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# Vitamin D and uterine fibroid growth, incidence, and loss: a prospective ultrasound study

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**Objective:** Fibroid treatments that have few side-effects and can preserve fertility are a clinical priority. We studied the association between serum vitamin D and uterine fibroid growth, incidence, and loss.
**Design:** A prospective community cohort study (enrollment 2010–2012) with 4 study visits over 5 years to conduct standardized ultrasounds, measure 25-hydroxyvitamin D (25(OH)D), and update covariates.
**Setting:** Detroit, Michigan area.
**Patients:** Self-identified African American or Black women aged 23–35 at enrollment without previous clinical diagnosis of fibroids.
**Intervention(s):** Serum 25(OH)D measured using immunoassay or liquid chromatography-tandem mass spectrometry.
**Main Outcome Measure(s):** The primary outcomes were fibroid growth, as measured by change in log volume per 18 months, and fibroid incidence (first detection of fibroid in previously fibroid-free uterus). Adjusted growth estimates from linear mixed models were converted to estimated difference in volume for high vs. low 25(OH)D. Incidence differences were estimated as hazard ratios from age-specific Cox regression. A secondary outcome fibroid loss (reduction in fibroid number between visits), was modeled using Poisson regression. Covariates (reproductive and hormonal variables, demographics, body mass index, current smoking) and 25(OH)D were modeled as time-varying factors.
**Result(s):** At enrollment among 1,610 participants with ≥1 follow-up ultrasound, mean age was 29.2 years, 73% had deficient vitamin D (<20ng/mL), and only 7% had sufficient vitamin D (≥30ng/mL). Serum 25(OH)D ≥20ng/mL compared with <20ng/mL was associated with an estimated 9.7% reduction in fibroid growth (95% confidence interval [CI]: -17.3%, -1.3%), similar to the minimally adjusted estimate -8.4% (95% CI: -16.4, 0.3). Serum 25(OH)D ≥30ng/mL compared with <30ng/mL was associated with an imprecise 22% reduction in incidence (adjusted hazard ratio=0.78; 95% CI: 0.47, 1.30), similar to the unadjusted estimate of 0.84 (95% CI: 0.51, 1.39). The >30ng/mL group also had a 32% increase in fibroid loss (adjusted risk ratio=1.32; 95% CI: 0.95, 1.83).
**Conclusion(s):** Our data support the hypothesis that high concentrations of vitamin D decrease fibroid development but are limited by the few participants with serum 25(OH)D ≥30ng/mL. Interventional trials that raise and maintain 25(OH)D concentrations >30ng/mL and then prospectively monitor fibroid development are needed to further assess supplemental vitamin D efficacy and determine optimal treatment protocols. (Fertil Steril® 2022;■:■–■. ©2022 by American Society for Reproductive Medicine.)

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Uterine fibroids are noncancerous tumors of the myometrium that cause significant morbidity including menorrhagia and pelvic pain, often requiring medical or surgical intervention (1). Estimates based on findings of ultrasound screening suggest that fibroids develop in >70% of women. Black women have a 10-year earlier tumor onset compared with White women (2); they also have larger tumors, more debilitating symptoms, and an increased need for surgery (3). Existing medical and surgical treatments for fibroids can impact childbearing goals, and apart

from hysterectomy, most treatments do not preclude fibroid recurrence (1). Most individuals with fibroids prefer noninvasive treatments and preservation of the uterus (4).

Despite the high prevalence of fibroids, few modifiable risk factors have been identified. However, none explains the differences in disease severity between Black and White individuals (3, 5). Vitamin D concentration, which is lower on average among self-identified Black women, has been proposed as a possible modifiable risk factor for the development of fibroids (6–9). In vitro and animal studies support a protective effect of vitamin D. In these models, vitamin D reduces fibroid tissue proliferation, changes the expression of estrogen and progesterone receptors, alters gene expression in proliferation and apoptosis pathways, and reduces expression of extracellular matrix proteins (8, 10–18). Human observational studies report lower fibroid prevalence among women with higher serum levels of vitamin D (6, 11). Most (19–22), but not all (23), human interventional studies document reduced fibroid growth when participants are treated with vitamin D, but studies were small (30–205 participants).

Although highly suggestive, the existing laboratory and epidemiologic literature has limitations. Animal and in vitro studies usually study the active form of vitamin D (1,25-dihydroxyvitamin D [1,25$(OH)_2$D]). These models may not reflect tissue concentrations in humans who obtain vitamin D through ultraviolet-B (UV-B) light exposure ($D_3$), diet ($D_2$ or $D_3$), or vitamin supplements ($D_2$ or $D_3$) with conversion to 25-hydroxyvitamin D (25(OH)D) in the liver and final conversion to 1,25$(OH)_2$D in the kidney or target tissue. In addition, most epidemiologic studies have relied on clinical diagnosis or ultrasound detection of prevalent fibroids, but initial fibroid onset occurs many years before clinical detection (6, 11).

We hypothesize that higher concentrations of serum 25(OH)D will be associated with reduced fibroid growth and incidence. We investigated this hypothesis with 4 repeated standardized ultrasound examinations over 5 years.

## METHODS

### Study Design

The *Study of Environment, Lifestyle and Fibroids* (SELF) is a prospective cohort study designed to evaluate both fibroid growth and incidence (24). Because of earlier fibroid onset in Black and African American women in the United States, SELF enrollment was limited to those who self-identified as 'Black or African American' among a list of racial and ethnic groups (2). Details of study recruitment and activities are provided in Appendix 1. Briefly, recruitment was conducted in the Detroit, Michigan area from 2010 to 2012 in collaboration with Henry Ford Health. Eligible participants were aged 23–34 years, who reported no previous diagnosis of fibroids at recruitment. The participants completed baseline questionnaires and attended a clinic visit that included an ultrasound examination, anthropometric measurements, and nonfasting blood collection. Of 1,693 participants enrolled, 1,610 (95%) attended $\geq$1 of the 3 follow-up visits during which the same study activities were completed. The 4 study visits occurred approximately 20 months apart and were completed in 2018. Participants who missed a visit were encouraged to attend the next. Visits for pregnant participants were delayed until 3–4 months postpartum for better ultrasound imaging. Study retention was high; 91% of the enrolled cohort attended the final visit, 95% attended $\geq$2 visits, and 79% attended all 4 study visits (Supplemental Fig. 1, available online). The 5% who only attended the enrollment visit tended to be older with lower income, lower body mass index (BMI) and were more likely to have had a pregnancy. Those participants who attended all 4 visits tended to be older and were more likely to be employed compared with those who attended only 2 or 3 visits, but otherwise the data showed no clear pattern of differences in baseline characteristics (Supplemental Table 1, available online).

The Study of Environment, Lifestyle and Fibroids was approved by the institutional review boards of the National Institutes of Health and Henry Ford Health. All participants provided written informed consent and received compensation.

### Measurement of 25(OH)D Concentration

Serum from each visit was aliquoted and stored at -80 °C until assayed. Analysis of 25(OH)D was conducted in 3 batches (serum from baseline, follow-up 1, and follow-up 2). Details of the vitamin D measurement and quality control procedures are described in Appendix 2. In brief, total serum 25(OH)D for the baseline visit was measured using LIAISON, a competitive chemiluminescence immunoassay (25, 26). For subsequent visits serum 25-hydroxyvitamin $D_2$ (25$(OH)D_2$) and 25-hydroxyvitamin $D_3$ (25$(OH)D_3$) were measured with liquid chromatography-tandem mass spectrometry and summed to create a total 25(OH)D measure. Blinded quality control serum samples were included in all assays, and based on these samples all intra-assay coefficients of variation were $<$5%, whereas inter-assay coefficients of variation were 9% for LIAISON and $<$5% for liquid chromatography-tandem mass spectrometry. The total 25(OH)D concentrations for 77 samples run on both platforms showed a mean difference of 0.6 ng/mL (25th–75th percentiles, −1.8 to 1.1). We did not adjust for different assay methods.

To account for seasonal variation in 25(OH)D, we estimated the overall annual average 25(OH)D using a cosinor model (27). We modeled the natural log of 25(OH)D using the sine and cosine of the day of the year of blood sample collection (first and second harmonic) as predictors (27, 28). We then created individual values for annual average 25(OH)D by adding the residual for each participant's visit to the model intercept and transforming back to the original scale (ng/mL). This season-adjusted 25(OH)D is denoted 25(OH)D hereafter.

For 90 samples, the serum volume was insufficient for the 25(OH)D assay (1.2%–2.6% of samples at a given visit). We imputed the majority (N = 80) of these values using the mean of measured values from adjacent visits, or carryover of the value from an adjacent visit if there was only 1. The remaining observations (N = 10) were dropped from the analyses.

We used clinically relevant cut points based on recommendations from the Institute of Medicine (IOM) (29) and the Endocrine Society (ES) (30) to categorize individual season-adjusted 25(OH)D values for analyses: <12 ng/mL (vitamin D deficient) (IOM and ES), <20 ng/mL (vitamin D inadequacy [IOM] or deficient [ES]), <30 ng/mL (vitamin D insufficient [ES]).

## Uterine Fibroid Assessment

Experienced sonographers, trained on the SELF protocol, used transvaginal ultrasound to count, localize, and measure fibroids ≥0.5 cm in diameter. Sonographers made 3 separate passes through the uterus, recording the 3 diameters of a given tumor at each pass. We calculated the fibroid volume for each pass with the ellipsoid formula, and the 3 volumes were averaged for analysis. Per protocol, sonographers noted any problems with visualization (e.g., calcifications, shadowing) that may have limited visualization. Video and still images were archived, and an 8% sample for each sonographer, oversampled for fibroid cases, was reviewed every month by the lead sonographer (details in Appendix 3).

The primary outcomes of interest were fibroid growth and fibroid incidence. Given the observed reduction in fibroid growth, fibroid loss was explored as a secondary outcome. Every fibroid outcome analysis had different eligibility criteria given that fibroid growth and loss require a fibroid to be detected and fibroid incidence is estimated among those who are fibroid-free at enrollment (Supplemental Fig. 2, available online).

**Fibroid growth.** The lead sonographer (TC) and 1 other investigator (DDB) identified fibroids that could be seen across 2 successive visits using archived images and fibroid locations. The growth analyses used data from 434 participants ($n$ = 1,357 interval growth measurements from successive visits). We defined fibroid growth as the change in the natural logarithm of the tumor volume (ln-volume). Change in ln-volume was scaled to a growth rate per 18 months (median time between visits =19 months; 25th–75th percentiles: 18–21) by calculating daily growth rates and multiplying by 540. To compare growth of fibroids from participants in high vs. low categories of 25(OH)D we estimated percent difference in volume per 18 months (Appendix 4).

**Fibroid incidence.** We identified incident fibroid cases (first appearance of any fibroids) among participants confirmed via ultrasound examination not to have fibroids at enrollment ($N$ = 1,246). If sonographers noted factors which impaired detection of fibroids (e.g., calcifications or shadowing, only a transabdominal ultrasound) the data were excluded from analysis (~0.5% of ultrasounds), resulting in incidence data for 1,232 participants. After excluding observations with insufficient serum for 25(OH)D, the incidence analysis included 1,230 participants (Supplemental Fig. 2).

**Fibroid loss.** Fibroid loss was assessed among participants with prevalent fibroids at enrollment that had not been clinically diagnosed previously, or participants who developed incident fibroids. For analysis of fibroid loss, defined as a decrease in fibroid number between 2 successive visits, we excluded intervals including, or after, a myomectomy, hysterectomy, or uterine artery embolization. When there are numerous fibroids, it can be difficult to accurately count tumors; therefore, we restricted this analysis to the 539 participants who had ≥2 successive visits with ≤4 fibroids at the earlier visit and no sonographer report of difficulty with visualization (Supplemental Fig. 2) (31). Fibroids that become undetectable by ultrasound between visits include those that shrink below the limit of ultrasound detection (0.5 cm for any diameter); therefore, this outcome cannot be interpreted as complete resolution of fibroids.

## Covariate Assessment

We identified covariates of interest based on prior fibroid research, previous work in this cohort, and availability of SELF data (5, 32, 33). We measured height during the first clinic visit, and weight at every visit to calculate BMI (kg/m$^2$) for each visit. We collected other covariate data via telephone or computer-assisted questionnaires at every visit including reproductive and hormonal variables (years since last birth, parity, age at menarche, years since last use of depot medroxyprogesterone acetate [DMPA]), demographic variables (household income, educational attainment, current employment), physical activity, and current smoking. All factors of interest were explored in adjusted models and those that did not affect observed associations between 25(OH)D and the fibroid outcome were not included in final models (34). Parameterization of covariates (categorization or continuous) for modeling is noted in the table footnotes for each fibroid outcome. Categorical covariates were usually modeled using indicator variables to allow for nonlinear associations. Covariates were updated at the beginning of each interval, except for years since last birth and parity, which were updated at the end of the interval between visits to incorporate events during the interval (35). There were minimal missing covariate data (<0.5%); therefore, when adjusting, we conducted complete-case analyses.

## Statistical Analyses

**Analysis of fibroid growth.** We used linear mixed models to account for correlated growth among fibroids from the same participant and for the same fibroid over time as previously described (32, 33, 36). Minimally adjusted growth models included time-varying 25(OH)D, continuous age, and categorical values for fibroid volume and number. Fully adjusted models included the minimal adjustment set plus age at menarche and time-varying measures of income, employment, BMI, years since last birth, and years since last DMPA use (Appendix 4).

**Analysis of fibroid incidence.** We used Cox regression with age as the time scale to estimate hazard ratios and 95% confidence intervals (95% CIs) for associations between 25(OH)D categories and fibroid incidence. Participants were included from the age at enrollment until the age at fibroid detection, with censoring for loss to follow-up, nonfibroid-related hysterectomy, or the final study visit, whichever occurred first.

Age-adjusted models examining the association between 25(OH)D and fibroid incidence were estimated first without covariates, and then were further adjusted for BMI, current smoking, income, parity, years since last birth, and years since last DMPA use. There was no evidence that the proportional hazards assumption was violated.

**Analysis of fibroid loss.** We used Poisson regression accounting for multiple observations per participant to estimate risk ratios and 95% CI with robust standard errors for fibroid loss. Minimally adjusted models included time-varying 25(OH)D, continuous age, months between visits, and categorical values for largest fibroid volume and number of

## TABLE 1

**Participant characteristics at enrollment, Study of Environment Lifestyle & Fibroids, Detroit, MI 2010–2012.**

| Characteristic[a] | Overall (N = 1,610) | 25(OH)D (<20 ng/mL) (N = 1179)[b] | 25(OH)D (≥20 ng/mL) (N = 424)[b] |
|---|---|---|---|
| Baseline[c] 25(OH)D (ng/mL) | | | |
| Median (IQR) | 15.3 (11.1–20.6) | 12.9 (10.2–16.2) | 25.2 (22.3–30.1) |
| Age (y) | | | |
| Mean ± SD | 29.2 ± 3.4 | 29.1 ± 3.4 | 29.5 ± 3.4 |
| Highest educational attainment[d] | | | |
| High school/general education diploma | 353 (22) | 288 (24) | 65 (15) |
| Some college/associates/technical | 807 (50) | 618 (52) | 186 (44) |
| Bachelors/masters/doctorate | 449 (28) | 272 (23) | 173 (41) |
| Currently employed | 1,002 (62) | 704 (60) | 292 (69) |
| Household income[d] | | | |
| <$20,000 | 721 (45) | 574 (49) | 145 (34) |
| $20,000–$50,000 | 605 (38) | 429 (36) | 172 (41) |
| >$50,000 | 272 (17) | 168 (14) | 103 (24) |
| Body mass index (kg/m$^2$) | | | |
| <25 | 318 (20) | 211 (18) | 106 (25) |
| 25–<30 | 331 (21) | 235 (20) | 96 (23) |
| 30–<35 | 310 (19) | 202 (17) | 107 (25) |
| 35–<40 | 267 (17) | 218 (18) | 48 (11) |
| ≥40 | 384 (24) | 313 (27) | 67 (16) |
| Current smoker | 310 (19) | 261 (22) | 48 (11) |
| Age at menarche (y) | | | |
| <11 | 297 (18) | 212 (18) | 82 (19) |
| 11 | 325 (20) | 235 (20) | 90 (21) |
| 12 | 430 (27) | 324 (27) | 103 (24) |
| 13 | 274 (17) | 199 (17) | 75 (18) |
| ≥14 | 284 (18) | 209 (18) | 74 (17) |
| Parity | | | |
| Never pregnant | 432 (27) | 320 (27) | 109 (26) |
| Prior pregnancy, no births | 192 (12) | 137 (12) | 55 (13) |
| 1–2 births | 708 (44) | 504 (43) | 201 (47) |
| ≥3 births | 278 (17) | 218 (18) | 59 (14) |
| Years since last birth (y) | | | |
| No birth | 624 (39) | 457 (39) | 164 (39) |
| <3 | 360 (22) | 256 (22) | 101 (24) |
| 3–4 | 207 (13) | 146 (12) | 61 (14) |
| 5–9 | 291 (18) | 226 (19) | 64 (15) |
| ≥10 | 128 (8) | 94 (8) | 34 (8) |
| Current OCP use | 183 (11) | 98 (8) | 85 (20) |
| Years since last use of DMPA[d] | | | |
| Never used DMPA | 920 (57) | 661 (56) | 253 (60) |
| <2 years | 183 (11) | 142 (12) | 41 (10) |
| ≥2 years | 506 (31) | 375 (32) | 130 (31) |
| Categorical baseline 25(OH)D (ng/mL)[a, b, c] | | | |
| <12 | 495 (31) | 495 (42) | — |
| 12–<20 | 684 (42) | 684 (58) | — |
| 20–<25 | 209 (13) | — | 209 (49) |
| 25–<30 | 108 (7) | — | 108 (25) |
| ≥30 | 107 (7) | — | 107 (25) |

*Note:* 25(OH)D = 25-hydroxyvitamin D; IQR = Interquartile range with 25th and 75th percentile shown; OCP = oral contraceptive pill, combined estrogen and progestin only; DMPA = depot medroxyprogesterone acetate (Depo-Provera), injection progestin-only contraceptive.

[a] N (%) unless otherwise specified.

[b] N = 7 participants are missing a baseline 25(OH)D measure.

[c] Season-adjusted 25(OH)D measure.

[d] Missing data: Education n = 1 missing (<20ng/mL); Income n = 12 missing (8 <20ng/mL, 4 ≥20ng/mL). Years since last use of DMPA n = 1 missing (<20 ng/mL).

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fibroids. Fully adjusted models included the minimal adjustment set plus time-varying education, BMI, years since last birth, and years since last DMPA use.

**Sensitivity analyses to evaluate potential biases.** For all outcomes, we removed observations with imputed serum 25(OH)D. Because use of estrogen-containing oral contraceptives is associated with increased serum concentrations of 25(OH)D (28), we explored possible confounding by including current use (yes, no) as a covariate. For fibroid incidence we set the incidence of fibroids to the midpoint of the interval rather than end. For fibroid growth and loss we removed covariates for fibroid number and fibroid volume to evaluate the possible influence of exposure-related differences in fibroid number and size. For fibroid growth we excluded statistical outliers (positive or negative growth beyond 3SD). (Details in Appendix 5). All analyses used SAS 9.4 (Cary, NC).

## RESULTS

At enrollment, among the 1,610 participants who had ≥1 follow-up visit, mean age was 29.2 ± 3.4 years, 78% had educational attainment beyond high school, 62% were employed, and 45% had a household income below $20,000. Almost a quarter (24%) had a BMI ≥40 kg/m$^2$ (Table 1). Participants had low 25(OH)D (median 25(OH)D=15.3 ng/mL, 25th–75th percentiles: 11.1–20.6). Compared to those with 25(OH)D <20ng/mL, those with 25(OH)D ≥20ng/mL had higher educational attainment and income, lower BMI, and were more likely to be using oral contraceptives and be nonsmokers (Table 1). There was no time trend in 25(OH)D concentrations across the study's duration (2010–2016) (Supplemental Fig. 3, available online). Median 25(OH)D concentrations were 15.3 ng/mL at enrollment, 14.8 ng/mL at follow-up 1, and 15.3 ng/mL at follow-up 2. Among 25(OH)D measures across the study, 32% were deficient (<12 ng/mL), 41% were 12–<20 ng/mL, 27% were ≥20 ng/mL. Only 7% exceeded the ES cut point for sufficiency (≥30 ng/mL) (Supplemental Fig. 4, available online) (30). Very few (1.7%) of the participants had concentrations ≥30 ng/mL at all 3 visits. Participants had a median length of study participation of 4.8 years (25th–75th percentiles: 4.7–5.0). Baseline characteristics were similar for the participants in each of the fibroid outcome groups (growth, incidence, and loss analyses), except for age, percent nulliparous, recency of birth, and use of DMPA. These are known factors associated with fibroid prevalence and are expected to differ because the growth and loss analyses require participants with fibroids, whereas the incidence analysis requires participants without fibroids (Supplemental Table 2, available online).

Most fibroids, including undiagnosed fibroids detected at enrollment and incident fibroids that developed during the study, were small. Median volume of the incident fibroids at time of first detection was 0.56 cm$^3$ (25th–75th percentiles: 0.2–1.4 cm$^3$). Median volume for those followed for growth was 2.2 cm$^3$ (25th–75th percentiles: 0.7–8.6 cm$^3$). At enrollment, 23% of participants had ≥1 fibroid (median: 1 fibroid, 75th percentile: 2 fibroids), whereas by the study's end, 32% had ≥1 fibroid (median: 2 fibroids, 75th percentile: 3 fibroids).

Overall estimated fibroid growth averaged 77.0% (95% CI: 68.6%, 85.9%) volume increase per 18 months. Serum 25(OH)D ≥20 ng/mL was associated with a reduced fibroid growth rate. Fibroids among those with 25(OH)D ≥20 ng/mL had 9.7% less growth per 18 months (95% CI: -17.3%, -1.3%) than fibroids from participants with 25(OH)D <20 ng/mL (Table 2).

Fibroid incidence averaged 9.6% within each study interval. Only at 25(OH)D concentrations ≥30 ng/mL was there any indication of a decrease in incidence (Table 3). Hazard ratios for serum 25(OH)D ≥30 ng/mL suggested an estimated 22% reduction in fibroid incidence (hazard ratio= 0.78, 95% CI: 0.47, 1.30) when compared with serum 25(OH)D <30 ng/mL. Associations were imprecise given the small number of 25(OH)D measures ≥30 ng/mL.

Fibroid loss during an interval was common (24.3% of eligible intervals had loss of at least one fibroid between study visits). Participants with 25(OH)D concentration ≥30 ng/mL had 32% higher fibroid loss in an observation interval than those with 25(OH)D <30 ng/mL (risk ratio= 1.32; 95% CI, 0.95, 1.83) (Table 4).

Sensitivity analyses showed very similar associations to those in the primary analyses (Appendix 4), (Supplemental Table 3, available online).

## DISCUSSION

Using prospectively collected ultrasound data and repeated measures of serum 25(OH)D, we observed slower fibroid growth in participants with 25(OH)D ≥20 ng/mL. For participants with serum 25(OH)D ≥30 ng/mL, we found evidence for a greater likelihood of fibroid loss, but only limited evidence for reduced incidence given the wide confidence intervals.

In this cohort, there were few 25(OH)D observations ≥20 ng/mL (27%) or ≥30 ng/mL (7%). Only 1.7% of participants maintained 25(OH)D concentrations ≥30 ng/mL for all 3 measures. Thus, we had limited power to detect associations at higher concentrations of 25(OH)D. The optimal range of 25(OH)D is debated (37). Although the IOM has identified ≥20 ng/mL as needed for skeletal health (29), the ES argues that concentrations ≥30 ng/mL are needed (30). Observational and clinical trials for improved reproductive outcomes including the success of fertility treatment, fecundability in natural conceptions, preterm birth, menstrual cycle characteristics, and breast cancer risk suggest that concentrations of 25(OH)D ≥40 ng/mL may be required (38–42).

Although most fibroids in this cohort were small and might not warrant immediate clinical attention, our study still has important clinical implications. Symptoms and major interventional treatments are more likely with larger fibroids (43,44). Therefore, minimizing fibroid growth and increasing fibroid loss when fibroids are small can delay severe morbidity and prevent the need for surgical or radiologic treatments.

Our findings for reduced growth of fibroids from participants with higher 25(OH)D levels are consistent with results of small clinical trials that treated participants with vitamin D supplements, although most of those studies included

## TABLE 2

**Serum 25(OH)D and fibroid growth, 434[a] participants from the Study of Environment, Lifestyle & Fibroids, Detroit, Michigan, 2010–2018**

| Categories of 25(OH)D ng/mL | No. growth intervals | Estimated % difference in growth (95% CI) | |
|---|---|---|---|
| | | Minimally adjusted[b] | Fully adjusted[c] |
| Four categories of 25(OH)D | | | |
| <12 | 394 | REF | REF |
| 12–<20 | 524 | 2.8 (−7.1, 13.7) | 4.7 (−5.0, 15.4) |
| 20–<30 | 321 | −6.6 (−16.9, 5.0) | −6.3 (−16.4, 5.0) |
| ≥30 | 118 | −7.7 (−21.9, 9.1) | −9.1 (−22.6, 6.8) |
| Two categories of 25(OH)D split at 20 ng/mL | | | |
| <20 | 918 | REF | REF |
| ≥20 | 439 | −8.4 (−16.4, 0.3) | −9.7 (−17.3, −1.3) |
| Two categories of 25(OH)D split at 30 ng/mL | | | |
| <30 | 1,239 | REF | REF |
| ≥30 | 118 | −6.5 (−19.6, 8.7) | −8.8 (−21.0, 5.4) |

*Note:* 25(OH)D = 25-hydroxyvitamin D; CI = confidence interval.
[a] Growth analyses were conducted among fibroids which could be matched across successive visits based on fibroid location. This includes fibroids from 434 participants with 1357 interval growth measurements. Participants could contribute multiple fibroids and fibroids could be followed across multiple intervals.
[b] Minimally adjusted model includes volume of fibroid (<0.5 $cm^3$,0.5–4.19 $cm^3$,4.2–14.0 $cm^3$,≥14.1 $cm^3$), number of fibroids (ordinal 1, 2, 3, ≥4), age (continuous).
[c] Fully adjusted models further adjust for years since last birth (<5 years, ≥5 years ago including no birth), years since last use of injection contraceptive (<2 years, ≥2 years/never), body mass index ($kg/m^2$) (<25, 25–<30, 30–<35, 35–<40, ≥40), income (<$20,000, $20–50,000, >$50,000), employment (employed yes/no), age at menarche (ordinal <11, 11, 12, 13, >13 years). Three observations excluded from analyses because of missing data on at least one covariate.

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participants with 25(OH)D concentrations ranging up to 30 ng/mL at enrollment, and treated with supplementation that would have resulted in higher concentrations than generally seen in our sample (19–22). Fibroid loss was not included as an outcome in the trials of fibroid growth. However, if our finding of reduced fibroid growth with higher vitamin D results in some fibroids shrinking below the ultrasound detection limit, increased fibroid loss would be expected.

Although the association between vitamin D and fibroid incidence has not been previously studied, a recent systematic review and additional observational studies report lower concentrations of 25(OH)D among women with prevalent fibroids than those without fibroids (6, 45–47). The observational studies rely on self-reported fibroid status, and this results in undiagnosed fibroids being included in the "noncase" group (5) as well as temporal misclassification of 25(OH)D concentrations. A large, randomized control trial using prospective ultrasound examinations is underway in China which will investigate the effects of daily vitamin D supplementation over 2 years on fibroid incidence in >2000 women, and fibroid growth in 360 women (48). Trials in other populations are warranted.

In vitro and animal models support our finding that vitamin D limits fibroid growth as reviewed recently by Vergara et al. (10). In vitro models including those using immortalized human uterine leiomyoma cells (HuLM) and cultured human cells from fibroid and adjacent myometrial tissue show that treatment with $1,25(OH)_2D$ inhibits their growth

## TABLE 3

**Serum 25(OH)D and fibroid incidence, 1230[a] participants from the Study of Environment, Lifestyle & Fibroids, Detroit, Michigan, 2010–2018**

| Categories of 25(OH)D (ng/mL) | Incident cases | Person-years | Hazard ratio (95% confidence interval) | |
|---|---|---|---|---|
| | | | Unadjusted[b] | Fully adjusted[c] |
| Four categories of 25(OH)D | | | | |
| <12 | 88 | 1,717 | REF | REF |
| 12-<20 | 119 | 2,213 | 1.06 (0.81, 1.40) | 1.03 (0.78, 1.37) |
| 20-<30 | 71 | 1,075 | 1.23 (0.90, 1.68) | 1.15 (0.83, 1.58) |
| ≥30 | 16 | 303 | 0.91 (0.53, 1.55) | 0.83 (0.48, 1.42) |
| Two categories of 25(OH)D split at 20 ng/mL | | | | |
| <20 | 207 | 3,930 | REF | REF |
| ≥20 | 87 | 1,377 | 1.11 (0.87, 1.43) | 1.05 (0.81, 1.36) |
| Two categories of 25(OH)D split at 30 ng/mL | | | | |
| <30 | 278 | 5,004 | REF | REF |
| ≥30 | 16 | 303 | 0.84 (0.51, 1.39) | 0.78 (0.47, 1.30) |

*Note:* 25(OH)D = 25-hydroxyvitamin D
[a] Incidence analyses were conducted among 1,230 participants who were fibroid-free at enrollment and had at least one follow-up ultrasound.
[b] Cox model with age as time scale (starting at age of enrollment), with no further adjustment.
[c] Cox model with age as time scale further adjusted for time-varying parity (0, 1-2 births, ≥3 births), years since last birth (within 4 years, ≥4 years ago including no births), years since last use of injection contraceptive (<2 years, ≥2 years/never), body mass index ($kg/m^2$) (<25, 25–<30, 30–<35, 35–<40, ≥40), current smoking (yes, no), household income (<$20,000, ≥$20,000). Twelve observations excluded from analyses because of missing data on at least one covariate.

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**TABLE 4**

**Serum 25(OH)D and fibroid loss in 539[a] participants in the Study of Environment, Lifestyle & Fibroids, Detroit, Michigan, 2010–2018**

| Categories of 25(OH)D (ng/mL) | Intervals with loss/eligible intervals (%) | Risk ratio (95% confidence interval) | |
|---|---|---|---|
| | | Minimally adjusted[b] | Fully adjusted[c] |
| Four categories of 25(OH)D | | | |
| <12 | 71/319 (22.3) | REF | REF |
| 12–<20 | 97/406 (23.9) | 1.04 (0.80, 1.35) | 1.06 (0.81, 1.38) |
| 20–<30 | 56/217 (25.8) | 1.04 (0.76, 1.42) | 1.14 (0.83, 1.57) |
| ≥30 | 24/77 (31.2) | 1.29 (0.88, 1.88) | 1.40 (0.95, 2.06) |
| Two categories of 25(OH)D split at 20 ng/mL | | | |
| <20 | 168/725 (23.2) | REF | REF |
| ≥20 | 80/294 (27.2) | 1.08 (0.86, 1.36) | 1.17 (0.92, 1.48) |
| Two categories of 25(OH)D split at 30 ng/ml | | | |
| <30 | 224/942 (23.8) | REF | REF |
| ≥30 | 24/77 (31.2) | 1.26 (0.90, 1.75) | 1.32 (0.95, 1.83) |

*Note:* 25(OH)D, 25-hydroxyvitamin D; HS/GED, High school/general education diploma.

[a] Loss analyses were conducted among 539 participants with 1-4 prevalent fibroids at the beginning of an observed interval. This includes participants with fibroids at enrollment and those who develop incident fibroids.

[b] Minimally adjusted model includes age (continuous), months between visits (continuous), number of fibroids (1, 2, ≥3) and volume of largest fibroid (<0.5 $cm^3$,0.5–4.19 $cm^3$,4.2–14.0 $cm^3$,≥14.1 $cm^3$).

[c] Fully adjusted model also includes years since last birth (<4 years, ≥4 years ago including no birth), years since last use of injection contraceptive (<2 years, ≥2 years/never), body mass index (kg/$m^2$) (<25, 25–<30, 30–<35, 35–<40, ≥40), education (HS/GED or less, >HS/GED). Four observations excluded from analyses because of missing data on at least one covariate.

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through changes in cell proliferation, apoptosis, extracellular matrix composition, Wnt/$\beta$-catenin, and TGF$\beta$3 expression, and down-regulation of estrogen and progesterone receptors (12, 13, 16, 18, 49–51). Treatment with 1,25$(OH)_2$D or an analogue in mice and Eker rats with fibroid tumors also results in decreased tumor size through such mechanisms (17, 50, 52).

Limited laboratory studies were conducted related to fibroid initiation and vitamin D. Most fibroids harbor specific somatic mutations in *HMGA2* or *MED12* (53). Fibroids have elevated DNA damage and vitamin D treatment of HuLM cells reduced the evidence of DNA damage with up-regulation of DNA-repair proteins (14). Improved detection and repair of DNA damage could prevent the proliferation of cells with mutations and thus limit fibroid initiation (54).

An inherent limitation to studying the growth of individual fibroids is that they must be observed over time, and lost fibroids are not included; to compensate, we examined fibroid loss as a separate outcome. There is also measurement error in ultrasound assessment of fibroid size, and very small fibroids may be missed. However, taking 3 separate passes through the uterus will maximize fibroid detection, and using the mean of 3 separate volume measures has less measurement error than a single measure. In addition, measurement error is greater for smaller fibroids (55), but we address this limitation by accounting for the differential error in our growth model (33). The most important limitation is that we had few participants with 25(OH)D concentrations >30 ng/mL which limits our power to find associations at the higher vitamin D concentrations recommended by the ES.

Our prospective study design with multiple standardized ultrasounds is the first that allows for the timely detection of incident fibroids, thus reducing the misclassification of fibroid status which is present in previous observational studies. Our multiple measures of serum 25(OH)D reduce the bias introduced when a single measure of 25(OH)D is assumed to be relevant for later timepoints. In addition, our focus on Black women allows us to examine individuals at high risk for both vitamin D deficiency and fibroid burden, although vitamin D deficiency among reproductive-aged women in the United States is widespread (7).

Vitamin D is well known to be critical for bone health (29) with growing evidence that higher concentrations of 25(OH)D improve reproductive outcomes (56) and reduce breast cancer risk (42). Our findings add support to the existing literature suggesting vitamin D may also reduce fibroid development. As compared with existing medical and surgical treatments for fibroids that have significant side-effects and impact fertility, vitamin D is safe and compatible with pregnancy. The high prevalence of vitamin D deficiency in the SELF cohort and reproductive-aged US women in general (47) suggests that further public health awareness about vitamin D is needed. An additional issue for US women is that elevated BMI is associated with lower 25(OH)D concentrations (57). Current dietary and supplement recommendations for vitamin D intake may not be sufficient for individuals with high BMI who require 2–3 times higher vitamin D supplementation to attain desired serum concentrations (58).

In conclusion, 25(OH)D concentrations >20 ng/mL were associated with reduced fibroid growth. Results for analyses examining 25(OH)D concentrations >30 ng/mL provided suggestive evidence for increased likelihood of fibroid loss and a possible reduction in fibroid incidence. Intervention trials with vitamin D supplementation to attain and maintain sufficient 25(OH)D will be needed to further assess the impact of vitamin D on fibroid development.

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