Maternal serum 25-hydroxyvitamin D and placental vascular pathology in a multicenter US cohort[1–3]

Alison D Gernand, Lisa M Bodnar, Mark A Klebanoff, W Tony Parks, and Hyagriv N Simhan

ABSTRACT

Background: Maternal vitamin D deficiency has been linked to fetal growth restriction, but the underlying mechanisms are unclear.

Objective: We tested the hypothesis that poor maternal 25-hydroxyvitamin D [25(OH)D] is associated with increased risk of placental vascular pathology.

Design: Maternal serum 25(OH)D was measured at ≤26 wk of gestation in a random subcohort of term, singleton infants in the Collaborative Perinatal Project (1959–1966; n = 2048). A dichotomous vascular construct was created from the presence of any of 12 pathologies identified on placental examinations, including evidence of placental abruption, infarction, hypoxia, decidual vasculopathy, or thrombosis of fetal vessels (n = 240 cases).

Results: The relation between 25(OH)D and vascular pathology was modified by infant sex (P = 0.003). A maternal 25(OH)D concentration ≥80 compared with <50 nmol/L was associated with 49% lower risk of pathology in boys [adjusted OR (95% CI): 0.27, 0.95] after conditioning on study site. No associations were observed between maternal 25(OH)D and pathology in mothers with female offspring. Subsequent analyses showed that, in pregnancies with a female fetus, vascular pathology was associated with a reduced birth-weight z score when the mother's 25(OH)D concentration was <30 nmol/L (β: −0.73; 95% CI: −1.17, −0.30). No association was observed between pathology and birth weight in mothers of female offspring with 25(OH)D concentrations ≥30 nmol/L or in boys, regardless of maternal 25(OH)D status.

Conclusions: Our findings suggest complex relations between vitamin D, placental vascular pathology, and birth weight that differ by infant sex. Maternal vitamin D status may be beneficial for male and female offspring through different mechanisms. *Am J Clin Nutr* 2013;98:383–8.

INTRODUCTION

Vitamin D deficiency, which has long been recognized as a cause of rickets and osteomalacia, has been given increased attention for its noncalcemic roles across the life span, including in pregnancy. Maternal vitamin D deficiency has been associated with many poor birth outcomes, including fetal growth restriction (1–3). A recent Cochrane Review showed that vitamin D supplementation in pregnancy reduces the incidence of low birth weight (<2.5 kg) by 52% (4). Studies of the biological marker of vitamin D status, serum 25-hydroxyvitamin D [25(OH)D][4] (5), and fetal growth also have contributed to the evidence of an association, although there have been inconsistencies in findings (6–11). In a large, multiethnic cohort in the Netherlands, Leffelaar et al (6) showed that risk of small-for-gestational age was 2.4 times higher for mothers with 25(OH)D concentrations <29.9 compared with ≥50 nmol/L in the first trimester. We recently reported that maternal 25(OH)D concentrations ≥37.5 compared with <37.5 nmol/L at ≤26 wk gestation was associated with a 46-g higher term birth weight and, in the first trimester, was associated with one-half the risk of small-for-gestational age at birth (12).

Mechanistic research is lacking to explain the inverse relation between maternal vitamin D status and fetal growth restriction. However, because the placenta can enzymatically convert 25(OH)D to its biologically active form and contains functional vitamin D receptors, there are numerous potential placental roles that remain unexplored (13, 14). The placenta plays a crucial role in fetal growth. The placenta is more than simply the physical link between maternal and fetal tissues, because it both protects and nourishes the fetus while constantly adapting to the maternal-fetal milieu. Placental pathology is a principal cause of poor fetal growth by disrupting the normal exchange of oxygen and nutrients and may be responsible for the majority of fetal growth restriction (15). A normal vasculature remodeling is essential for placental function (16), and maternal vascular lesions in the placenta are most commonly associated with poor fetal growth (17). Our objective was to examine the association between maternal vitamin D status at ≤26 wk gestation and placental vascular pathology in a multicenter US cohort of singleton, term births.

[1] From the Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA (ADG and LMB); the Department of Obstetrics and Gynecology, School of Medicine, University of Pittsburgh, Pittsburgh, PA (LMB and HNS); the Departments of Pediatrics and of Obstetrics and Gynecology, Ohio State University College of Medicine, Columbus, OH (MAK); The Research Institute, Nationwide Children's Hospital, Columbus, OH (MAK); and the Department of Pathology (WTP) and Division of Maternal-Fetal Medicine (HNS), Magee-Womens Hospital, Pittsburgh, PA.

[2] Supported by NIH grant HD056999 (principal investigator: LMB).

[3] Address correspondence to LM Bodnar, University of Pittsburgh Graduate School of Public Health, A742 Crabtree Hall, 130 DeSoto Street, Pittsburgh, PA. E-mail: bodnar@edc.pitt.edu.

[4] Abbreviations used: CPP, Collaborative Perinatal Project; HIF1α, hypoxia-inducible factor 1α; 1,25$(OH)_2$D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

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SUBJECTS AND METHODS

The study was part of a larger, ongoing investigation on vitamin D and adverse pregnancy outcomes. The analysis was deemed exempt from ethnical approval by the Institutional Review Board at the University of Pittsburgh because deidentified data were used. Data and blood samples were from the Collaborative Perinatal Project (CPP; 1959–1966), which enrolled a cohort of pregnant women from 12 medical centers across the United States and sampled nonfasting venous blood every 8 wk (n = 55,908 births) (18, 19). Extensive interviews on participant demographics and medical histories were conducted during pregnancy. A labor and delivery summary was recorded by the obstetrician responsible for each patient's care. After delivery, structured gross and microscopic placental examinations were conducted by pathologists at each site.

Pregnancies were eligible for the parent study if they met the following criteria: singleton gestation; white, black, or Puerto Rican maternal race; no pregestational diabetes or hypertension; entry to prenatal care at ≤26 wk; available stored serum sample at ≤26 wk; and gestational age at birth 20–42 wk (n = 28,429). Of these eligible pregnancies, 3074 were randomly selected for maternal 25(OH)D assessment. If a woman had multiple blood samples available at ≤26 wk, one sample was selected at random for 25(OH)D measurement. For the current investigation, we included only term births (n = 2413) because we were interested in the contribution of pathology to fetal growth and not pathology that affects premature delivery. We excluded subjects without gross and microscopic placental pathology examinations (n = 287) and pregnancies for which the selected serum was unsuitable for vitamin D measurement (n = 79). The final analytic sample was 2048 singleton, term infants born to 2030 mothers (n = 18 women with one repeated pregnancy).

Vitamin D status

Circulating 25(OH)D is considered the best biomarker of vitamin D nutritional status (5). We measured total serum 25(OH)D [$25(OH)D_2$ plus $25(OH)D_3$] by using liquid-chromatography–tandem mass spectrometry with a lower detection limit of 1 ng/mL and no upper limit. No total 25(OH)D concentrations fell below the detectable range. The intraassay CV was 8.2% for $25(OH)D_2$ and 5.9% for $25(OH)D_3$. Although samples were stored at −20°C for over 40 y, we and other authors have shown that such long-term storage is unlikely to cause significant degradation in 25(OH)D (20, 21).

Placental vascular pathology

Pathologists conducted a gross examination of freshly delivered placentas. A full-thickness placental sample was taken from a representative block of the central portion of tissue ~3–4 in from the cord insertion. One umbilical cord sample, one membrane roll sample, and any significant gross abnormalities were also taken for microscopic examination. There were multiple pathologists at each CPP site, most of whom were blinded to the clinical course or outcome; however, in 2% of gross (n = 49) and 3% of microscopic (n = 61) cases, examiners were aware of the outcome (normal or abnormal) for the mother or infant. We created a dichotomous maternal placental vascular construct from the presence of one or more of 12 pathologies identified on gross and microscopic examinations, including evidence of placental abruption, infarction, hypoxia, decidual vasculopathy, or thrombosis of fetal vessels (**Table 1**).

Covariates

We calculated a birth-weight z score by using the mean and SD of birth weight at each week of gestation from a large birth weight reference (22). Mothers were white, black, or Puerto Rican. We classified the season of blood draw as winter and spring (December through May) or summer and fall (June through November). We also assessed gestational age at blood draw, prepregnancy BMI (in kg/m^2) (self-reported weight divided by measured square of height), height, parity (0 compared with ≥1), smoking at registration (yes or no), marital status (married or not married), socioeconomic status [continuous scale as described previously (23)], maternal age (continuous), study site (which also accounted for latitude), and infant sex.

Statistical analysis

We used Lowess plots to guide the specification of vitamin D in models and tested for a departure from linear relations with

TABLE 1
Pathologies in placental vascular construct

Component	Placental examination
Maternal	
Depressed area on maternal surface caused by hemorrhage	Gross
Hemorrhage (old or abruption) on maternal surface, not <0.5 cm from peripheral edge of hemorrhage to placental margin	Gross
Infarcts on cut surface (>3 or ≤3 with at least one measuring ≥3 cm)	Gross
Infarct(s) on maternal floor	Gross
Atheroma in decidual vessels	Microscopic
Fibrinoid in decidual vessels	Microscopic
Marked necrosis in decidua basalis	Microscopic
Microinfarcts at terminal villi	Microscopic
Nucleated red blood cells in fetal circulation at terminal villi	Microscopic
Fetal	
Thrombosed fetal vessels present on membranes/fetal surface	Gross
Thrombosis of cord vessels present	Microscopic
Thrombosis and/or necrosis of fetal surface membranes	Microscopic

splines. We explored 30, 50, and 80 nmol/L as clinically meaningful cutoffs to dichotomize vitamin D because there is controversy about defining vitamin D deficiency (24). We tested the independent association between maternal 25(OH)D and placental vascular pathology by using multivariable conditional logistic regression (conditioned on study site). We used a directed acyclic graph (25) to identify potential confounders (maternal race, season of blood draw, gestational age at blood draw, prepregnancy BMI, height, parity, smoking, marital status, socioeconomic status, and age). We assessed effect-measure modification on the multiplicative scale by trimester at maternal blood draw, maternal race, and infant sex by using the likelihood ratio test ($\alpha = 0.10$) in full models with all potential confounders. We tested if any of the potential confounders changed the main effect by >10% by removing them from full models. In this phase of the analysis, we included pregnancies with complete information on covariates. No covariates met our confounder criterion, and therefore, they were dropped from the final model [thus, women with covariate data missing (eg, for BMI) were included]. We showed no meaningful difference after fitting generalized estimating equation regression models to account for the clustering of infants within women ($n = 18$ mothers with repeated pregnancies), and thus, we ignored the clustering. We conducted analyses with STATA 12.1 software (StataCorp).

RESULTS

At entry into the study, women were parous, young, and married; almost one-half of women were smokers (**Table 2**). For maternal vitamin D status, we showed 22.3% of women had 25(OH)D concentrations <30 nmol/L, 55.4% of women had 25(OH)D concentrations <50 nmol/L, and 86.3% of women had 25(OH)D concentrations <80 nmol/L. There were 5 female and 3 male stillbirths. Male infants were heavier and longer than female infants at birth (**Table 3**), but maternal 25(OH)D did not differ by infant sex ($P = 0.59$). Placental vascular pathology was present in 11.7% of pregnancies ($n = 240$); incidence appeared higher in boys than girls, but the difference was not statistically significant (13.1% compared with 10.4%, respectively; $P = 0.058$).

The association between maternal serum 25(OH)D and placental vascular pathology was modified by infant sex ($P = 0.003$). In conditional logistic regression models, we conditioned on study site and included 25(OH)D, infant sex, and the interaction of the 2 variables. In boys, for each SD increase in maternal 25(OH)D (1 SD: 27.0 nmol/L), there was 25% decreased risk of vascular lesions (95% CI: 0.61, 0.93). After dichotomizing at an insufficiency point of 50 nmol/L, we showed that vitamin D–sufficient compared with –insufficient mothers had 44% reduced risk of placental vascular pathology (95% CI: 0.45, 0.97). In addition, for mothers with 25(OH)D concentrations ≥80 compared with <50 nmol/L, there was 49% decreased risk (95% CI: 0.27, 0.95) of pathology in pregnancies with male offspring (**Table 4**).

In female infants, there was no association between maternal 25(OH)D and placental vascular pathology [adjusted OR: 1.15 (95% CI: 0.95, 1.40) per SD increase in 25(OH)D]. Although there was an apparent trend when 25(OH)D concentrations were divided at 50 and 80 nmol/L into 3 categories, such that higher maternal 25(OH)D appeared to increase risk of pathology, associations were not significant in conditional logistic regression models (Table 4).

TABLE 2
Characteristics of pregnant women randomly selected for vitamin D assessment from the eligible cohort, the Collaborative Perinatal Project (1959–1966)[1]

	Values
Race [*n* (%)]	
White	1110 (54.2)
Black	790 (38.6)
Puerto Rican	148 (7.2)
Socioeconomic index category [*n* (%)][2]	
1	113 (5.6)
2	496 (24.6)
3	666 (33.0)
4	440 (21.8)
5	305 (15.1)
Prepregnancy BMI [*n* (%)]	
<18.5 kg/m^2	196 (10.2)
18.5–24.9 kg/m^2	1382 (72.2)
25–29.9 kg/m^2	242 (12.6)
≥30 kg/m^2	94 (4.9)
Nulliparous (at enrollment) [*n* (%)]	690 (33.7)
Maternal age [*n* (%)]	
<20 y	431 (21.0)
20–29 y	1282 (62.6)
≥30 y	335 (16.4)
Married [*n* (%)]	1690 (82.5)
Smoking at study entry [*n* (%)]	976 (47.8)
Latitude of study site [*n* (%)]	
≥41°N (7 sites)	1367 (66.8)
36–40°N (3 sites)	551 (26.9)
≤35°N (2 sites)	130 (6.4)
Gestational age at blood sampling (wk)	20.7 (15.9, 23.4)[3]
Season of blood sampling [*n* (%)]	
Winter	492 (24.0)
Spring	529 (25.8)
Summer	504 (24.6)
Fall	523 (25.5)
Maternal serum 25(OH)D (nmol/L)[4]	51.2 ± 27.2[5]

[1] $n = 2048$ pregnancies; $n = 2030$ women (18 women with one repeated pregnancy). Missing data are as follows: socioeconomic index ($n = 28$), BMI ($n = 134$), parity ($n = 3$), and smoking ($n = 7$).
[2] Socioeconomic index categories are lowest (1) to highest (5).
[3] Median; IQR in parentheses. Range was 4.1–26.0 wk.
[4] 25(OH)D, 25-hydroxyvitamin D.
[5] Mean ± SD.

Of note, there was no overall association between 25(OH)D concentrations ≥30 compared with <30 nmol/L and pathology in models adjusted for infant sex and conditioned on study site (adjusted OR: 0.87; 95% CI: 0.62, 1.22) or an effect modification by infant sex when 25(OH)D was dichotomized at the deficiency concentration of 30 nmol/L ($P = 0.51$). There was no meaningful difference in any of the aforementioned results after adjustment for gestational age at blood draw, season at blood draw, maternal race, prepregnancy BMI, smoking, and socioeconomic status or after dropping extreme outliers for 25(OH)D (>167 nmol/L) or stillbirths (data not shown).

To further explore vitamin D and differences by infant sex in post hoc analyses, we examined the relation between placental vascular pathology and birth weight. In mothers who carried a male infant, there was no association between the placental vascular pathology and birth-weight z score, regardless of 25(OH)D status. However, in mothers with 25(OH)D concentrations <30 nmol/L who were carrying a female fetus, vascular pathology was associated

TABLE 3
Characteristics of singleton term infants and placentas of pregnancies randomly selected for vitamin D assessment from the eligible cohort, the Collaborative Perinatal Project (1959–1966)[1]

	Male (n = 1017)	Female (n = 1031)
Gestational age (wk)[2]	39.6 ± 1.2	39.7 ± 1.3
Birth weight (g)[2]	3234 ± 396	3139 ± 424
Length (cm)[2]	50.3 ± 2.2	49.8 ± 2.4
Placental weight (g)	439 ± 91	436 ± 87

[1] All values are means ± SDs. n = 2048 pregnancies; n = 2030 women (18 women with one repeated pregnancy).
[2] $P < 0.05$ for difference by using the t test or Kruskal-Wallis test.

with a lower birth-weight z score ($\beta = -0.73$; 95% CI: -1.17, -0.30), which corresponded to a 298-g lower birth weight (adjusted for study site). There was no relation between placental vascular pathology and birth weight in nondeficient mothers [25(OH)D concentration ≥30 nmol/L] who were carrying a female fetus. When we used a sufficiency cutoff of 50 nmol/L, results were similar but attenuated ($\beta = -0.31$; 95% CI: -0.58, -0.03). Results were similar after controlling for gestational age at blood draw, season at blood draw, maternal race, prepregnancy BMI, smoking, and socioeconomic status.

DISCUSSION

Poor fetal growth continues to be a major public health issue worldwide (26). Inadequate growth in utero is not only linked to immediate morbidity and mortality risk of offspring but also lifelong risk of chronic disease (27, 28). Maternal vitamin D deficiency has been associated with poor fetal growth in terms of a reduced term birth weight and higher risk of small-for-gestational age, including in the current multicenter US cohort, in whom we showed an association between maternal 25(OH)D and both term birth weight and small-for-gestational age (12). However, the literature that may explain mechanisms that underlie the vitamin D and fetal growth connection is sparse.

TABLE 4
Associations between maternal 25(OH)D at ≤26 wk of gestation and placental vascular pathology by infant sex in pregnancies randomly selected for vitamin D assessment from the eligible cohort, the Collaborative Perinatal Project (1959–1966)[1]

25(OH)D	No pathology	Placental vascular pathology	Adjusted OR (95% CI)[2]
	n (%)	n (%)	
Overall			
≥80 nmol/L	248 (88.3)	33 (11.7)	0.89 (0.59, 1.35)
50–79 nmol/L	557 (88.1)	75 (11.9)	0.94 (0.69, 1.28)
<50 nmol/L	1003 (88.4)	132 (11.6)	1 (reference)
Male[3]			
≥80 nmol/L	126 (90.7)	13 (9.4)	0.51 (0.27, 0.95)
50–79 nmol/L	267 (87.8)	37 (12.2)	0.74 (0.48, 1.12)
<50 nmol/L	491 (85.5)	83 (14.5)	1 (reference)
Female[3]			
≥80 nmol/L	122 (85.9)	20 (14.1)	1.58 (0.90, 2.78)
50–79 nmol/L	290 (88.4)	38 (11.6)	1.26 (0.80, 1.99)
<50 nmol/L	512 (91.2)	49 (8.7)	1 (reference)

[1] n = 2048 pregnancies; n = 2030 women (18 women with one repeated pregnancy). 25(OH)D, 25-hydroxyvitamin D.
[2] Conditional logistic regression models were conditioned on the Collaborative Perinatal Project study site and adjusted for infant sex; there was no meaningful difference after adjustment for gestational age at blood draw, season at blood draw, maternal race, prepregnancy BMI, smoking, and socioeconomic status.
[3] $P = 0.016$ for effect modification by infant sex in adjusted models with 25(OH)D categorized as shown in the table.

We sought to examine the association between maternal vitamin D status and the development of placental vascular pathology to potentially explain part of the connection with fetal growth. The placenta plays a critical role in fetal health, and it is well known that placental pathologies, specifically vascular lesions, negatively affect fetal growth (29). We showed clear differences by infant sex, such that higher maternal 25(OH)D was associated with lower risk of pathology in pregnancies with male fetuses and no association in females. In mothers with male offspring and vitamin D concentrations ≥80 nmol/L [≥75 nmol/L is considered an optimal concentration by the Endocrine Society (30)] compared with <50 nmol/L [considered insufficient per the Institute of Medicine (24)] had 49% reduced risk of developing a vascular pathology in the placenta. However, because the development of vascular lesions may have occurred in early pregnancy and overlapped our vitamin D measurement at a median of 20 wk of gestation, the directionality of the association cannot be certain.

The CYP27B1 enzyme [which activates 25(OH)D] (31) and the vitamin D receptor (32) are both expressed and functional in the human placenta, which allow diverse biological roles for the active 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] locally. In cultured human syncytiotrophoblasts, it has been shown that 1,25$(OH)_2$D stimulates the secretion of estradiol (33), and estradiol affects neovascularization and placental blood flow (34). Although there is a paucity of biological basic data regarding the effect of vitamin D on placental vasculature, there are data from other model systems that have supported a plausible connection between vitamin D and vascular function. Vitamin D_3 administration improves endothelium- and smooth muscle–dependent systemic arterial vasorelaxation in a rat model (35), and a similar phenomenon has been observed in women as well, with lower 25(OH)D concentrations associated with poorer systemic arterial relaxation (36). Another important component of vascular function in the placenta, which is potentially influenced by vitamin D, is angiogenesis. In vitro, vitamin D improves the angiogenic properties of endothelial progenitor cells (37), and 1,25$(OH)_2$D increases vascular endothelial growth factor secretion in fibroblasts directly through the activation of the vascular endothelial growth factor promoter and induction of gene expression (38). To our knowledge, this is the first study to examine the relations between maternal 25(OH)D and placental vascular pathology. More work is needed to understand these relations.

To further explore the finding of an association between 25(OH)D and pathologies that differed by infant sex, we studied how vitamin D status modified the vascular pathology and birthweight relation in pregnancies with male and female infants separately. We observed that female fetuses exhibited reduced birth weight in the setting of placental vascular pathology and maternal vitamin D deficiency, but no association between pathology and birth weight was observed in girls without vitamin D deficiency or in boys. We postulate that the relation between placental vascular lesions and birth weight is modified by vitamin D only in girls because of sex-specific responses to hypoxia and undernutrition. There have been several examples of a sex-differential

response of fetuses to hypoxia. In a rat model, fetal brain (39) and aorta (40) responses to hypoxia differed by fetal sex. In an elegant murine model of maternal undernutrition, Ito et al (41) showed a significant increase in the heart weight to body weight ratio in female fetuses but not in male fetuses. They showed that the expression of hypoxia-inducible factor 1α (HIF1α), which is a protein that plays an essential role in cellular and systemic responses to hypoxia, was increased $\sim$1.3-fold in the male fetal heart under maternal undernutrition but remained unchanged in the female heart. Moreover, maternal undernutrition increased the messenger RNA concentration of prolyl hydroxylase 1, which contributes to the degradation of HIF1α, in the male but not female heart. These results suggested that maternal undernutrition may induce HIF1α expression in the fetal heart through distinct mechanisms depending on the sex of the fetus. In a rat model of intrauterine hypoxia, Patterson et al (42) showed sex differences in the vulnerability of the fetal heart to ischemia. Other authors have reported similar findings that male fetuses appear less sensitive to insults during pregnancy (43). Had we not examined the modification of the relation by infant sex, we would have reported that placental vascular pathology was associated with a modest reduction in the birth weight z score overall (-0.18, $P = 0.004$), similar to the findings of others (44).

We excluded preterm births from our investigation because fetal growth restriction is more difficult to accurately assess in premature infants, and we were interested in exploring underlying causes of the relation between maternal vitamin D and fetal growth restriction. Thus, although our study used data from >40 y ago when smoking was common and obesity was uncommon, there was a higher threshold for medically indicated preterm births that allowed more deliveries to naturally reach term. Also, the CPP conducted pathology examinations without regard to clinical outcomes, which made it is a rich source of data on placentas, mothers, and infants in a diverse US sample. Although we cannot be certain that serum 25(OH)D concentrations did not change because of long-term storage, we expect that any misclassification of vitamin D status would not have differed based on vascular pathology status and would have biased our results toward the null, which, in effect, would have made our observed associations more conservative (20).

In conclusion, in this pregnancy cohort from the 1960s with a diverse US population of women and racial representation, we showed that higher maternal circulating vitamin D was associated with lower risk of placental vascular pathology in pregnancies of boys. Future studies should confirm or refute these relations in modern populations. Vitamin D responsive genes should be explored in boys and girls to investigate mechanisms. Our findings may help to explain the apparent protective effect of maternal vitamin D in fetal growth restriction but differently for male and female offspring. More evidence is needed to understand biologic mechanisms that underlie the relation between vitamin D, placental vascular pathologies, and fetal growth. Our study supports the theory that boys and girls respond differently to maternal nutritional stimuli, including maternal vitamin D status. Infant sex differences should be explored in vitamin D research.

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The authors' responsibilities were as follows—ADG, HNS, WTP, and LMB: conducted the research; ADG: analyzed data, wrote the manuscript, and had primary responsibility for the final content of the manuscript; HNS, MAK, WTP, and LMB: provided critical input on the writing of the manuscript; and all authors: participated in the research design and read and approved the final manuscript. None of the authors reported a conflict of interest.

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