GYNECOLOGY

A blinded, randomized controlled trial of high-dose vitamin D supplementation to reduce recurrence of bacterial vaginosis

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OBJECTIVE: Low serum vitamin D levels have been associated with increased prevalence of the reproductive tract condition bacterial vaginosis (BV). The objective of this trial was to evaluate the effect of high-dose vitamin D supplementation on BV recurrence.

STUDY DESIGN: This randomized, placebo-controlled, double-blinded trial enrolled 118 women with symptomatic BV from an urban sexually transmitted disease clinic (clinicaltrials.gov registration NCT01450462). All participants received 500 mg of oral metronidazole twice daily for 7 days. Intervention participants (n = 59) also received 9 doses of 50,000 IU of cholecalciferol (vitamin D3) over 24 weeks; control women (n = 59) received matching placebo. Recurrent BV was assessed via Nugent scoring after 4, 12, and 24 weeks. We assessed the effect of the intervention using an intention-to-treat approach, fitting Cox proportional hazards models to evaluate recurrent BV over the follow-up period.

RESULTS: Most participants (74%) were black, with a median age of 26 years. Median presupplementation serum 25-hydroxyvitamin D [25(OH)D] was similar across randomization arms: 16.6 ng/mL in the vitamin D arm and 15.8 ng/mL in the control arm. At trial completion, median 25(OH)D among women receiving vitamin D was 30.5 ng/mL, vs 17.8 ng/mL in control women; 16% of women receiving vitamin D and 57% receiving placebo remained vitamin D deficient (<20 ng/mL). BV prevalence among women randomized to vitamin D was very similar to those randomized to placebo at the 4- and 12-week visits, but by the 24-week visit, BV prevalence was 65% among women in the vitamin D arm and 48% among control women. BV recurrence was not reduced by vitamin D supplementation (intention-to-treat hazard ratio, 1.11; 95% confidence interval, 0.68—1.81). Among women experiencing recurrent BV, median time to recurrence was 13.7 weeks in the vitamin D arm and 14.3 weeks in the control arm.

CONCLUSION: Women receiving vitamin D experienced significant increases in serum 25(OH)D, but this increase was not associated with decreased BV recurrence in this high-risk sexually transmitted disease clinic population.

Key words: bacterial vaginosis, randomized controlled trial, sexually transmitted disease clinic, vitamin D

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Bacterial vaginosis (BV), the most common vaginal infection worldwide among reproductive-age women,[1] is associated with increased acquisition and transmission of human immunodeficiency virus (HIV)[2,3] and numerous obstetric complications.[4,5] BV is highly persistent and recurrent after treatment.[6] However, the etiology of BV is not clear. The most consistent risk factor for

EDITORS' ★ CHOICE

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Bio-Tech Pharmacal (Fayetteville, AR) donated the vitamin D and placebo products used in this trial. Bio-Tech Pharmacal had no involvement in any other aspect of the research, including protocol development, data collection, analysis, manuscript writing, or any other component of trial implementation or interpretation. The authors have otherwise no commercial or other association that might pose a conflict of interest.

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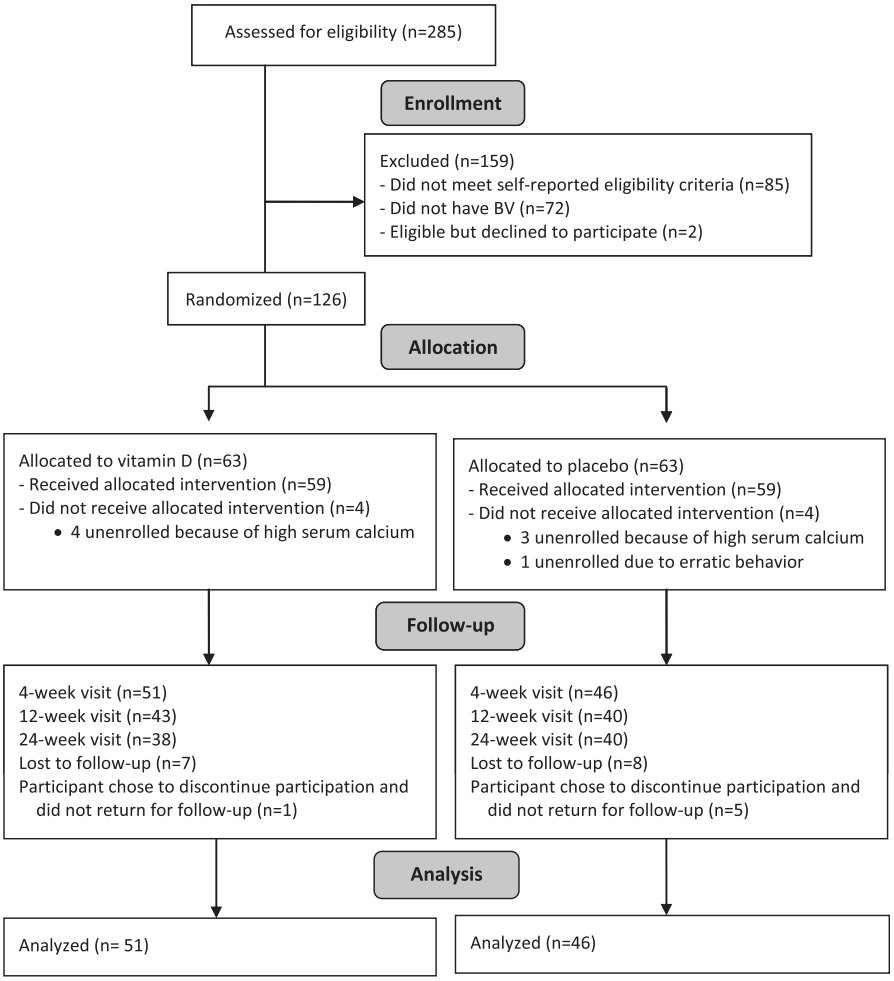
FIGURE 1
Flow diagram of screening, enrollment, follow-up, and analysis of study participants



BV, bacterial vaginosis.

Turner. Vitamin D and bacterial vaginosis. Am J Obstet Gynecol 2014.

BV in US populations is non-white race, an association that persists after adjustment for all known confounding factors.[7] According to the US National Health and Nutrition Examination Survey (NHANES), 52% of black women have BV compared with 23% of white women.[8]

Vitamin D is essential to immune function, both stimulating mechanisms associated with pathogen elimination and regulating immune response.[9] Vitamin D levels, quantified clinically as serum 25-hydroxyvitamin D [25(OH)D], also vary by race: 90% of US blacks, Hispanics, and Asians have vitamin D levels below the threshold of sufficiency set by the US Institute of Medicine (IOM) (30 ng/mL), compared with 75% of non-Hispanic whites.[10-12] Thus, we hypothesized that the link between race and BV could be a conflation of 2 other associations: (1) race and vitamin D, and (2) vitamin D and BV.

Several cross-sectional studies report that vitamin D insufficiency is associated with BV in pregnant women.[13-16] In contrast, 2 vitamin D trials in pregnant women examined BV as a secondary endpoint[17,18]; neither found an effect of daily vitamin D supplementation on BV.[19] Two studies examined the correlation between vitamin D and BV in nonpregnant women. One reported no association.[16] In the other, vitamin D deficiency was associated with BV in HIV-positive women but not HIV-negative women.[20] A case-crossover study of nonpregnant women used season as a proxy for vitamin D level, and found no evidence of increased BV prevalence during seasons when vitamin D levels are expected to be lowest.[21]

We conducted a randomized controlled trial of high-dose vitamin D supplementation in nonpregnant, BV-positive women recruited from a sexually transmitted disease (STD) clinic. We aimed to quantify the effect of high-dose vitamin D supplementation on BV recurrence.

Materials and Methods

Study design and setting

This randomized, placebo-controlled, double-blinded, single-site trial was conducted in a public STD clinic. Trial design and results are reported according to CONSORT guidelines.[22,23] Participants attended 4 visits: enrollment and after 4, 12, and 24 weeks (Figure 1). The study was approved by the Ohio State University Institutional Review Board on May 25, 2011 (protocol 2011H0089) and was registered at clinicaltrials.gov (NCT01450462).

Recruitment, eligibility, and informed consent

All female patients received study information at registration. Women who expressed interest were screened for self-reported eligibility criteria. Eligible women provided written consent.

Eligible women were 18-50 years old; premenopausal; had at least 1 ovary; spoke English; and were BV-positive on clinical examination by modified Amsel criteria (vaginal pH >4.5; thin, homogenous discharge; and positive "whiff" test).[24] Excluded women were pregnant (by report or rapid urine test) or planning pregnancy within 6 months; breastfeeding; currently menstruating; or self-reporting kidney disease or kidney stones, hypercalcemia, hypercalciuria, sarcoidosis, histoplasmosis, thyroid disease, lymphoma, or tuberculosis. Concomitant infection with HIV or other STDs was not an exclusion criterion.

Treatment

All participants received standard BV therapy at no cost: 500 mg of metronidazole orally twice daily for 7 days. Women also received no-cost treatment for other infections diagnosed at enrollment according to normal clinic procedures.

Randomization

Using a permuted block design developed using software (SAS, version 9.2; SAS Institute Inc, Cary, NC), participants were randomized into 4 equal-sized treatment arms labeled by color. Two arms received vitamin D and 2 received placebo. The code linking which color corresponded to which product was not known to participants, staff, investigators, or data analysts. Randomization assignments were placed in individual opaque envelopes, each labeled by participant identification number (PIN). Sealed envelopes were stored in PIN order in a locked cabinet. At enrollment, each participant, together with staff, opened the envelope corresponding to the next available PIN to reveal her color group assignment.

Intervention

Women received their assigned study product in prefilled pill boxes. Women in the vitamin D groups received 9 capsules, each containing 50,000 IU of vitamin D_3 (cholecalciferol) (BioTech Pharmacal, Fayetteville, AR) and women in the control groups received 9 placebo capsules with identical appearance to the vitamin D. Women were instructed to take the capsules 1, 2, 3, 4, 8, 12, 16, 20, and 24 weeks after enrollment. Participants received a pill schedule at enrollment, a duplicate schedule via post 1 week later, and text message reminders each day a capsule was to be taken. The target date for each capsule was also written in permanent marker directly on pill compartments. Women who reported at enrollment that they regularly took vitamin D supplements were requested to stop for the duration of the trial.

Specimen collection, processing, and testing

At the enrollment and 24-week visits, women underwent comprehensive clinical examinations with collection of blood and vaginal and cervical swabs for STD and reproductive tract infection testing. Chlamydia and gonorrhea were diagnosed by nucleic acid amplification testing; syphilis through rapid plasma reagin testing and confirmed by *Treponema pallidum* particle agglutination assay; HIV by rapid testing on plasma; trichomoniasis by microscopy and culture; and yeast by microscopy. Total serum calcium was assessed within 7 days of enrollment, and women with levels above the normal range—for whom vitamin D supplementation could be unsafe—were unenrolled. At the 4- and 12-week visits, women self-collected vaginal swabs, which were used to create slides of vaginal material. Slides from all 4 visits were stored and underwent Gram staining and Nugent scoring for BV detection after trial conclusion; technicians scoring the slides were blinded to treatment assignment. Stored serum from each visit was used to quantify 25(OH)D at the end of the trial, using the Liaison 25 OH vitamin D total assay (DiaSorin, Saluggia, Italy). Pregnancy was assessed via urine testing at each visit. Women found to be pregnant remained in the study but had their assigned study product discontinued immediately.

Questionnaire

At enrollment and each follow-up visit, women underwent face-to-face interviews to collect data on demographics, sexual behavior, adherence, and other information. Women were asked about 19 individual side effects previously associated with vitamin D supplementation or metronidazole treatment. Endorsement of any side effect was recorded as an adverse event (AE).

Safety

Participants' serum calcium levels were checked at enrollment, 4 weeks, and 24 weeks. Safety was further monitored by an independent safety committee, which reviewed AEs by blinded study arm after half the anticipated person-time for the trial had been accrued.

Statistical analysis

All analyses were conducted using software (SAS Institute). We first examined changes in serum 25(OH)D levels by randomization group over time. Next, we examined simple BV prevalence at each visit, by randomization group. Nugent score of 7-10 on Gram-stained vaginal smear was interpreted as BV.[25] We estimated BV-free survival time using the Kaplan-Meier method.[26] We tested the homogeneity of survival functions across randomization groups using the log rank test. Our primary intention-to-treat analysis used Cox proportional hazards models to estimate the hazard ratio (HR) and 95% confidence interval (CI) for the effect of randomization assignment on time to BV recurrence. Our secondary analysis used extended Cox proportional hazard models, and modeled the effect of time-varying 25(OH)D level (rather than randomization assignment) on time to BV recurrence; that analysis also controlled for age and race. In all Cox models, women who missed a visit but returned for later visits were assumed to have remained BV-negative during the missing visit.

We performed 3 exploratory sensitivity analyses. First, using a threshold of $\alpha = 0.10$ to classify imbalance in participant characteristics at enrollment, we ran an adjusted Cox model controlling for those factors which, due to chance, were not evenly balanced by randomization. Second, we limited the analysis to women who had 25(OH)D levels <20 ng/mL (the IOM threshold for vitamin D deficiency) at enrollment, to determine whether the effect of vitamin D supplementation on BV recurrence was different for this subgroup. Third, we assessed the impact of using Amsel criteria to diagnosis BV at enrollment, by restricting the analysis population to women also determined to be BV-positive at enrollment by Nugent scoring.

Power

We anticipated 20% loss to follow-up and BV recurrence in the placebo arm of 50% within 24 weeks.[6,27] Under these assumptions, with $\alpha = 0.05$, we estimated that 120 women (60 per group) would yield 80% power to detect an absolute difference in BV recurrence of

TABLE 1
Demographic, behavioral, and clinical characteristics of participants at enrollment, overall and by randomization group

Characteristic	Vitamin D, n = 59		Control, n = 59		Total, n = 118		*P* value
	n	(%)	n	(%)	n	(%)	
Race/ethnicity[a]							
Black	44	(75)	43	(73)	87	(74)	.84
White	15	(25)	16	(27)	31	(26)	.84
American-Indian/Alaskan Native	3	(5)	3	(5)	6	(5)	1.00
Asian/Pacific-Islander	0	(0)	1	(2)	1	(1)	.32
Hispanic	3	(5)	4	(7)	7	(6)	.70
Education							
Finished high school	52	(88)	49	(83)	101	(86)	.43
Finished college	8	(14)	6	(10)	14	(12)	.57
Main partner is:[b]							
Man	34	(58)	31	(53)	65	(55)	
Woman	2	(3)	2	(3)	4	(3)	.85
No main partner	23	(39)	26	(44)	49	(42)	
Season of enrollment							
Fall (September-November)	19	(32)	21	(36)	40	(34)	
Winter (December-February)	16	(27)	14	(24)	30	(25)	.85
Spring (March-May)	19	(32)	19	(32)	38	(32)	
Summer (June-August)	5	(50)	5	(50)	10	(8)	
Cohabitate with main partner[c]	10	(28)	11	(33)	21	(30)	.75
Employed full- or part-time	40	(68)	31	(53)	71	(60)	.09
Food insecure in last year[d]	19	(32)	19	(32)	38	(32)	1.00
Housing insecure in last year[e]	25	(42)	23	(39)	48	(41)	.71

Turner. Vitamin D and bacterial vaginosis. Am J Obstet Gynecol 2014. *(continued)*

24%, comparing the vitamin D and placebo arms.

RESULTS

Screening

From September 2011 through January 2013, 285 women underwent screening and 85 were ineligible for at least 1 reason (Figure 1). Of 200 women eligible by self-reported criteria, 128 were BV-positive by modified Amsel criteria and 126 enrolled. Following randomization, 1 woman began acting erratically and was immediately unenrolled. Seven women (6%) had abnormal serum calcium levels and were unenrolled. The final sample included 59 women randomized to vitamin D and 59 randomized to placebo. In all, 97 participants (82%) returned after enrollment: 51 (86%) in the vitamin D arm and 46 (78%) in the placebo arm returned after 4 weeks, and 38 women (64%) in the vitamin D arm and 40 control women (68%) returned after 24 weeks (Figure 1). Among returning participants, median follow-up was 24.0 weeks in the vitamin D arm and 24.8 weeks in the control arm.

Participant characteristics at enrollment

Most participants (n = 87; 74%) were black, with a median age of 26 years (Table 1). Women's median lifetime number of male sex partners was 10 (interquartile range [IQR], 5–20 partners). A large minority (n = 48; 41%) reported lifetime experience with anal sex, and 32 (27%) reported sex with women. All women were BV-positive by modified Amsel criteria, and when vaginal smears from enrollment were Nugent scored at the end of the trial, 80% of the sample was found to be BV-positive at enrollment by Nugent score (Table 1).

In all, 91 women (77%) reported a past BV diagnosis before enrollment. Several participants tested positive for STDs at enrollment (Table 1), but only trichomoniasis varied significantly by randomization group ($P = .05$).

TABLE 1
Demographic, behavioral, and clinical characteristics of participants at enrollment, overall and by randomization group *(continued)*

Characteristic	Vitamin D, n = 59		Control, n = 59		Total, n = 118		*P* value
	n	(%)	n	(%)	n	(%)	
Self-rated health							
Excellent	7	(12)	7	(12)	14	(12)	
Very good	17	(29)	19	(32)	36	(31)	
Good	26	(44)	20	(34)	46	(39)	.70
Fair	9	(15)	11	(19)	20	(17)	
Poor	0	(0)	2	(3)	2	(2)	
Depression screen positive[f]	25	(42)	28	(47)	53	(45)	.72
Ever pregnant	39	(66)	40	(68)	79	(67)	.84
Current user of vitamin D supplements	11	(19)	6	(10)	18	(15)	.20
Currently using any method of contraception[g]	23	(39)	26	(44)	49	(42)	.58
Hormonal methods (oral contraceptive pills, implants, injectable, patch, ring, hormonal IUD)	6	(26)	7	(27)	13	(27)	.95
Male condoms	14	(61)	13	(50)	27	(55)	.44
Female condoms	1	(4)	1	(4)	2	(4)	1.00
IUD (copper or unknown type)	1	(4)	1	(4)	2	(4)	1.00
Sterilization	3	(13)	9	(35)	12	(24)	.10
Other (diaphragms, spermicides, douching, withdrawal, rhythm, abstinence, morning after pills)	1	(4)	3	(12)	4	(8)	.61
Condom use, last 3 mo							
0% of vaginal acts with men	24	(41)	16	(27)	40	(34)	
>0% and <100% of vaginal acts with men	24	(41)	32	(54)	56	(47)	.32
100% of vaginal acts with men	7	(12)	6	(10)	13	(11)	
Ever anal sex	23	(39)	25	(42)	48	(41)	.55
Ever sex with women	18	(31)	14	(24)	32	(27)	.41
Concurrent sexual partnerships in last 3 mo[h]	11	(19)	20	(34)	31	(26)	.03
Feminine hygiene products before, during, or after sex in last 3 mo	24	(41)	20	(34)	44	(37)	.58
Ever douched	50	(85)	41	(69)	91	(77)	.09
Ever past BV diagnosis (self-reported, prior to enrollment)	48	(81)	43	(73)	91	(77)	.23
Nugent score							
Normal flora (Nugent 0-3)	3	(5)	3	(5)	6	(5)	
Intermediate (Nugent 4-6)	7	(12)	10	(17)	17	(14)	.58
BV (Nugent 7-10)	49	(83)	45	(76)	94	(80)	
Insufficient specimen (unscorable)	0	(0)	1	(2)	1	(1)	

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TABLE 1
Demographic, behavioral, and clinical characteristics of participants at enrollment, overall and by randomization group *(continued)*

Characteristic	Vitamin D, n = 59		Control, n = 59		Total, n = 118		*P* value
	n	(%)	n	(%)	n	(%)	
Infections							
Chlamydia	5	(8)	6	(10)	11	(9)	.75
Gonorrhea	3	(5)	6	(10)	9	(8)	.30
Trichomoniasis	6	(10)	14	(24)	20	(17)	.05
Syphilis	0	(0)	0	(0)	0	(0)	1.00
Yeast	4	(7)	4	(7)	8	(7)	1.00
Prevalent HIV (not new diagnoses)	2	(3)	0	(0)	2	(2)	
	Median	**(IQR)**	**Median**	**(IQR)**	**Median**	**(IQR)**	
Age, y	28	(22–36)	25	(22–32)	26	(22–33)	.45
Age of main partner, y[c]	35	(25.5–42)	28	(23–31)	30	(22–38)	.01
BMI	28.0	(24.6–32.1)	25.8	(22.9–32.0)	27.3	(23.3–32.1)	.21
Gravidity	1	(0–3)	1	(0–3)	1	(0–3)	.72
Age at first sex, y	16	(14–17)	15	(14–17)	16	(14–17)	.46
Lifetime no. male partners	11	(6–20)	8	(5–15)	10	(5–20)	.10
No. male partners, last 3 mo	1	(1–2)	1	(1–2)	1	(1–2)	.97
No. vaginal sex acts with men, last 3 mo	15	(5–36)	10	(3–24)	11	(4–30)	.11
Lifetime no. female partners[i]	3	(2–3)	2	(2–8)	3	(2–6)	.65

BMI, body mass index; *BV*, bacterial vaginosis; *HIV*, human immunodeficiency virus; *IQR*, interquartile range; *IUD*, intrauterine device.

[a] Some women reported >1 race, so totals sum to >100%. Race and ethnicity were queried together: "What race and ethnicity do you consider yourself? If you consider yourself to be in >1 group, please tell me all the groups that you are part of." Response options, which were read aloud to participant, were: black/African American, white, American Indian/Alaskan Native, Asian/Pacific Islander, Hispanic or Latino(a), other race/ ethnicity (with response recorded by interviewer); [b] Women were considered to have main partner if they answered "yes" to question, "Do you have a main partner right now? By 'main partner,' I mean a spouse or other committed partner whom you are romantically involved with."; [c] Among those reporting main partner; [d] Women were considered food insecure if they answered "yes" to question, "In the last year, have you ever been concerned about having enough food for yourself or your family?"; [e] Women were considered housing insecure if they answered "yes" to question, "In the last year, have you ever been concerned about having a place to live for yourself or your family?"; [f] No. and proportion answering "yes" to either or both questions on 2-question Patient Health Questionnaire; [g] Denominator for proportions of women using each contraceptive method type is total no. of women reporting use of any method. Because women could report multiple methods, proportions sum to >100%; [h] Women were considered to have concurrent sexual partnerships if they answered "yes" to question, "In the past 3 months, did you have sex with 1 partner while involved in a sexual relationship with another partner during the same period of time? This may include times when you did not consider yourself to be in a committed relationship."; [i] Among women who report any lifetime sex with women.

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Participant characteristics were generally balanced between randomization groups, but we also observed some important differences. Women randomized to the control arm were more likely to report concurrent partnerships in the last 3 months ($P = .03$). These women were also somewhat less likely than women in the vitamin D arm to be employed ($P = .09$) and to have ever douched ($P = .09$). Control women had somewhat fewer lifetime male sexual partners than women randomized to vitamin D ($P = .10$). Among women with main partners, control women reported significantly younger partners than women in the vitamin D arm ($P = .01$) (Table 1).

We also compared the characteristics of women who completed the trial (n = 78) with those who failed to complete the final visit (n = 40). Very few differences were observed (Table 2). Women who completed the trial were somewhat more likely to have ever douched ($P = .07$), and they also reported higher median numbers of lifetime male partners ($P = .03$) and somewhat higher median numbers of sex acts with men in the last 3 months ($P = .09$).

Vitamin D levels

Presupplementation vitamin D levels were similar: women randomized to the vitamin D arm had median 25(OH)D of 16.6 ng/mL (IQR, 12.1–21.2 ng/mL), vs 15.8 ng/mL (IQR, 11.8–23.3 ng/mL) for control women. Vitamin D deficiency at enrollment [25(OH)D <20 ng/mL] was present in 71% of women randomized to vitamin D and 68% of control women. Throughout follow-up, women in the vitamin D arm had significantly higher median vitamin D levels than control women (Figure 2). By the final visit, the median 25(OH)D level in the vitamin D

TABLE 2
Selected demographic, behavioral, and clinical characteristics of participants, comparing women completing trial (n = 78) with those lost to follow-up (n = 40)

Characteristic	Completed trial, n = 78		Lost to follow-up, n = 40		*P* value
	n	(%)	n	(%)	
Randomization group					
Vitamin D	38	(64)	21	(36)	.70
Placebo	40	(68)	19	(32)	
Race/ethnicity[a]					
Black	56	(72)	31	(78)	.51
White	22	(28)	9	(23)	.51
American-Indian/Alaskan Native	3	(4)	3	(8)	.40
Asian/Pacific-Islander	0	(0)	1	(3)	.34
Hispanic	4	(5)	3	(8)	.69
Education					
Finished high school	67	(86)	34	(85)	.90
Finished college	11	(14)	3	(8)	.29
Employed full- or part-time	48	(62)	23	(58)	.67
Current user of vitamin D supplements	12	(15)	5	(13)	.69
Currently using any method of contraception[b]	31	(40)	18	(45)	.58
Hormonal methods (oral contraceptive pills, implants, injectable, patch, ring, hormonal IUD)	7	(23)	6	(33)	.41
Male condoms	17	(55)	10	(56)	.96
Female condoms	1	(3)	1	(6)	.69
IUD (copper or unknown type)	2	(6)	0	(0)	.52
Sterilization	8	(26)	4	(22)	.78
Other (diaphragms, spermicides, douching, withdrawal, rhythm, abstinence, morning after pills)	4	(13)	0	(0)	.28
Condom use, last 3 mo					
0% of vaginal acts with men	29	(40)	11	(31)	
>0% and <100% of vaginal acts with men	37	(51)	19	(53)	.46
100% of vaginal acts with men	7	(10)	6	(17)	
Ever anal sex	33	(42)	15	(38)	.69
Ever sex with women	22	(28)	10	(25)	.71
Concurrent sexual partnerships in last 3 mo[c]	23	(30)	8	(22)	.37
Ever douched	65	(83)	26	(68)	.07
Ever past BV diagnosis (self-reported, prior to enrollment)	63	(82)	28	(76)	.44
Nugent score at enrollment					
Normal flora (Nugent 0-3)	4	(5)	2	(5)	
Intermediate (Nugent 4-6)	12	(15)	5	(13)	.81
BV (Nugent 7-10)	62	(79)	32	(82)	
Insufficient specimen (unscorable)	0	(0)	1	(3)	

Turner. Vitamin D and bacterial vaginosis. Am J Obstet Gynecol 2014. *(continued)*

TABLE 2
Selected demographic, behavioral, and clinical characteristics of participants, comparing women completing trial (n = 78) with those lost to follow-up (n = 40) *(continued)*

Characteristic	Completed trial, n = 78 n	(%)	Lost to follow-up, n = 40 n	(%)	*P* value
Infections					
Chlamydia	8	(10)	3	(8)	.63
Gonorrhea	7	(9)	2	(5)	.44
Trichomoniasis	14	(18)	6	(15)	.68
Syphilis	0	(0)	0	(0)	1.00
Yeast	5	(6)	3	(8)	.82
Prevalent HIV (not new diagnoses)	2	(3)	0	(0)	
	Median	**(IQR)**	**Median**	**(IQR)**	
Age, y	28	(22–35)	24.5	(22–31)	.33
Age of main partner, y[d]	31	(24–38)	28	(23–34.5)	.36
Gravidity	1	(0–3)	1	(0–3)	.49
Lifetime no. male partners	10	(7–20)	8.5	(4–13.5)	.03
No. vaginal sex acts with men, last 3 mo	12	(5–39)	7.5	(2.5–24)	.09

BV, bacterial vaginosis; *HIV*, human immunodeficiency virus; *IQR*, interquartile range; *IUD*, intrauterine device.

[a] Some women reported >1 race, so totals sum to >100%; [b] Denominator for proportions of women using each contraceptive method type is total no. of women reporting use of any method. Because women could report multiple methods, proportions sum to >100%; [c] Women were considered to have concurrent sexual partnerships if they answered "yes" to question, "In the past 3 months, did you have sex with 1 partner while involved in a sexual relationship with another partner during the same period of time? This may include times when you did not consider yourself to be in a committed relationship."; [d] Among those reporting main partner.

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arm was 30.5 ng/mL, vs 17.8 ng/mL in the control arm.

Self-reported adherence to metronidazole therapy

At the 4-week visit, women self-reported their compliance with metronidazole therapy, including the number of doses taken and the timing of treatment. Among women randomized to vitamin D, 81% reported taking metronidazole as prescribed. Among control women, 80% reported taking metronidazole as prescribed.

BV prevalence over follow-up, by randomization group

BV prevalence increased over follow-up in both groups (Figure 3). At the 4-week and 12-week visits, BV prevalence was similar between the vitamin D and control arms. By the 24-week visit, 65% of women randomized to vitamin D had BV, compared to 48% of control women.

Infections diagnosed during follow-up

We observed no meaningful differences between groups in STD and reproductive tract infections during follow-up: trichomoniasis (4 women in the vitamin D arm vs 6 in the control arm); chlamydia (2 vs 4 cases); gonorrhea (0 vs 2 cases); syphilis (0 vs 2 cases); and yeast infections (10 vs 9 cases) were each similar by intervention arm.

The effect of vitamin D on BV recurrence

BV-free survival time was very similar by randomization group (Figure 4). The log rank test was nonsignificant ($P = .86$). Among those experiencing BV recurrence, median time to recurrence was 13.7 weeks in the vitamin D arm and 14.3 weeks in the control arm.

The intention-to-treat HR for the effect of vitamin D supplementation on BV recurrence was 1.11 (95% CI, 0.68–1.81) (Table 3). In the secondary analysis examining the effect of time-varying 25(OH)D on BV recurrence, the adjusted HR for BV per 1-unit increase in 25(OH)D also demonstrated no significant effect of vitamin D level on BV (HR, 1.02; 95% CI, 0.99–1.04).

Exploratory sensitivity analyses

Following adjustment for BV risk factors not balanced by randomization, the HR for the effect of vitamin D on BV recurrence was 0.71 (95% CI, 0.40–1.25). When restricting the analysis to women with deficient serum 25(OH)D at enrollment (<20 ng/mL), the HR was 1.10 (95% CI, 0.62–1.97). Exclusion of

participants who had normal or intermediate vaginal flora at enrollment, as determined by Nugent scoring, led to an HR of 0.96 (95% CI, 0.55–1.66) (Table 3).

Adverse events

A total of 975 AEs occurred during follow-up; 948 (97%) were considered possibly, probably, or definitely related to study products (Table 4). For nearly all categories, the number of AEs among women randomized to vitamin D was lower than, or similar to, control women. The only AEs seen substantially more often among women in the vitamin D arm were dry mouth and vaginal discharge or itching. Two ectopic pregnancies occurred, both in the vitamin D arm; 1 occurred after enrollment but before the participant had begun vitamin D supplementation. Two miscarriages occurred, 1 in the vitamin D arm and 1 in the placebo arm (Table 4).

Comment

In this randomized trial of STD clinic patients with symptomatic BV, supplementation with high-dose vitamin D in addition to standard metronidazole therapy did not reduce BV recurrence. Adjustment for adherence, and sensitivity analyses to test the robustness of the primary finding, reinforced the lack of association between vitamin D supplementation and BV recurrence.

Several cross-sectional studies suggested an association between low vitamin D levels and BV prevalence. One study reported a significant 65% increase in BV prevalence in pregnant black women with serum 25(OH)D <20 ng/mL, compared to those with levels >32 ng/mL.[13] Another found that BV predicted vitamin D deficiency [25(OH)D <20 ng/mL] in pregnant black adolescents (odds ratio [OR], 4.4; $P = .02$).[14] An NHANES analysis reported increased odds of BV in pregnant women with vitamin D levels <30 ng/mL (adjusted OR, 2.9; 95% CI, 1.1–7.3).[16] A case-control study that examined 25(OH)D levels during pregnancy reported significantly lower vitamin D levels in BV-positive compared to BV-negative participants (18.0 vs 24.3 ng/mL, $P = .04$).[15]

In contrast, 2 randomized trials of vitamin D_3 supplementation in pregnant women examined BV as a secondary endpoint.[17,18] When the trials were combined and after adjusting for study and race, neither the 2000 IU daily dose nor the 4000 IU daily dose significantly affected BV ($P = .75$ for 2000 IU vs control; $P = .34$ for 4000 IU vs control).[19] The existing literature is also mixed on the association between vitamin D and BV in nonpregnant women. In the NHANES analysis, 25(OH)D <30 ng/mL in nonpregnant

FIGURE 2
Median and interquartile range of serum 25-hydroxyvitamin D [25(OH)D] levels over follow-up period, by randomization group

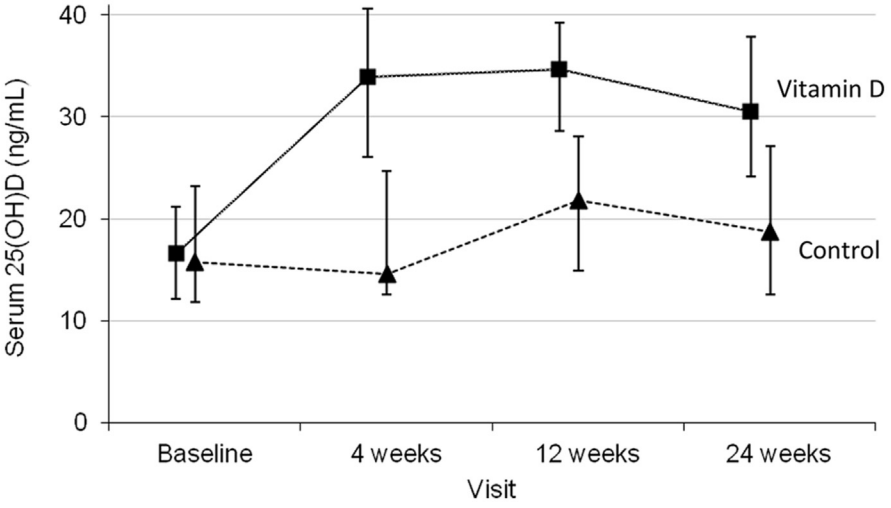

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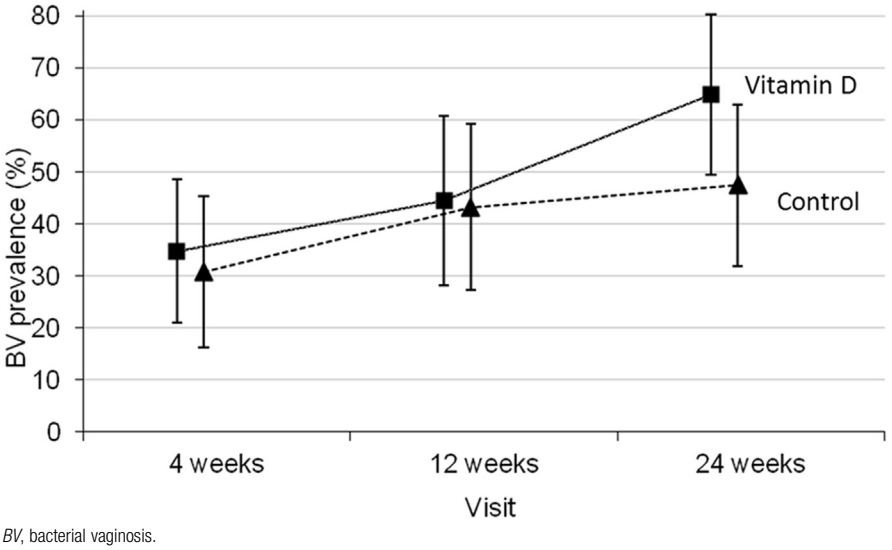
FIGURE 3
Prevalence and 95% confidence intervals of BV over follow-up period, by randomization group



BV, bacterial vaginosis.

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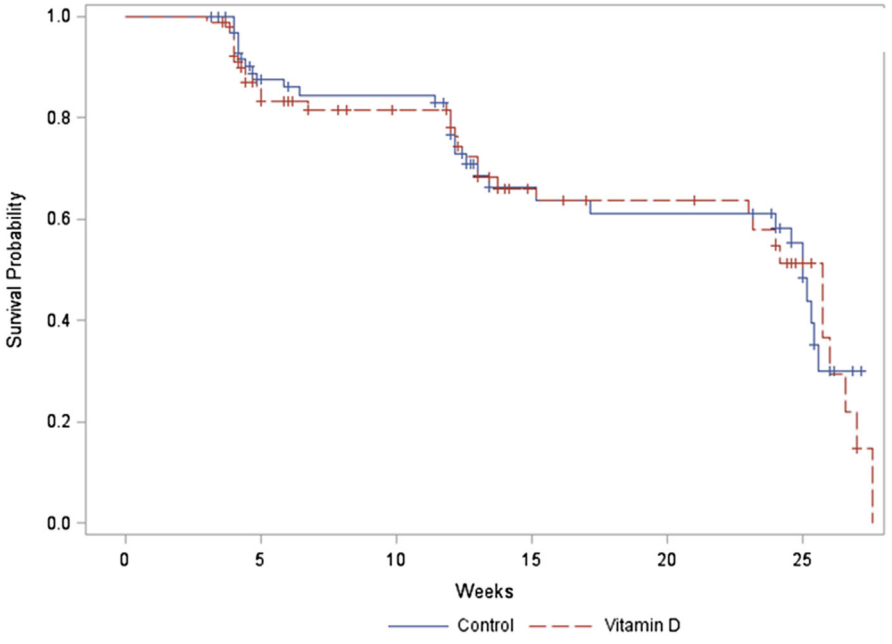
FIGURE 4
Kaplan-Meier survival curve of time to bacterial vaginosis recurrence, by randomization group



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women was associated with BV in unadjusted but not adjusted analyses.[16] One cross-sectional analysis reported an adjusted OR of 3.1 (95% CI, 1.2−8.4) for the effect of 25(OH)D <20 ng/mL on BV prevalence in HIV-positive women, but an adjusted OR of 1.1 (95% CI, 0.2−5.1) in HIV-negative women.[20]

As a small trial conducted in a busy STD clinic, our study had several limitations. We assessed only 3 of the 4 Amsel criteria because of inadequate time for microscopy. Some studies demonstrate that BV diagnosis using only 2 Amsel criteria (vs 4) has similar sensitivity and specificity compared to Nugent scoring.[28,29] Other studies indicate that the sensitivity of Amsel criteria can be low compared with Nugent scoring.[30,31] In this study, the variability in the 2 diagnostic methods was clearly demonstrated: all women had symptomatic BV at enrollment by modified Amsel criteria, but only 80% had BV according to Nugent scoring.

Despite the randomized design, some variables were unbalanced at enrollment.

TABLE 3
Multivariable modeling of effect of high-dose vitamin D supplementation on recurrence of bacterial vaginosis

Analysis description	n[a]	Exposure	Adjustment variables	HR	95% CI
Primary analysis: intention-to-treat	97	Randomization group	None	1.11	0.68−1.81
Secondary analysis: using women's measured 25(OH)D levels as primary exposure, instead of randomization group	97	Time-varying serum 25(OH)D level	Age, race	1.02[b]	0.99−1.04
Sensitivity analysis 1: controlling for factors not balanced by randomization at enrollment	92	Randomization group	Employment status, sterilization for contraception, concurrent partnerships in last 3 mo, ever douched, lifetime male partners, trichomoniasis status	0.71	0.40−1.25
Sensitivity analysis 2: restricted to women with deficient 25(OH)D (<20 ng/mL) at enrollment	64	Randomization group	None	1. 10	0.62−1.97
Sensitivity analysis 3: restricted to women with BV at enrollment by Nugent scoring	76	Randomization group	None	0.96	0.55−1.66

BV, bacterial vaginosis; *CI*, confidence interval; *HR*, hazard ratio; *25(OH)D*, 25-hydroxyvitamin D.

[a] Primary and secondary analyses included person-time from all women who returned for any follow-up visit (n = 97; 51 randomized to vitamin D and 46 to placebo). Sensitivity analysis 1 excluded 5 women who had missing data for ≥1 variables in adjustment set, leading to analysis sample of n = 92 (49 in vitamin D group and 43 in placebo group). Sensitivity analysis 2 was restricted to subgroup of women with 25(OD)D levels <20 ng/mL at enrollment and who returned for at least 1 follow-up visit (n = 64 women: 36 in vitamin D group and 28 in placebo group). Sensitivity analysis 3 was restricted to subgroup of women who had BV according to Nugent scoring (Nugent 7-10) at enrollment and who returned for at least 1 follow-up visit (n = 76 women: 42 in vitamin D group and 34 in placebo group); [b] HR for this model is interpreted as increased hazard for BV per 1-ng/mL increase in serum 25(OH)D.

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TABLE 4
Related adverse events by randomization group

Variable	Vitamin D (n = 442)	Control (n = 506)	Total (n = 948)
No. of AEs			
4 wk	124	126	250
12 wk	80	99	179
24 wk	110	121	231
Via telephone	1	3	4
Interim visits	127	157	284
Expected events[a]			
Headache	61	65	126
Increased thirst	40	44	84
Nausea	35	46	81
Fatigue	27	29	56
Dry mouth	26	7	33
Constipation	24	21	45
Dizziness/fainting/weakness	17	38	55
Increased urination	19	34	53
Itchy skin	21	26	47
Behavior change/increased irritability	19	26	45
Loss of appetite	17	13	30
Vomiting	16	25	41
Ringing in ears	14	12	26
Muscle or bone pain	12	14	26
Metallic taste	12	10	22
Weight loss	11	8	19
Confusion	7	14	21
Abdominal pain	6	2	8
Back pain	3	7	10
Muscle weakness	2	3	5
Rash	2	2	4
High serum calcium	1	2	3
Kidney stone	0	1	1
Unexpected and possibly related events[b]			
Yeast infection	10	9	19
Vaginal discharge/itching	10	1	11
Chest pain	6	10	16
Trichomoniasis	4	6	10
Irregular heartbeat	3	2	5
Shortness of breath	2	10	12
Chlamydia	2	4	6

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However, sensitivity analyses demonstrated that this imbalance is unlikely to explain our primary findings. In addition, we did not conduct tests of cure following completion of metronidazole treatment, so BV diagnosed at the 4-, 12-, and 24-week visits was likely a mixture of persistent BV that never cleared and recurrent BV following successful metronidazole treatment. However, for a polymicrobial clinical syndrome such as BV, where the division between health and disease is a continuum rather than a sharp line, the distinction between prevalent and reemergent disease may be less important. Finally, we had higher than expected loss to follow-up at 24 weeks, leading to lower than anticipated statistical power. However, given the consistency of the observed effects in both intention-to-treat and sensitivity analyses, we do not believe that low statistical power is masking a significant effect of vitamin D on BV recurrence.

Our trial also has important strengths. Unlike many interventions that rely on self-reports to assess protocol adherence, the demonstrated rise in 25(OH)D among women randomized to vitamin D compared to control women is strong evidence of compliance: median serum 25(OH)D levels in intervention women had cleared the 30-ng/mL threshold after just 4 weeks. Nevertheless, 30 ng/mL may not be a meaningful threshold for BV recurrence, and a higher target level may be more appropriate. Considerable debate exists about the appropriate dose, schedule, and target serum 25(OH)D level for optimal human health.[10,32,33]

Earlier studies primarily examined prevalent BV rather than recurrent BV, our trial endpoint. Whether sufficient vitamin D may prevent the initial development of BV remains an unanswered question, but our results suggest that short-term, high-dose vitamin D in women with existing BV is not effective in reducing BV recurrence. It is also possible that vitamin D's effect on BV requires >24 weeks to be observed. Although serum 25(OH)D levels of women in the randomization arm rose rapidly, whether related immune

TABLE 4
Related adverse events by randomization group *(continued)*

Variable	Vitamin D (n = 442)	Control (n = 506)	Total (n = 948)
Urinary tract infection	2	1	3
Ectopic pregnancy	2	0	2
Miscarriage	1	1	2
Low calcium	1	1	2
Bladder infection	1	0	1
Bleeding following cervical swab	1	0	1
Cellulitis	1	0	1
Mucopurulent cervicitis	1	0	1
Pressure in arm	1	0	1
Genital warts	1	1	2
Pelvic inflammatory disease	1	1	2
Gonorrhea	0	2	2
Syphilis	0	2	2
Herpes	0	1	1
Pelvic pain	0	3	3
Throat swelled	0	1	1

AE, adverse event.

[a] Events that were known side effects of vitamin D supplementation or metronidazole, as described in informed consent form;
[b] Events that were possibly, probably, or definitely related to study products or procedures.

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parameters increased at the same pace is not known. Despite high-dose supplementation, nearly 50% of women in the vitamin D arm had 25(OH)D levels lower than the 30-ng/mL threshold for sufficiency at trial end. Finally, most earlier data on the vitamin D-BV association focused on pregnant women. Perhaps an association between vitamin D and prevalent BV exists in pregnant women because of unique conditions of pregnancy (eg, a high estrogen state, compromised immunity), and that low serum vitamin D does not have the same impact on BV in nonpregnant women.

A recent evaluation confirmed that black Americans have lower serum 25(OH)D levels than whites, but that blacks also have reduced levels of vitamin D-binding protein, resulting in similar concentrations by race of bioavailable 25(OH)D.[34] Three-quarters (74%) of trial participants self-identified as black; their measured 25(OH)D levels may be systematically lower than their bioavailable 25(OH)D. However, this limitation has no impact on the primary intention-to-treat analysis, in which each woman's randomized treatment assignment, not her measured 25(OH)D level, is the primary exposure.

In summary, previous studies suggested that low vitamin D levels may be associated with increased BV prevalence. Our trial explored a related question: does increasing vitamin D level, in addition to metronidazole therapy, lead to reduced BV recurrence? Our findings suggest that short-term, high-dose vitamin D supplementation does not reduce BV recurrence in nonpregnant women. Given the established associations between BV and negative health outcomes, effective interventions to reduce BV's impact continue to be urgently needed. ■

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