JOURNAL OF WOMEN'S HEALTH
Volume 27, Number 10, 2018

DOI: 10.1089/jwh.2017.6804

# Serum 25-Hydroxyvitamin D and Risk of Self-Reported Bacterial Vaginosis in a Prospective Cohort Study of Young African American Women

Kristen R. Moore, PhD, Quaker E. Harmon, MD, PhD, and Donna D. Baird, PhD

## Abstract

***Background:*** Bacterial vaginosis (BV), the leading cause of vaginal discharge, is associated with multiple adverse health outcomes; however, its etiology is unknown. BV treatment is not very effective, thus prevention approaches are needed. Studies investigating the impact of vitamin D on the risk of BV have had mixed findings, including two studies reporting increased risk of recurrent BV for women with higher vitamin D.
***Materials and Methods:*** Participants were nonpregnant women in a prospective fibroid study of African Americans (ages 23–34 years) from the Detroit area. The exposure was seasonally adjusted annual mean serum 25-hydroxyvitamin D [25(OH)D] at enrollment. The outcome was self-reported doctor-diagnosed BV over ~20 months between baseline and follow-up. Multivariable-adjusted binomial regression models estimated the risk of BV for a doubling of 25(OH)D and sufficient (≥20 ng/mL) versus deficient (<20 ng/mL) 25(OH)D.
***Results:*** In total, 1459 women were included. Median 25(OH)D was 15.2 ng/mL and 73% were deficient. Sixteen percent of participants reported BV diagnoses over follow-up, 78% of whom had recurrent BV. In multivariable-adjusted analyses, a doubling of 25(OH)D was associated with an increased, rather than the hypothesized decreased, risk of self-reported BV (risk ratio [RR] 1.22, 95% confidence interval 1.02–1.48). Sufficient women also had a significantly higher, rather than lower, risk of self-reported BV (RR 1.31). Results were robust to sensitivity analyses, and *post hoc* analyses showed no evidence of reverse causation.
***Conclusions:*** Overall, our findings do not support vitamin D deficiency as a risk factor for BV in these young, nonpregnant African American women.

**Keywords:** bacterial vaginosis, vitamin D, prospective

## Background

Bacterial vaginosis (BV) is the leading cause of vaginal discharge[1] and disproportionately burdens African American women compared with white women (51% vs. 23% in the National Health and Nutrition Examination Survey, respectively).[2] BV also may increase the risk of sexually transmitted infections, human immunodeficiency virus (HIV), pelvic inflammatory disease, spontaneous abortion, and preterm birth.[3–8] However, the etiology of BV is largely unknown. Due to its high recurrence and lack of effective treatment options, approaches to prevent BV are needed.[9] Similar to BV, vitamin D deficiency is highly prevalent in African American women with over 80% of African Americans under the Institute of Medicine's (IOM) 20 ng/mL vitamin D sufficiency threshold[10] compared with 30% of white women.[11] Recently, vitamin D, an immune regulator,[12] has been studied in relation to BV with the theory that vitamin D deficiency could be a reason for racial disparity in BV prevalence.[13] Vitamin D deficiency has been found to be associated with numerous diseases such as cancer,[14] autoimmune disease,[15] and respiratory diseases such as asthma,[16] as well as some infectious diseases,[17] including tuberculosis[18] and HIV.[19] Possible mechanisms by which vitamin D might decrease risk of BV include (1) antimicrobial activity through regulation of cathelicidin, a defense peptide found in the genital tract[20]; (2) regulation of immune response to infection[21,22]; or (3) increased anti-inflammatory cytokine expression.[23]

Studies on the association between vitamin D and BV have had mixed findings. Among pregnant women, one observational study found a null association,[24] but most have found a higher prevalence of BV among those who are vitamin D deficient or insufficient.[25–28] In addition, a randomized controlled trial (RCT) found a trend of lower rates of BV with

Epidemiology Branch A3-05, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

increasing doses of vitamin D, although they were nonsignificant.[29] One of these studies used wet prep and/or culture[26] to diagnose BV and the rest used Nugent scoring.

Among nonpregnant women, null associations between vitamin D concentrations and BV have been reported in observational studies among nationally representative women with vitamin D < 30 ng/mL[28] (BV diagnosed by Nugent scoring) and HIV-negative women with vitamin D < 20 ng/mL[30] (BV diagnosed by Amsel). In contrast, an observational study of HIV-positive women found a higher prevalence of symptomatic BV (by Amsel criteria) for those with vitamin D deficiency[30]; and an RCT among women in Iran who had to have asymptomatic BV (diagnosed by Nugent scoring) and vitamin D deficiency to be enrolled, reported a higher BV cure rate in the vitamin D treatment arm.[31]

Two studies that evaluated recurrent BV had unexpected findings. A case–crossover study found an increased risk of recurrent BV (by Nugent scoring) in summer/fall versus winter/spring months[32] and a null association for those with no BV at enrollment. An RCT of African Americans who had to have symptomatic BV (by Amsel criteria) to be enrolled also suggested an increased risk of recurrent BV (after standard treatment) for those in the vitamin D arm,[13] although it was nonsignificant.

The inconsistency of associations found in the literature does not appear to be due to differences in diagnostic methods or whether participants were enrolled based on symptoms; however, the two studies that evaluated recurrent BV found unexpected findings and the number of studies focusing on symptomatic BV are limited. To contribute to the literature on nonpregnant women with symptomatic BV, the aim of this study was to prospectively examine the risk of self-reported BV in relation to 25-hydroxyvitamin D [25(OH)D] concentration in serum among a cohort of young African American women with predominantly recurrent BV diagnoses.

## Materials and Methods

We used serum samples and self-reported questionnaire data from participants in the ongoing National Institute of Environmental Health Sciences (NIEHS) Study of Environment, Lifestyle and Fibroids, described previously.[33] In brief, starting in 2010, the study enrolled a volunteer sample of 1695 African American women (ages 23–34 years) living in the Detroit, Michigan, area. Follow-up visits continue approximately every 20 months for 5 years. Women were ineligible if they had previously been diagnosed with fibroids; had a hysterectomy; had lupus, Grave's disease, Sjogren's scleroderma, or multiple sclerosis requiring medication; or ever had any type of cancer treated with radiation or chemotherapy. The study was approved by the institutional review boards of NIEHS and Henry Ford Health System.

### *Measurement of 25(OH)D*

The exposure of interest was the 25(OH)D concentration measured at baseline. Circulating concentrations of 25(OH)D are an established biomarker of vitamin D status from both ultraviolet (UV) and dietary exposure.[10] A nonfasting blood sample for analysis of 25(OH)D was drawn using a red-top vacutainer without additive or preservative and processed. Details on measurement of 25(OH)D were described previously.[34] In brief, the serum was aliquoted and stored at −80°C within 24 hours (90% within 5 hours of blood draw). Analysis of 25(OH)D was conducted at Heartland Laboratories using the LIAISON 25 OH Vitamin D Total assay, a competitive chemiluminescence immunoassay.[35,36] Based on blinded controls, the intra- and interassay coefficients of variation were 2.9% and 8.6%, respectively. Measurements of enrollment 25(OH)D were available for 1662 women. Serum concentrations of 25(OH)D vary seasonally due to differences in UV exposure, and participants provided serum samples year-round. Our outcome of interest was BV diagnosis between enrollment and the first follow-up visit [median 19 months (interquartile range [IQR]: 18–20 months)]. Therefore, to avoid misclassification by season of enrollment, we used enrollment serum concentrations to estimate an annual mean 25(OH)D using a cosinor model[34,37,38] denoted as $25(OH)D_{ANN}$ in this study.

Doctor diagnosis of BV (yes/no) was self-reported at the first follow-up visit (for BV diagnosed between baseline and the first follow-up). This analysis was performed among women who completed both baseline and follow-up 1 data collection and had a measure of 25(OH)D at enrollment ($n = 1459$; 86%).

### *Statistical analyses*

Binomial regression models were used to compute risk ratios (RRs) and 95% confidence intervals (CIs) to evaluate the risk of self-reported BV. We modeled $25(OH)D_{ANN}$ continuously on the $\log_2$ scale, as done previously.[37] The exponentiated regression coefficient from this model represented the association between doubling of $25(OH)D_{ANN}$ and risk of self-reported BV. We also modeled $25(OH)D_{ANN}$ as a binary variable (sufficient vs. deficient) based on the IOM cut point of 20 ng/mL; however, the median $25(OH)D_{ANN}$ in this population was 15.2 ng/mL, thus most of the population was deficient (73%). Therefore, in this population, doubling of $25(OH)D_{ANN}$ can be loosely interpreted as changing a woman from deficient to sufficient.

Potential baseline covariates (age, parity, alcohol use [within past year], body–mass index [BMI], smoking, current hormonal contraceptive [HC] use, education, douching, marital status, income, and sexual behavior variables [number of lifetime sex partners, herpes serostatus, chlamydia serostatus, and age at first sex]) were determined based on a review of both the BV and vitamin D literature. Then, we examined their association with self-reported BV at the first follow-up using RRs and 95% CIs and $25(OH)D_{ANN}$ using medians and IQRs.

The following baseline covariates were associated with both BV and $25(OH)D_{ANN}$ and were included in the full model: education (high school, some college, bachelors, or higher), marital status (never, current, or previous), BMI (<35 or ≥35), and alcohol (drinking level in the past year: low/moderate or heavy). Age (continuous in years) was not significantly associated with both outcome and exposure, but was included in the full model *a priori*.

### *Sensitivity analyses*

Because vitamin D status could change during the ~20 months between baseline and follow-up 1, we conducted an analysis limited to those with fairly stable concentrations (baseline and follow-up values for 25(OH)D concentrations differed by no more than 7 ng/mL), $n = 1011$; 70% of sample.

HC use has been associated with reduced BV,[39] and exogenous estrogen has been associated with increased 25(OH)D concentrations.[34] Thus, we wanted to ensure that a change in the use of HCs between baseline and follow-up 1 did not impact the results. Based on questionnaire responses, we categorized HC use over follow-up by hormonal component (estrogen-containing, progestin-only, or no use) and months of use over follow-up. As a sensitivity analysis, we excluded all women who began using any estrogen-containing contraceptive (combination contraceptive pill, contraceptive patch, or ring) and used it for at least half of follow-up time ($n=34$) and those who stopped using an estrogen-containing contraceptive over follow-up ($n=46$).

We also ran the analyses by both adjusting for and stratifying by a measure of financial difficulty in visiting a doctor for a routine health exam (yes/no) to ensure that access to care was not impacting findings. Women with better access to care may be more likely to have had a physician diagnosis of BV and may also have higher 25(OH)D concentrations.

In addition, to focus on extreme vitamin D deficiency, we analyzed the association for those sufficient (≥20 ng/mL) versus extremely deficient (≤10 ng/mL).

Table 1. Annual Mean 25(OH)D Median by Participant Characteristics ($N=1459$)

| *Characteristic* | n | % | *Median 25(OH)D (ng/mL)* | *IQR* |
|---|---|---|---|---|
| Self-reported BV at follow-up 1 | | | | |
| No | 1222 | 84 | 14.9 | 10.9–20.2 |
| Yes | 237 | 16 | 16.2 | 12.3–22.2 |
| Baseline characteristics | | | | |
| Age, years | | | | |
| 23–26 | 435 | 30 | 14.5 | 11.0–20.1 |
| 27–30 | 511 | 35 | 15.2 | 11.1–20.7 |
| 31–35[a] | 513 | 35 | 15.6 | 11.0–21.1 |
| Season (of blood draw)[b] | | | | |
| Winter | 307 | 21 | 13.4 | 9.6–18.5 |
| Spring | 334 | 23 | 14.2 | 10.4–20.0 |
| Summer | 434 | 30 | 18.9 | 14.3–23.7 |
| Fall | 384 | 26 | 14.6 | 11.3–20.8 |
| Education | | | | |
| ≤High school/GED | 315 | 22 | 13.4 | 10.0–18.4 |
| Some college or technical | 739 | 51 | 14.6 | 11.1–19.4 |
| ≥Bachelors | 404 | 28 | 17.7 | 12.6–24.6 |
| Missing | 1 | | | |
| Marital status | | | | |
| Never married | 855 | 59 | 14.2 | 10.8–20.1 |
| Currently married | 399 | 27 | 16.4 | 11.5–21.4 |
| Previously married | 205 | 14 | 16.6 | 11.9–20.9 |
| Body–mass index[c] | | | | |
| 15–24 | 291 | 20 | 17.3 | 12.6–23.3 |
| 25–29 | 300 | 21 | 16.0 | 11.5–21.8 |
| 30–34 | 271 | 19 | 16.9 | 12.2–22.8 |
| 35–80 | 597 | 41 | 13.4 | 10.3–17.8 |
| Alcohol use (in past year)[d] | | | | |
| None | 420 | 29 | 15.1 | 11.3–21.2 |
| Moderate | 760 | 52 | 15.7 | 11.4–21.5 |
| Heavy | 279 | 19 | 13.7 | 10.3–18.3 |
| Smoking | | | | |
| Nonsmoker | 1073 | 74 | 15.7 | 11.4–21.7 |
| Former | 108 | 7 | 15.5 | 11.3–19.0 |
| Current | 278 | 19 | 13.5 | 9.8–17.5 |
| Current hormonal contraception use | | | | |
| None | 1039 | 71 | 14.4 | 10.8–19.3 |
| Any estrogen | 197 | 14 | 19.5 | 13.6–25.3 |
| Progesterone-only | 221 | 15 | 15.7 | 11.3–21.3 |
| Missing | 2 | | | |

[a]No one over 34 was recruited, but some 34-year-olds had turned 35 by the time they had their visit.
[b]Concentrations of 25(OH)D in this row are the unadjusted measured values; the other rows of the table show the average annual mean 25(OH)D.
[c]Body–mass index was calculated as weight (kg)/height $(m)^2$
[d]Heavy drinkers were those who usually drank 6 or more drinks on days when they drank or drank 4+ drinks per sitting at least 2–3 times a month. Moderate drinkers were all others.
BV, bacterial vaginosis; GED, general education development; IQR, interquartile range.

Table 2. $25(OH)D_{ANN}$ Concentration and Risk of Self-Reported Bacterial Vaginosis Among 1459 23 to 34-Year-Old African American Women in the Study of Environment, Lifestyle and Fibroids, Detroit, MI Metropolitan Area, 2010–2012: Risk Ratios and 95% Confidence Intervals

| | | *Unadjusted risk ratio (95% CI)* | | *Multivariable-adjusted risk ratio[a] (95% CI)* | |
|---|---|---|---|---|---|
| | N | *For a doubling of 25(OH)$D_{ANN}$* | *For sufficient 25(OH)$D_{ANN}$ (compared with deficient)[b]* | *For a doubling of 25(OH)$D_{ANN}$* | *For sufficient 25(OH)$D_{ANN}$ (compared with deficient)[b]* |
| BV | | | | | |
| No | 1222 | 1.00 | 1.00 | 1.00 | 1.00 |
| Yes | 237 | 1.28 (1.08–1.53) | 1.38 (1.08–1.76) | 1.24 (1.04–1.49) | 1.34 (1.04–1.71) |

Annual mean 25(OH)D concentration.
[a]Adjusted for age, education, marital status, body–mass index, and alcohol; $n = 1221$—one participant had missing data on education.
[b]For $25(OH)D_{ANN}$ ≥20 ng/mL (sufficient) compared with <20 ng/mL (deficient).
CI, confidence interval.

We also conducted *post hoc* analyses to evaluate possible reverse causation. Our BV cases diagnosed during follow-up include both women who were experiencing a first diagnosis of BV and women who were experiencing a recurrence. We were concerned that women experiencing a recurrence may have been taking vitamin D supplements to help prevent BV. Because the number of women with a first diagnosis was small ($n = 53$), we focused on women who had a recurrence of BV during follow-up ($n = 184$) and compared them with women who had not been diagnosed with BV during follow-up ($n = 1222$), and then further limited the sample to those who were not using supplements ($n = 849$), thus eliminating the mechanism for reverse causation.

## Results

Of the 1695 women enrolled, 236 were ineligible for inclusion in the analyses, resulting in 1459 eligible participants. Ineligible participants included 34 without a 25(OH)D measurement and 202 who did not attend the follow-up 1 visit. The median $25(OH)D_{ANN}$ concentration was 15.2 ng/mL (IQR: 11.1–20.6 ng/mL), and 73% ($n = 1060$) were below the 20 ng/mL IOM cut point for sufficiency.

Sixteen percent ($n = 237$) of participants reported at least one BV diagnosis over follow-up. Most participants ($n = 184$, 78%) reported a recurrent diagnosis (they had reported a previous diagnosis at enrollment). Median $25(OH)D_{ANN}$ tended to be lower among women who reported lower education, higher BMI, never being married, not using an estrogen-containing HC, current smoking, and high alcohol use (Table 1). The median (IQR) $25(OH)D_{ANN}$ concentration was 14.9 ng/mL (10.9–20.2) for those with no self-reported doctor-diagnosed BV and 16.2 ng/mL (12.3–22.2) for those with a self-report of BV diagnosis (Table 1). In unadjusted analyses, a doubling of $25(OH)D_{ANN}$ was associated with an increased, not decreased, risk of self-reported BV (RR 1.28,

Table 3. Sensitivity Analyses for $25(OH)D_{ANN}$ and Risk of Self-Reported Bacterial Vaginosis Among 23 to 34-Year-Old African American Women in the Study of Environment, Lifestyle and Fibroids, Detroit, MI Metropolitan Area, 2010–2012: Risk Ratios and 95% Confidence Intervals

| | | *Multivariable-adjusted risk ratio[a] (95% CI)* | |
|---|---|---|---|
| | N | *For a doubling of 25(OH)$D_{ANN}$* | *For sufficient 25(OH)$D_{ANN}$ (compared with deficient)[b]* |
| BV | | | |
| Restrict to women who had within a 7-ng/mL change in 25(OH)D from baseline to follow-up 1 | | | |
| No | 857 | 1.00 | 1.00 |
| Yes | 154 | 1.37 (1.05–1.78) | 1.40 (1.00–1.94) |
| Remove participants who stopped estrogen HC use over follow-up or started estrogen HC use (for at least 50% of interval) | | | |
| No | 1154 | 1.00 | 1.00 |
| Yes | 215 | 1.22 (1.01–1.48) | 1.31 (1.01–1.70) |
| Adjust for financial difficulty to see a doctor for a routine physical[c] | | | |
| No | 1219 | 1.0 | 1.0 |
| Yes | 236 | 1.25 (1.04–1.50) | 1.35 (1.06–1.73) |
| Lower vitamin D deficient cut point to 10 ng/mL[d] | | | |
| No | 544 | — | 1.00 |
| Yes | 108 | — | 1.77 (1.16–2.70) |

Annual mean 25(OH)D concentration.
[a]Adjusted for age, education, marital status, body–mass index, and alcohol.
[b]For $25(OH)D_{ANN}$ ≥20 ng/mL (sufficient) compared with <20 ng/mL (deficient).
[c]Binary variable (not difficult or at least a little difficult) with three missing values.
[d]For $25(OH)D_{ANN}$ ≥20 ng/mL (sufficient) compared with ≤10 ng/mL (deficient); $n = 807$ missing where $25(OH)D_{ANN}$ was between 10 and 20 ng/mL.
HC, hormonal contraceptive.

95% CI 1.08–1.53) and the increased risk was also seen in women who were sufficient (≥20 ng/mL) versus deficient (Table 2). These results were slightly attenuated, but remained significant, in the multivariable-adjusted analysis (aRR: 1.22, 95% CI 1.02–1.48) (Table 2). Results were similar across the sensitivity analyses (Table 3). When stratifying by financial difficulty in seeing a doctor, we found that those with at least some difficulty accessing care had a higher risk of BV with a doubling of $25(OH)D_{ANN}$ (aRR: 1.65, 95% CI 1.21–2.26) compared with those with no difficulty (aRR: 1.08, 95% CI 0.86–1.36), with a p for interaction = 0.08. Results were similar for sufficient versus deficient $25(OH)D_{ANN}$. We would have expected the group with more difficulty accessing care to be less likely to report a diagnosis of BV, but we see the opposite.

When we examined the association of 25(OH)D with the risk of BV for the sample of women who had recurrent BV, the associations were even stronger (aRR: 1.30, 95% CI 1.05–1.60, for a doubling of $25(OH)D_{ANN}$ and aRR: 1.52, 95% CI 1.15–2.01, for sufficient vs. deficient 25(OH)D). Further restricting to those who were not using supplements, there was still a significant positive association between 25(OH)D and BV risk (aRR: 1.31, 95% CI 0.93–1.84, for a doubling of $25(OH)D_{ANN}$ and aRR: 1.78, 95% CI 1.17–2.71, for sufficient vs. deficient 25(OH)D). Reverse causation due to selective supplement use cannot explain our results.

## Discussion

In this sample of young, nonpregnant African American women, lower concentrations of 25(OH)D were not associated with an increased risk of self-reported predominantly recurrent BV; instead, the opposite was seen. The association of increased 25(OH)D with increased risk remained consistent after multivariable adjustment and across sensitivity analyses. We also did not find evidence for reverse causation due to selective vitamin D supplement use among participants with recurrent BV.

Although vitamin D deficiency has been associated with an increased risk of BV among pregnant women in some studies, overall the literature has been inconsistent. Two prior studies, like ours, found associations that were opposite to the hypothesized result (an increased risk of BV with increased vitamin D) and only among women with recurrent BV. One longitudinal study that used a case–crossover design only among women who had Nugent-based BV at some, but not all, visits ($n = 1335$) compared each woman's BV status in spring, summer, and fall with her own status in winter. They found that BV was statistically significantly more common in the summer and fall when 25(OH)D is expected to be highest, but only among those BV positive at enrollment ($n = 639$).[32] For women who were BV negative at enrollment ($n = 689$), there was a null association between BV and the season. The second study, an RCT among predominantly African American women with symptomatic Amsel-based BV, found that providing nine doses of high-dose vitamin D over 24 weeks in addition to standard therapy (metronidazole twice daily for 7 days) did not decrease the risk of Nugent-based BV recurrence.[13] Instead they found a higher proportion of BV recurrence (65%) and shorter time to recurrence (13.7 weeks) among women randomized to vitamin D compared with control women (48% and 14.3 weeks), although they were nonsignificant.[13] The mechanisms for these disparate findings for recurrent BV are unclear.

Our study has several limitations. Self-reported doctor-diagnosed BV may be subject to recall error and may underestimate the dysbiotic changes in the vaginal flora. Self-report of vaginal symptoms is typically what leads to screening for BV. However, as many as 40% of women with BV based on Nugent scoring do not report experiencing any symptoms such as odor or discharge.[40,41] Thus, the BV may go undiagnosed.

We did not use the gold standard assay of liquid chromatography–tandem mass spectrometry (LC-MS/MS) to measure 25(OH)D. The concentrations from the LIASION assay we used have been shown to underestimate the concentrations from LC-MS/MS.[42] Using National Institute of Standards and Technology samples, we estimate underestimation of 2–5 ng/mL across the range of values measured in our study. However, given this possible measurement error, our population would still have low concentrations of 25(OH)D, and the continuous values represent the relationship of the concentrations between individuals. However, given the overall low concentrations of 25(OH)D in this cohort, these findings may not be generalizable to populations with sufficient concentrations of 25(OH)D.

Our study did have the strengths of having extensive data to assess confounding, minimal missing data, and a sample size sufficient to provide good precision for the main hypothesis. However, if high 25(OH)D (≥30 ng/mL or even ≥40) is required for any risk reduction, our sample lacked sufficient numbers in that range for assessment.

Our findings do not support a role of vitamin D deficiency in the increased risk of BV in our population of young, African American nonpregnant women. Given the large public health burden and lack of information regarding the etiology and prevention of BV, further studies are needed to better understand the natural history of BV and to develop potential interventions to reduce its initiation and recurrence.

## Acknowledgments

The authors thank Drs. Srishti Shrestha and Christine Parks for review of a draft of the manuscript. The authors also thank their collaborators and study staff at Henry Ford Health System in Detroit, MI, and Social and Scientific Systems in Research Triangle Park, NC.

## Financial Support

The research was supported by the Intramural Research Program of the National Institute of Health (NIH), National Institute of Environmental Health Sciences (10-E-N044). Funding also came from the American Recovery and Reinvestment Act funds designated for NIH research. 25(OH)D measurements were funded by the Office of Disease Prevention of NIH.

## Author Disclosure Statement

No competing financial interests exist.

## References

1. Sobel JD. Vaginitis. N Engl J Med 1997;337:1896–1903.
2. Koumans EH, Sternberg M, Bruce C, et al. The prevalence of bacterial vaginosis in the United States, 2001–2004;

associations with symptoms, sexual behaviors, and reproductive health. Sex Transm Dis 2007;34:864–869.
3. Atashili J, Poole C, Ndumbe PM, Adimora AA, Smith JS. Bacterial vaginosis and HIV acquisition: A meta-analysis of published studies. AIDS 2008;22:1493–1501.
4. Brotman RM, Klebanoff MA, Nansel TR, et al. Bacterial vaginosis assessed by gram stain and diminished colonization resistance to incident gonococcal, chlamydial, and trichomonal genital infection. J Infect Dis 2010;202:1907–1915.
5. Cherpes TL, Meyn LA, Krohn MA, Lurie JG, Hillier SL. Association between acquisition of herpes simplex virus type 2 in women and bacterial vaginosis. Clin Infect Dis 2003;37:319–325.
6. Haggerty CL, Totten PA, Tang G, et al. Identification of novel microbes associated with pelvic inflammatory disease and infertility. Sex Transm Infect 2016;92:441–446.
7. Klebanoff MA, Hillier SL, Nugent RP, et al. Is bacterial vaginosis a stronger risk factor for preterm birth when it is diagnosed earlier in gestation? Am J Obstet Gynecol 2005; 192:470–477.
8. Nelson DB, Hanlon AL, Wu G, Liu C, Fredricks DN. First trimester levels of BV-associated bacteria and risk of miscarriage among women early in pregnancy. Mater Child Health J 2015;19:2682–2687.
9. Bradshaw CS, Morton AN, Hocking J, et al. High recurrence rates of bacterial vaginosis over the course of 12 months after oral metronidazole therapy and factors associated with recurrence. J Infect Dis 2006;193:1478–1486.
10. Institute of Medicine. Dietary reference intakes for calcium and Vitamin D. In: Ross AC, Taylor CL, Yaktine AL, Del Valle HB, eds. Washington (DC): The National Academies Press, 2011:487.
11. Forrest KY, Stuhldreher WL. Prevalence and correlates of vitamin D deficiency in US adults. Nutr Res 2011;31: 48–54.
12. Cantorna MT, Zhu Y, Froicu M, Wittke A. Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system. Am J Clin Nutr 2004;80:1717S–1720S.
13. Turner AN, Carr Reese P, Fields KS, et al. A blinded, randomized controlled trial of high-dose vitamin D supplementation to reduce recurrence of bacterial vaginosis. Am J Obstet Gynecol 2014;211:479 e471–479 e413.
14. Moukayed M, Grant WB. The roles of UVB and vitamin D in reducing risk of cancer incidence and mortality: A review of the epidemiology, clinical trials, and mechanisms. Rev Endocr Metab Disord 2017;18:167–182.
15. Rosen Y, Daich J, Soliman I, Brathwaite E, Shoenfeld Y. Vitamin D and autoimmunity. Scand J Rheumatol 2016;45: 439–447.
16. Han YY, Forno E, Celedon JC. Vitamin D insufficiency and asthma in a US nationwide study. J Allergy Clin Immunol Pract 2017;5:790–796.
17. Watkins RR, Lemonovich TL, Salata RA. An update on the association of vitamin D deficiency with common infectious diseases. Can J Physiol Pharmacol 2015;93: 363–368.
18. Huang SJ, Wang XH, Liu ZD, et al. Vitamin D deficiency and the risk of tuberculosis: A meta-analysis. Drug Design Dev Ther 2017;11:91–102.
19. Pinzone MR, Di Rosa M, Malaguarnera M, et al. Vitamin D deficiency in HIV infection: An underestimated and undertreated epidemic. Eur Rev Med Pharmacol Sci 2013;17: 1218–1232.
20. Cole AM. Innate host defense of human vaginal and cervical mucosae. Curr Top Microbiol Immunol 2006;306: 199–230.
21. Hoe E, Nathanielsz J, Toh ZQ, et al. Anti-inflammatory effects of vitamin D on human immune cells in the context of bacterial infection. Nutrients 2016;8:806–819.
22. Chesney RW. Vitamin D and the magic mountain: The anti-infectious role of the vitamin. J Pediatr 2010;156:698–703.
23. Azizieh F, Alyahya KO, Raghupathy R. Association between levels of vitamin D and inflammatory markers in healthy women. J Inflamm Res 2016;9:51–57.
24. Turner AN, Carr Reese P, Chen PL, et al. Serum vitamin D status and bacterial vaginosis prevalence and incidence in Zimbabwean women. Am J Obstet Gynecol 2016;215:332 e331–332 e310.
25. Bodnar LM, Krohn MA, Simhan HN. Maternal vitamin D deficiency is associated with bacterial vaginosis in the first trimester of pregnancy. J Nutr 2009;139:1157–1161.
26. Davis LM, Chang SC, Mancini J, Nathanson MS, Witter FR, O'Brien KO. Vitamin D insufficiency is prevalent among pregnant African American adolescents. J Pediatr Adolesc Gynecol 2010;23:45–52.
27. Dunlop AL, Taylor RN, Tangpricha V, Fortunato S, Menon R. Maternal vitamin D, folate, and polyunsaturated fatty acid status and bacterial vaginosis during pregnancy. Infect Dis Obstet Gynecol 2011;2011:216217.
28. Hensel KJ, Randis TM, Gelber SE, Ratner AJ. Pregnancy-specific association of vitamin D deficiency and bacterial vaginosis. Am J Obstet Gynecol 2011;204:41 e41–49.
29. Wagner CL, McNeil R, Hamilton SA, et al. A randomized trial of vitamin D supplementation in 2 community health center networks in South Carolina. Am J Obstet Gynecol 2013;208:137 e131–113.
30. French AL, Adeyemi OM, Agniel DM, et al. The association of HIV status with bacterial vaginosis and vitamin D in the United States. J Womens Health (Larchmt) 2011;20: 1497–1503.
31. Taheri M, Baheiraei A, Foroushani AR, Nikmanesh B, Modarres M. Treatment of vitamin D deficiency is an effective method in the elimination of asymptomatic bacterial vaginosis: A placebo-controlled randomized clinical trial. Indian J Med Res 2015;141:799–806.
32. Klebanoff MA, Turner AN. Bacterial vaginosis and season, a proxy for vitamin D status. Sex Transm Dis 2014;41:295–299.
33. Baird DD, Harmon QE, Upson K, et al. A prospective, ultrasound-based study to evaluate risk factors for uterine fibroid incidence and growth: Methods and results of recruitment. J Womens Health (Larchmt) 2015;24:907–915.
34. Harmon QE, Umbach DM, Baird DD. Use of estrogen-containing contraception is associated with increased concentrations of 25-Hydroxy vitamin D. J Clin Endocrinol Metab 2016;101:3370–3377.
35. Ersfeld DL, Rao DS, Body JJ, et al. Analytical and clinical validation of the 25 OH vitamin D assay for the LIAISON automated analyzer. Clin Biochem 2004;37:867–874.
36. Wagner D, Hanwell HE, Vieth R. An evaluation of automated methods for measurement of serum 25-hydroxyvitamin D. Clin Biochem 2009;42:1549–1556.
37. Jukic AM, Upson K, Harmon QE, Baird DD. Increasing serum 25-hydroxyvitamin D is associated with reduced odds of long menstrual cycles in a cross-sectional study of

African American women. Fertil Steril 2016;106:172–179 e172.
38. Sachs MC, Shoben A, Levin GP, et al. Estimating mean annual 25-hydroxyvitamin D concentrations from single measurements: The multi-ethnic study of atherosclerosis. Am J Clin Nutr 2013;97:1243–1251.
39. Vodstrcil LA, Hocking JS, Law M, et al. Hormonal contraception is associated with a reduced risk of bacterial vaginosis: A systematic review and meta-analysis. PLoS One 2013;8:e73055.
40. Klebanoff MA, Schwebke JR, Zhang J, Nansel TR, Yu KF, Andrews WW. Vulvovaginal symptoms in women with bacterial vaginosis. Obstet Gynecol 2004;104:267–272.
41. Nelson DB, Bellamy S, Odibo A, Nachamkin I, Ness RB, Allen-Taylor L. Vaginal symptoms and bacterial vaginosis (BV): How useful is self-report? Development of a screening tool for predicting BV status. Epidemiol Infect 2007;135: 1369–1375.
42. Farrell CJ, Martin S, McWhinney B, Straub I, Williams P, Herrmann M. State-of-the-art vitamin D assays: A comparison of automated immunoassays with liquid chromatography-tandem mass spectrometry methods. Clin Chem 2012;58: 531–542.

Address correspondence to:
*Kristen R. Moore, PhD*
*Epidemiology Branch A3-05*
*National Institute of Environmental Health Sciences*
*Research Triangle Park, NC 27709*

*E-mail:* kristen.moore@nih.gov