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Polymorphisms at Cytokine Genes May Determine the Effect of Vitamin E on Cytokine Production in the Elderly[1–3]

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Abstract

Vitamin E has been shown to affect cytokine production. However, individual response to vitamin E supplementation varies. Previous studies indicate that cytokine production is heritable and common single nucleotide polymorphisms (SNP) may explain differences in cytokine production between individuals. We hypothesize that the differential response to the immunomodulatory actions of vitamin E reflects genetic differences among individuals, including SNP at cytokine genes that modulate cytokine production. We used data from a double-blind, placebo-controlled 1-y vitamin E (182 mg d,l-α-tocopherol) intervention study in elderly men and women (mean age 83 y) to test this hypothesis (vitamin E, n = 47; placebo, n = 63). We found that the effect of vitamin E on tumor necrosis factor (TNF)-α production in whole blood stimulated for 24 h with lipopolysaccharide (1.0 mg/L) is dependent on TNFα -308G > A. Participants with the A/A and A/G genotypes at TNFα -308G > A who were treated with vitamin E had lower TNFα production than those with the A allele treated with placebo. These observations suggest that individual immune responses to vitamin E supplementation are in part mediated by genetic factors. Because the A allele at TNFα has been previously associated with higher TNFα levels in whole blood and isolated immune cells, our observations suggest that the antiinflammatory effect of vitamin E is specific to those genetically predisposed to higher inflammation. Further studies are needed to determine the biological mechanism driving the interaction between vitamin E treatment and TNFα -308G > A and its implications for disease resistance. J. Nutr. 139: 1855–1860, 2009.

Introduction

Vitamin E has been shown in a number of studies to enhance the immune response and resistance to infection (1–4). However, not all participants respond positively to vitamin E supplementation and the variability in response is not completely explained by change in blood vitamin E levels (5). It is likely that the variation in the immunological effectiveness of vitamin E reflects in part the genetic differences among the participants studied.

Studies have shown that the immunomodulatory effect of vitamin E is in part mediated via its effect on cytokine production (5–9). Interleukin (IL)[7]-1β, tumor necrosis factor (TNF)-α, and IL-6 are critical to mounting an effective immune response to respiratory infection and promoting the clearance of pathogens (10). Production of these cytokines is altered in the aged (11–14). Recent studies indicate that single nucleotide polymorphisms (SNP) located at the genes for IL-1β, IL-6, and TNFα are associated with cytokine production (15–17).

Given the variability in response to vitamin E supplementation, studies that elucidate the genetic differences underlying immunologic response to vitamin E are necessary. This study sought to identify the impact of common genetic differences at the genes coding for IL-1β, IL-6, and TNFα on the ability of vitamin E to alter cytokine production in the elderly by using

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[3] Supplemental Tables 1–3 are available with the online posting of this paper at jn.nutrition.org.

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[7] Abbreviations: Ab, antibody; ANCOVA, analysis of covariance; IL, interleukin; LPS, lipopolysaccharide; MAb, monoclonal antibodies; SNP, single nucleotide polymorphism; TNF, tumor necrosis factor.

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data from an vitamin E intervention trial in a nursing home population (2). The goals of this study were to test if specific SNP at cytokine genes are associated with ex vivo cytokine production in the elderly and determine whether the effect of vitamin E supplementation on cytokine production is associated with specific SNP.

Participants and Methods

Study population. Between 1998 and 2001, elderly volunteers living in long-term care facilities were recruited to participate in a year-long randomized, double-blind, placebo-controlled vitamin E intervention trial (2). The Tufts Medical Center institutional review board approved the study protocol and informed consent form. Volunteers were recruited from 33 long-term care facilities in the Boston metropolitan area. Eligibility criteria for inclusion in the study included age of ≥65 y, life expectancy >6 mo, no anticipated discharge from facility in the next 3 mo, not room-bound in past 3 mo, no active neoplastic diseases, no tube feeding, no dialysis, no i.v. or urethral catheters in past 30 d, no tracheotomy or chronic ventilators, free of antibiotics for >2 wk, no chronic steroid use >10 mg/d, no use of immunosuppressive medications, BMI >18 kg/m^2, serum albumin of at least 0.3 g/L, ability to swallow pills, no use of micronutrient supplements (vitamins E, C, and B-6, selenium, zinc, β-carotene) greater than the recommended daily allowance or use of fish oil supplements, willing to receive influenza vaccination, and willing to provide informed consent (for patients with dementia, family members provided informed consent). Participants were randomized to receive either vitamin E (182 mg/d d,l-α-tocopherol) or placebo. In addition, all participants received a daily multivitamin/mineral capsule that contained one-half the recommended daily allowance of essential vitamins and minerals (18), including <4 mg of vitamin E (**Supplemental Table 1**).

Of the 617 volunteers enrolled in the study, 451 completed the study. Ex vivo production of cytokines was measured in 110 participants who enrolled during the second year of the parent study. This number of participants was determined to be adequate for the detection of significant changes in cytokine production due to vitamin E treatment based on power calculations done prior to the study (see Statistical analysis, below). Participants with a history of difficult blood draws and those who did not complete the study were excluded from the subgroup selected for cytokine production measurement. The study population characteristics have been previously reported (19). Participants were mostly white/non-Hispanic in ethnicity (95%), aged 66–100 y, and there were slightly more women (56%) than men.

Nutrient status and blood count differentials. Blood samples collected from fasting participants at enrollment and completion of the study were used to measure clinical chemistries, blood cell differentials, and plasma nutrient status for select nutrients including vitamin E as previously described (20–22).

Ex vivo cytokine production. Secreted ex vivo cytokine production was assessed from heparinized whole blood collected following an overnight fast. Using whole blood to determine cytokine production decreases processing of samples and the production closely models in vivo environment with values that correlate closely to production measured from peripheral blood mononuclear cells subfractions (23). To account for day to day variation (**Supplemental Table 2**), cytokine production was measured in 2 blood samples collected on 2 separate days at enrollment and at completion of the intervention. The mean cytokine production from the 2 d at each phase was used in data analysis.

Whole blood was diluted in complete RPMI at a 1:4 ratio and divided into aliquots in 24-well flat-bottom plates. IL-1β, IL-6, and TNFα production in response to lipopolysaccharide (LPS) (either 1.0 or 0.01 mg/L) was measured after 24-h incubation. Following incubation, plates were centrifuged and supernatants were collected and stored (−70°C) for analysis. Secreted cytokine protein levels were measured by ELISA. TNFα protein was detected using mouse anti-human monoclonal antibodies (MAb) and biotinylated mouse anti-human antibodies (Ab) specific for TNFα (BD PharMingen). IL-6 was detected using rat anti-human IL-6 MAb and biotinylated rat anti-human IL-6 Ab (BD PharMingen). IL-1β was detected using mouse anti-human IL-1β MAb and biotinylated anti-human IL-1β Ab (R&D Systems).

Cytokine production as a function of the number of monocytes and lymphocytes was calculated using blood count information obtained from samples taken concurrently to the samples taken for the cytokine production assay.

DNA isolation and genotyping. Among those participants selected for in vitro immunological measures, 100 participants consented to DNA analysis. DNA was isolated from blood samples using spin-prep kits according to the manufacturer's instructions (QIAamp DNA Blood Mini kit, Qiagen). The following locations were investigated: IL-1β -1473G > C (rs1143623), IL-1β -511G > A (rs16944), IL-1β 3954C > T (rs1143634), IL-1β 6054G > A (rs1143643), IL-6 174C > G (rs1800795), and TNFα -308G > A (rs1800629). These SNP were selected from among numerous other SNP based on previous reports that they were associated with altered cytokine production in younger populations or in vitro (15–17,24–27). Genotyping was performed with Taqman 5′ nuclease allelic discrimination using Validated ABI Assays according to the manufacturer's instructions (Applied Biosystems). The primers and probes used for genotyping are listed in **Supplemental Table 3**.

Statistical analysis. Sample size calculations for the effect of vitamin E treatment on cytokine production were based on previous studies considering the mean change in cytokine production induced by vitamin E. These calculations assumed a 30% change in cytokine production due to vitamin E treatment (5), except for IL-6, which assumed a 50% change (28). These calculations indicated the number of participants that were required to observe a difference in IL-6 production (53 per group) based on a projected mean ± SD of 2150 ± 1960 ng/L (2-sided test, α = 0.05).

Power calculations for the association between SNP and baseline cytokine production were based on previously reported mean differences (and SD) in production at each SNP using Student's *t* test. These indicated we had >80% power (2-sided, α = 0.05) to detect significant differences in mean cytokine production for each SNP assuming the following differences: *1*) 400 ± 285 ng/L (16) TNFα between 27 A/A and A/G, and 68 G/G participants at TNFα -308G > A; *2*) 1700 ± 1310 ng/L (15) IL-1β between 6 A/A, and 93 A/G and G/G participants at IL-1β -511G > A; and *3*) 4050 ± 900 ng/L (17) IL-6 between 15 G/G, and 85 C/G and C/C participants at IL-6 174C > G.

SNP were tested for Hardy-Weinberg Equilibrium using Statistical Genetics Utility programs (29) and calculations for each of the SNP were previously reported (19). Genotype

distributions within each treatment group did not deviate from Hardy-Weinberg Equilibrium. Linkage disequilibrium between SNP was assessed as Lewontin's D' and the square of the correlation between loci using Haploview 4.0 (Broad Institute, MIT).

The remaining analyses were performed by using SAS statistical software package version 9.1.2 (SAS Institute). Analysis used list-wise deletion for missing data. Significance was determined at $P < 0.05$ using 2-sided significance tests. The distribution of continuous variables was examined and transformed using a logarithm or square-root transformation as needed. Descriptive statistics are reported as nontransformed data. Baseline characteristics of each treatment group were compared using Student's *t* test for continuous variables or chi-square or Fisher's exact test for categorical variables.

Analyses first examined the main effect of supplementation on cytokine production. Student's *t* test for independent samples was used to determine whether there was a significant difference in cytokine production between vitamin E treatment groups at the beginning and the end of the study. Student's *t* tests for paired data were used to determine whether there was a significant change in production from baseline to follow-up within treatment groups. Analysis of covariance (ANCOVA) was used to test the effect of vitamin E treatment on follow-up cytokine production while adjusting for baseline cytokine production. These models were then adjusted for baseline production. Separate ANCOVA models were used to adjusted for baseline smoking, diabetes mellitus, dementia, albumin level, hemoglobin level, obstructive lung diseases, BMI, cardiovascular disease, hypertension, history of malignancy, gender, and age.

Analyses then examined the effect of genotype on baseline cytokine production. Baseline differences in cytokine production by genotype were analyzed using ANOVA. Initial tests examined the relationship between IL-6 174G > C and IL-6 production, TNFα -308G > A and TNFα production, and the SNP at IL-1β and IL-1β production. Because the data suggested a relationship between TNFα production and TNFα -308G > A, the relationship between TNFα -308G > A and production of IL-1β and IL-6 was explored. Group means for each genotype did not indicate clear patterns of dominance at baseline; therefore, analysis without grouping by previously reported dominance is reported for baseline data. Secondary analyses tested the interaction between genotype and sex for cytokine production at baseline and found no significant patterns. The analyses for interactions and main effects were then adjusted for *1*) sex and baseline cytokine production; and *2*) all of the independent factors adjusted for in models of the main effect of vitamin E listed above. If the main effect of genotype on baseline cytokine production was significant, a post-hoc test using Tukey's adjustment was applied to determine differences between groups.

The effect of vitamin E treatment and SNP on follow-up cytokine production was examined using ANCOVA. In these analyses, the first model tested the interaction between vitamin E and SNP and the second models tested the main effects were tested without an interaction. The interactions between genotype and treatment for follow-up cytokine production were tested in separate models for each SNP. Secondary analyses also tested the interaction between genotype and sex for cytokine production at follow-up and found no significant patterns. The analyses for interactions and main effects were then adjusted for sex and baseline cytokine production and all of the independent factors adjusted for in models of the main effect of vitamin E listed above. Because adjusting for these independent factors had a negligible effect on significant values in both the tests of the main effects and tests of interactions with genotype and treatment, unadjusted means are reported. If the interaction between SNP and vitamin E was significant, differences between treatment groups within each genotype group were explored in separate analyses by group.

Results

The study population characteristics have been previously reported (19). The frequency of malignancy at baseline was higher in the placebo group than in the vitamin E treatment group ($P = 0.04$); however, there were no differences between those with and without history of malignancy in baseline or follow-up IL-1β, IL-6, or TNFα production from whole blood (data not shown). There were no other differences in the baseline characteristics tested between the treatment groups.

The effect of vitamin E on cytokine production in the whole population. There were no overall differences with respect to treatment group for any of the cytokines. Cytokine production levels in whole blood elicited with LPS (1.0 mg/L) decreased ($P < 0.05$ for all) from baseline to follow-up in both treatment groups (**Table 1**). Cytokines elicited with 0.01 mg/L LPS did not show a significant change with time.

The effect of genotype on baseline cytokine production. TNFα -308G > A had a significant effect on TNFα production at baseline in whole blood elicited with 0.01 mg/L LPS ($P = 0.03$) but not on TNFα production elicited with 1.0 mg/L LPS ($P = 0.03$). IL-6–174G > C on IL-6 production, or any of the IL-1β SNP on IL-1β production in whole blood elicited with LPS at baseline (**Table 2**).

The interaction between vitamin E and genotype for cytokine production. There was an interaction between the TNFα -308 G > A and vitamin E treatment for follow-up TNFα production in whole blood elicited with LPS (**Table 3**). Participants with the A/A and A/G genotype at TNFα -308G > A who were treated with vitamin E had lower TNFα production than

TABLE 1 Whole blood cytokine production in response to 24-h LPS treatment at the beginning and end of a 1-y vitamin E supplementation trial in elderly nursing home residents[1]

Cytokine	LPS	Group	*n*	Baseline	Follow-up
IL-1β	*mg/L*			*ng/L*	
	1.0	Vitamin E	47	545 ± 384	461 ± 377*
		Placebo	63	547 ± 290	426 ± 232*
	0.01	Vitamin E	47	287 ± 231	254 ± 204
		Placebo	63	276 ± 179	237 ± 166
IL-6					
	1.0	Vitamin E	47	8780 ± 5720	7787 ± 4319*
		Placebo	63	9045 ± 4812	7600 ± 4182*
	0.01	Vitamin E	47	5367 ± 3542	5084 ± 2569
		Placebo	63	5596 ± 2883	5059 ± 3051
TNFα					
	1.0	Vitamin E	44	1476 ± 1884	769 ± 766*
		Placebo	61	1211 ± 1002	685 ± 638*
	0.01	Vitamin E	36	419 ± 464	389 ± 496
		Placebo	55	346 ± 317	329 ± 422

[1] Data are unadjusted means ± SD. *Different from baseline, $P < 0.05$ (Student's *t* test of log-transformed data).

TABLE 2 Whole blood cytokine production in response to 24-h LPS treatment at baseline in elderly nursing home residents by cytokine genotype group[1]

Cytokine	Genotype	*n*	LPS, *1.0 mg/L*	LPS, *0.01 mg/L*
TNFα	TNFα -308G > A[2]		*ng/L*	
	G/G	71	1127 ± 1013	358 ± 390[a,b]
	A/G	21	2060 ± 2463	615 ± 694[b]
	A/A	6	1008 ± 1161	269 ± 338[a]
	P[3]		0.10	0.03
IL-6	IL-6–174C > G			
	C/C	43	9571 ± 5564	5758 ± 3336
	G/C	42	9082 ± 5608	5836 ± 3574
	G/G	15	7195 ± 3751	4356 ± 1827
	P		0.24	0.18
IL-1β	IL-1β -1473G > C			
	G/G	59	568 ± 367	286 ± 217
	C/G	38	514 ± 284	284 ± 196
	C/C	2	769 ± 760	333 ± 303
	P		0.73	0.94
	IL-1β -511G > A			
	G/G	45	597 ± 387	292 ± 224
	A/G	48	514 ± 279	286 ± 194
	A/A	6	511 ± 476	253 ± 233
	P		0.25	0.25
	IL-1β 3954G > A			
	G/G	63	540 ± 367	271 ± 203
	A/G	33	574 ± 282	316 ± 212
	A/A	3	550 ± 562	287 ± 316
	P		0.55	0.46
	IL-1β 6054G > A			
	G/G	37	533 ± 324	275 ± 190
	A/G	44	509 ± 266	273 ± 192
	A/A	18	694 ± 501	342 ± 276
	P		0.37	0.77

[1] Data are unadjusted means ± SD. Means in a column with superscripts without a common letter differ, $P < 0.05$. (Tukey's post hoc test). The effect of genotype was assessed using ANOVA on log-transformed data. *P*-values are unadjusted.
[2] The number of observations at baseline for TNFα stimulated with 0.01 mg/L of LPS was G/G $n = 62$, A/G $n = 20$, A/A $n = 5$. All other SNP had equal numbers in each stimulation condition.

those with the A allele treated with placebo ($P = 0.03$). The interaction between vitamin E treatment and TNFα -308G > A was not predictive of IL-1β or IL-6 production at follow-up. There was no evidence of an interaction between vitamin E treatment and any of the other SNP examined for follow-up cytokine production.

Cytokine response in our study was quantified from whole blood cultures. The number of cytokine-producing cells, particularly monocytes and lymphocytes, could affect the cytokine response in culture. The interaction between vitamin E treatment and TNFα production was essentially the same when cytokine production per monocyte and lymphocyte was examined (data not shown).

Discussion

Our data suggests that TNFα -308G > A modulates TNFα response to vitamin E supplementation in the elderly. Previous studies have not provided a clear picture of the effect of

TABLE 3 Whole blood TNFα production in response to 24-h LPS treatment at the end of a 1-y vitamin E supplementation trial in elderly nursing home residents by TNFα -308G > A genotype and supplementation group[1]

LPS	Group	*n*	G/G	*n*	A/G, A/A	*P*-interaction[2]
mg/L			*ng/L*		*ng/L*	
1.0						
	Vitamin E	22	876 ± 840	17	749 ± 752[a]	0.04
	Placebo	46	631 ± 643	10	967 ± 728[b]	
0.01						
	Vitamin E	18	495 ± 615	14	340 ± 346	0.18
	Placebo	40	327 ± 470	10	379 ± 313	

[1] Data are unadjusted means ± SD. Means in a column with superscripts without a common letter differ, $P < 0.05$ (Stratified analysis).
[2] *P*-values for the interaction between vitamin E treatment and TNFα -308G > A, adjusted for baseline TNFα production.

supplemental vitamin E on TNFα production. Animal studies have indicated that vitamin E supplementation prior to infection was associated with decreased TNFα production relative to placebo treatment in mice infected with LP-BM5 (30) and influenza virus (1). However, results from human studies are varied. High-dose vitamin E supplementation (1200 mg/d for 8 wk) has been associated with decreased ex vivo TNFα production from monocytes of adults (7). Several small studies in adult men have shown higher doses of 402 mg/d (31) or 600 mg/d (32) of vitamin E lowered ex vivo TNFα production but did not change circulating TNFα levels (32,33). A study in healthy men reported that vitamin E supplementation (728 mg/d for 48 d) did not have a statistically significant effect on circulating or ex vivo secreted TNFα compared with placebo treatment (28). The interaction between vitamin E and TNFα -308G > A may explain why vitamin E treatment was not significantly different from placebo overall in this study. Furthermore, it may in part explain null results reported in previous human studies (28).

If vitamin E acted as a general antioxidant to reduce inflammation equally in all people, one may expect that supplementation with vitamin E would decrease TNFα production regardless of TNFα -308G > A genotype. The majority of participants in our study were G/G genotype at TNFα -308G > A. Participants with the A/A and A/G genotype at TNFα -308G > A who were treated with vitamin E had significantly lower TNFα production compared with participants with the A allele treated with placebo.

The A allele at TNFα -308G > A has been associated with increased TNFα production in vitro and ex vivo in healthy adults compared with the G allele (16,24). Previous studies reported that vitamin E treatment was most beneficial for improving cardiovascular outcomes, which are related to inflammation and oxidative stress, among diabetics who were genetically predisposed to increased oxidative stress (34). This interaction for TNFα production may offer further evidence that the antiinflammatory action of vitamin E is specific to people who are predisposed to increased inflammation. Further studies will be needed to determine the biological mechanism driving the interaction between vitamin E and TNFα -308G > A. Such studies may include functional assays in cell culture to specifically examine the role of oxidative stress and inflammation. Additional studies may also consider a more comprehensive analysis of the loci surrounding TNFα -308G > A. The gene coding for TNFα is located on chromosome 6 (35) in the MHC3, in proximity to genes coding for other immunoregulatory

proteins. Therefore, it is plausible that the association observed between TNFα -308G > A and vitamin E treatment could be due to linkage between TNFα -308G > A and other loci. Further, studies with different doses of LPS or means of eliciting TNFα may help determine why the interaction between vitamin E and TNFα -308G > A was not significant for TNFα elicited with a lower dose of LPS.

Our study focused on ex vivo cytokine production. However, TNFα -308G > A has also been investigated relative to numerous diseases, including heart disease, diabetes, sepsis (36–38), respiratory syncytial virus infection (39), and bacterial bronchopneumonia and chronic obstructive bronchitis (40). Based on our study, the clinical implications of the interaction between vitamin E and TNFα -308G > A are not clear. Future studies are needed to investigate if the observed interaction between vitamin E treatment and TNFα -308G > A is related to susceptibility to other diseases.

In contrast, the other SNP we investigated did not account for our observation that vitamin E treatment did not have a significant impact on IL-1β or IL-6 production overall in our study. The absence of an interaction between IL-1β -511G > A and vitamin E may be due in part to the low frequency of people with the A/A genotype within each treatment. Our observations for the overall effect of vitamin E contrast with those of previous studies that reported supplemental vitamin E lowered IL-1β and IL-6 production elicited with LPS (8,28,31). However, these previous studies used doses that were 3 (31), 4 (28), and 6 (8) times the dose used in our research. Therefore, it is possible that these discrepant findings for the effect of vitamin E on IL-1β and IL-6 are due to higher doses used in the other studies.

It is interesting to note that average cytokine production declined from baseline to follow-up within both treatment groups. This decrease is likely not due to seasonal variation, as this was a 1-y study and the baseline and follow-up measurements were performed in the same month of the year. Further, our assessment of delayed hypersensitivity response in this population (our unpublished results) indicates that this decrease is likely not due to a general decline in immune response in these participants. It is possible that this decrease is related to the consumption of the multivitamin/mineral supplement that all participants received. However, it is not possible to verify this speculation with the information available from this study.

Previously, researchers have reported associations between genotype at the SNP we investigated and ex vivo cytokine production. For example, participants with the A allele at TNFα -308G > A had higher TNFα production compared with the participants with the G allele (16); participants with the G allele at IL-6–174C > G had higher IL-6 production compared with participants with the C allele (17). Previous reports of these SNP at IL-1β indicated that participants with the G allele at IL-1β -1473G > C had higher IL-1β production compared with the participants with the C allele (25); participants with the T allele at IL-1β 3954C > T had higher IL-1β production compared with the participants with the C allele (26,27); and participants with the A allele at IL-1β -511G > A had higher IL-1β secretion compared with the participants with the G allele (15).

However, we observed no significant association between these common IL-1β and IL-6 SNP and ex vivo cytokine production at baseline. Many of these previous studies examined different study populations, such as younger (15–17,25,27) or non-Caucasian people (25), or used different cell types (27) or elicitation conditions (16,17,27) to measure cytokine production. These differences in study design may account in part for the differences between their observations and ours.

In conclusion, research that specifically examines interactions between vitamin E and gene variants is sparse. Recent reports have shown that response to vitamin E supplementation in diabetics may be modulated by variants of HaptoglobulinA (34,41,42) and plasminogenA-1 (43,44) genes. These reports support the idea that genetic factors modulate responses to supplemental E. We have observed that the effect of vitamin E supplementation on TNFα production was dependent on TNFα -308G > A. A better understanding of this interaction will allow us to determine the importance of this observation in future recommendations of supplemental vitamin E for immune function. As such, future studies will determine the clinical implications and mechanisms underlying this interaction between genotype and E. Further, replication of our observations in other populations is necessary to confirm the findings reported here and determine applicability to other age and ethnic groups. In total, our observations and those of others suggest that genetic factors should be considered when designing vitamin E interventions in humans.

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S.N.M. and J.M.O. designed the research; S.E.B., J.D.-L., L.S. L., and S.N.M. conducted the research; S.E.B. analyzed the data; and S.E.B., P.F.J., S.N.M., and J.M.O. wrote the paper. S. N.M. had primary responsibility for final content. All authors read and approved the final manuscript.

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