L, and male and female patients), the Benjamini and Hochberg false discovery rate method was considered to adjust the p values as previously described [17]. The false recovery rate was set at 5%. We performed all analyses using IBM SPSS Statistics version 23 (IBM Corporation, Armonk, NY, USA).

Results

Baseline Characteristics

Most patients were male and almost all patients were affected either by dilated or ischemic cardiomyopathy (Table 1). The majority of patients had left ventricular ejection fraction values below 30%. The prevalence of diabetes mellitus and chronic kidney disease stage III or higher was 23 and 28% respectively. Medication prescription was in line with current guidelines [16], thereby reflecting the severity of the disease. More than 50% of patients were taking lipid-lowering drugs (statins: >99%; omega-3-fatty acids or fibrates: <5%).

Vitamin D Effects on CVD Parameters

At baseline, median total- and LDL-cholesterol concentrations were within the reference range in both study groups, whereas median HDL-cholesterol concentrations were below and triglycerides concentrations above its respective reference range (Table 2). Initially, the median ratio of total- to HDL-cholesterol was slightly above its reference range, whereas the LDL- to HDL-cholesterol ratio was within its reference range in both study groups. Baseline parameters of VC were within its reference range in both study groups.

There were no significant differences in concentrations of lipid parameters and concentrations of VC risk markers between the vitamin D and placebo group at

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Table 1. Baseline characteristics of the study groups

Parameter	Vitamin D group (n = 80)	Placebo group (n = 81)	p value
Age, years	55.5 (48.0–61.0)	54.0 (46.0–58.0)	0.078
Gender, males, n (%)	68 (85.0)	763 (77.8)	0.239
BMI, kg/m^2	28.5 (25.2–31.4)	28.0 (25.5–31.4)	0.981
Diagnosis, n (%)			
Dilated cardiomyopathy	33 (41.3)	40 (49.4)	0.300
Ischemic cardiomyopathy	44 (55.0)	35 (43.2)	0.135
Others	3 (3.8)	6 (7.4)	0.312
LVEF <30%, n (%)	41 (51.2)	46 (56.8)	0.481
Arterial hypertension, n (%)	20 (25.0)	25 (30.9)	0.407
Diabetes mellitus, n (%)	23 (28.7)	14 (17.3)	0.084
eGFR <60 mL/min/1.73 m^2, n (%)	26 (32.5)	20 (24.7)	0.273
25OHD, nmol/L	31.5 (22.0–47.4)	34.7 (30.0–46.4)	0.312
Medications, n (%)			
Beta-blockers	77 (96.3)	79 (97.5)	0.639
ACE-inhibitors/ARB-blockers	77 (96.3)	80 (98.8)	0.305
Aldosterone-antagonists	64 (80.0)	70 (86.4)	0.276
Loop diuretics	66 (82.5)	71 (87.7)	0.359
Thiazide-diuretics	21 (26.3)	23 (28.4)	0.760
Digoxin	25 (31.3)	33 (40.7)	0.210
Calcium-antagonists	3 (3.8)	1 (1.2)	0.305
Lipid-lowering drugs	46 (57.5)	45 (55.6)	0.803
Calcium supplement use	1 (1.3)	2 (2.5)	0.567
Vitamin D supplement use	0	0	>0.99

LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; 25OHD, 25-hydroxyvitamin D; ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker; BMI, body mass index.

study termination (Table 2). Moreover, vitamin D did not influence the percentage of patients with non-physiological HDL-cholesterol and triglycerides concentrations. In detail, the percentage of patients with HDL-cholesterol concentrations below the reference range was in the vitamin D and placebo group 93.8 and 85.2%, respectively, at baseline (p = 0.077) and was 92.5 and 90.1%, respectively, at study termination (p = 0.593). The corresponding values for elevated triglycerides concentrations were 61.3 and 54.3%, respectively, at baseline (p = 0.373), and were 68.8 and 65.4%, respectively, at study termination (p = 0.654).

Subgroup Analyses

The effect of vitamin D supplementation on CVD risk markers in patients with baseline 25OHD concentrations <30 nmol/L, diabetic patients, and non-users of lipid-lowering drugs is presented in online supplementary Table 1–3 (for all online suppl. material, see www.karger.com/doi/10.1159/000495662). In none of the 3 subgroups and in none of the risk markers assessed, vitamin D achieved a significant treatment effect. Regarding the use/non-use of lipid-lowering drugs, it is noteworthy that baseline total-cholesterol and LDL-cholesterol concentrations were both significantly higher in non-users than in users of lipid-lowering drugs (total-cholesterol, median, 25th and 75th percentiles: 185, 160–215 and 155, 133–177 mg/dL; respectively, p < 0.001; LDL-cholesterol, median, 25th and 75th percentiles: 109, 93–129 and 84, 66–98 mg/dL; respectively, p < 0.001). The corresponding values for the baseline total-cholesterol/HDL-cholesterol ratios were 4.3 (3.7–5.2) and 3.8 (3.1–4.5) respectively (p < 0.001). Baseline 25OHD concentrations did not differ significantly between male and female patients (p = 0.828), and there were no sex-specific effects of vitamin D supplementation on lipid parameters or VC markers (online suppl. Tables 4, 5).

Since there were no significant treatment effects of vitamin D, neither in the entire study cohort nor in the sub-

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Table 2. Vitamin D effects on CVD parameters in study participants

Parameter	Baseline	Follow-up	Treatment effect	*p* value
Total cholesterol, mg/dL				
Vitamin D (*n* = 80)	157 (136 to 189)	171 (140 to 195)	–0.5 (–14.5 to 13.4)	0.939
Placebo (*n* = 81)	169 (144 to 196)	177 (149 to 203)		
LDL-cholesterol, mg/dL				
Vitamin D (*n* = 80)	91 (71 to 110)	97 (76 to 117)	0.60 (9.14 to 10.33)	0.904
Placebo (*n* = 81)	95 (76 to 115)	102 (80 to 123)		
HDL-cholesterol, mg/dL				
Vitamin D (*n* = 80)	41 (33 to 47)	41 (34 to 48)	–1.25 (–4.14 to 1.6)	0.395
Placebo (*n* = 81)	43 (36 to 50)	43 (34 to 51)		
Total-cholesterol/HDL-cholesterol (ratio)				
Vitamin D (*n* = 80)	4.12 (3.47 to 4.76)	4.11 (3.36 to 4.95)	0.05 (–24 to 0.35)	0.722
Placebo (*n* = 81)	4.02 (3.27 to 4.54)	4.22 (3.64 to 4.84)		
LDL-cholesterol/HDL-cholesterol (ratio)				
Vitamin D (*n* = 80)	2.26 (1.83 to 2.80)	2.24 (1.89 to 2.92)	0.07 (–0.15 to 0.29)	0.522
Placebo (*n* = 81)	2.28 (1.78 to 2.79)	2.37 (1.99 to 2.91)		
Triglycerides, mg/dL				
Vitamin D (*n* = 80)	198 (125 to 263)	182 (118 to 255)	–15.1 (–55.5 to 25.4)	0.463
Placebo (*n* = 81)	165 (122 to 218)	181 (130 to 227)		
Fetuin A, g/L				
Vitamin D (*n* = 80)	0.50 (0.40 to 0.60)	0.50 (0.40 to 0.60)	0.07 (–0.12 to 0.25)	0.481
Placebo (*n* = 81)	0.50 (0.40 to 0.60)	0.50 (0.40 to 0.60)		
dp-ucMGP, pmol/L[a]				
Vitamin D (*n* = 38)	337 (290 to 764)	313 (290 to 670)	–63 (–3,120 to 183)	0.610
Placebo (*n* = 32)	290 (290 to 633)	302 (290 to 599)		

[a] Based on 70 blood samples (38 samples in patients assigned to placebo and 32 samples in patients assigned to vitamin D).

LDL, low density lipoprotein; HDL, high density lipoprotein; dp-ucMGP, non-phosphorylated undercarboxylated matrix gla protein; CVD, cardiovascular disease.

groups, it was not necessary to apply the Benjamini and Hochberg false discovery rate method to adjust the *p* values.

Discussion

The present investigation indicates that long-term supplementation with a moderately high daily dose of vitamin D does not significantly influence lipid parameters and VC markers in patients with advanced heart failure. Similar results were achieved in the subgroups of nonusers of lipid-lowering drugs, patients with baseline 25OHD concentrations <30 nmol/L, and patients with diabetes mellitus. In addition, there were no sex-specific vitamin D effects.

Our data on lipid parameters are in general agreement with results of the aforementioned meta-analysis by Wang et al. [4], with the exception of the effect on LDL-cholesterol. The meta-analysis reported a significant increase in LDL-cholesterol concentrations by vitamin D supplementation. It is however noteworthy that in our study the mean treatment effect on LDL-cholesterol of –0.5 mg/dL was within the 95% CI of the LDL-cholesterol change in the meta-analysis, if our results are compared with the subgroup of studies with an intervention duration >1 year. Moreover, a recent individual patient data meta-analysis of RCTs reported a small but significant decrease in LDL-cholesterol concentrations with vitamin D supplementation with no changes in HDL-cholesterol and triglycerides concentrations [18].

Obviously, vitamin D supplementation was also ineffective in reducing lipid parameters in nonusers of lipid-lowering drugs, although this subgroup had significantly higher baseline total- and LDL-cholesterol concentrations than users of these drugs and a baseline total-cholesterol/HDL-cholesterol ratio of >4. Therefore, it is rather unlikely that patients with dyslipoproteinemia will benefit from vitamin D supplementation. In addition, vitamin D was ineffective with regard to the lipid profile in

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patients who had 25OHD concentrations <30 nmol/L, which are frequently used to classify vitamin D status as deficient [19, 20]. Similar results as in our study were reported on the lipid profile of apparently healthy adults who received 800 IU vitamin D daily for 12 weeks and had median baseline 25OHD concentrations <40 nmol/L [21].

Our data do not support the results of the aforementioned meta-analysis in 1,365 diabetic patients [5], indicating a significant reduction in total- and LDL-cholesterol concentrations by vitamin D supplementation. However, in our subgroup analysis of diabetic patients, the number of subjects was relatively small (n = 37) and the mean treatment effects and its 95% CIs covered a wide range. For definitive answers to be given, large, well-designed RCTs in vitamin D deficient diabetic patients with dyslipoproteinemia are needed.

Available data regarding the effects of vitamin D supplementation on the VC risk markers fetuin-A and dp-ucMGP are scarce: In hemodialysis patients, administration of the vitamin D analog paricalcitol for 8 weeks was associated with an increase in fetuin-A concentrations, but no control group without paricalcitol administration was available [22]. Weekly supplementation with 25,000 IU vitamin D for 13 weeks also increased fetuin-A concentrations in an RCT in hemodialysis patients [23]. Our data do not support these earlier results. Notably, hemodialysis is associated with a high risk of CVD [8] and it cannot be ruled out that the vitamin D effect on fetuin-A is a counter regulatory mechanism in these patients. Again, further studies are needed to explain potential differences between the earlier studies in patients with hemodialysis and our study in patients with advanced HF.

Similar to the bone protein osteocalcin, MGP belongs to a group of proteins that depend on vitamin K-mediated gammacarboxylation. While undercarboxylation of the bone protein osteocalcin is a risk factor for osteoporotic fractures [24], dp-ucMGP is a risk marker for heart failure severity [11]. Vitamin D status is an important determinant of circulating undercarboxylated osteocalcin in elderly institutionalized people and in vitro studies indicate that the active hormonal form of vitamin D, 1,25-dihydroxyvitamin D, decreases dose-dependently the secretion of undercarboxylated osteocalcin [24]. However, similar to findings, which show that in the clinical setting vitamin D supplementation does not alter the concentration of undercarboxylated osteocalcin [25], our data indicate that vitamin D supplementation also does not influence the concentration of dp-ucMGP.

We were unable to show beneficial effects of vitamin D supplementation on cardiovascular risk markers, but it should also be underlined that our results provide important safety information regarding relatively high vitamin D doses supplemented for 3 years. Such safety data are, in our opinion, very useful regarding public health considerations to improve vitamin D status in the general population such as systematic vitamin D food fortification [26].

Our study has both strengths and limitations. Strengths include the study design of an RCT, the study duration of 3 years, the high cumulative vitamin D dose, the homogenous group of patients, and the various subgroup analyses. One limitation is the relatively high dropout rate and the restriction of our analysis to survivors. However, this is primarily due to the severity of the disease. Another limitation is that due to insufficient sample volume the number of dp-ucMGP analyses was relatively small. Therefore, these results should be interpreted with caution.

Conclusion

In summary, our data indicate that it is unlikely to improve the lipid profile and to influence the calcification parameters fetuin-A and dp-ucMGP in patients with advanced heart failure and inadequate vitamin D status by vitamin D supplementation.

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Ethics Statement

All subjects gave their written informed consent before study enrolment. The study was approved by the Ethics Committee of the Medical Council Westphalia-Lippe, Germany (No. 2010-052-f-A), and was registered at EudraCT as 010-020793-42 and clinicaltrials.gov as NCT01326650.

Disclosure Statement

A.Z.: has received speaker honoraria from DiaSorin, Germany. J.B.E., S.P., U.F., J.D., J.K., C.K., J.B., H.K.B., S.P., I.G.-B., and J.F.G.: declare that they have no conflict of interest.

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Authors Contribution

A.Z. and J.B.E.: conceived and designed the study. A.Z., J.B.E., S.P., U.F., J.K., J.D., C.K., J.F.G., I.G.-B., H.K.B., and S.P.: performed data acquisition, analysis, and data interpretation. A.Z.: drafted the manuscript. S.P., S.P., I.G.-B., H.K.B., C.K., and J.F.G.: critically revised the manuscript for important intellectual content. A.Z.: obtained funding. J.K., J.D., C.K., and J.F.G.: provided administrative, technical, or material support. A.Z., J.B.E., S.P., U.F., and J.B.E.: supervised the study. All authors read and approved the final manuscript.

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