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

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Abstract

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 four study visits over five years to conduct standardized ultrasounds, measure 25-hydroxyvitamin D (25(OH)D), and update covariates.

Subjects: Self-identified African American or Black women aged 23–35 at enrollment without previous clinical diagnosis of fibroids from the Detroit, Michigan area.

Exposure: Serum 25(OH)D measured using immunoassay or liquid chromatography tandem mass-spectrometry.

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Attestation statements:

• Data on the association between vitamin D and fibroid development have not been previously published other than in abstract form.

• Data, with appropriate protection for potentially identifiable information, can be made available to the editors of the journal for review or query upon request.

Capsule: Higher concentrations of serum vitamin D are associated with lower fibroid growth and the suggestion of increased fibroid loss and reduced fibroid incidence.

Outcome Measures: 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 (HR) 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.

Results: At enrollment among 1610 participants with at least one 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 to <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 to <30ng/ml was associated with an imprecise 22% reduction in incidence [adjusted HR=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).

Conclusions: Our data support the hypothesis that higher 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 above 30ng/ml and then prospectively monitor fibroid development are needed to further assess supplemental vitamin D efficacy and determine optimal treatment protocols.

Introduction

Uterine fibroids are non-cancerous tumors of the myometrium that cause significant morbidity including menorrhagia and pelvic pain, often requiring medical or surgical intervention (1). Estimates based on ultrasound screening suggest that fibroids develop in over 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 non-invasive treatments and preservation of the uterus (4).

Despite the high prevalence of fibroids, few modifiable risk factors have been identified. None explain 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 (8, 10, 11), changes the expression of estrogen and progesterone receptors (12), alters gene expression in proliferation and apoptosis pathways (13–15), and reduces expression of extracellular matrix proteins (16–18). Human observational studies report lower fibroid prevalence in women with higher vitamin D (6, 11). Most (19–22) but not all (23) human intervention studies document reduced fibroid

growth when participants are treated with vitamin D, but studies were small (30 to 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 ($1,25(OH)_2D$)]. 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 $1,25(OH)_2D$ 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 four repeated standardized ultrasound examinations over five years.

Methods

Study Design

The Study of Environment, Lifestyle and Fibroids (SELF) is a prospective cohort study designed to evaluate fibroid growth and incidence (24). Due to earlier fibroid onset in Black/African American women in the United States (2), SELF enrollment was limited to those who self-identified as ‘Black or African American’ among a list of racial and ethnicity categories. 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 (HFH). Eligible participants were aged 23–34, who reported no prior diagnosis of fibroids at recruitment. The participants completed baseline questionnaires and attended a clinic visit that included an ultrasound examination, anthropometric measurements, and non-fasting blood collection. Of 1693 participants enrolled, 1610 (95%) attended ≥1 of the three follow-up visits during which the same study activities were completed. The four 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 post-partum for better ultrasound imaging. Study retention was high; 91% of the enrolled cohort attended the final visit, 95% attended at least 2 visits, and 79% attended all 4 study visits (Supplemental Figure 1). The 5% who only attended the enrollment visit tended to be older with lower income, lower body mass index and were more likely to have had a pregnancy. Those participants who attended all 4 visits tended to older and were more likely to be employed compared to those who attended only 2 or 3 visits, but otherwise the data showed no clear pattern of differences in baseline characteristics (Supplemental Table 1).

SELF was approved by the institutional review boards of the National Institutes Health and HFH. All participants provided written informed consent and received compensation.

Measurement of 25(OH)D Concentration

Serum from each visit was aliquoted and stored at −80C until assayed. Analysis of 25(OH)D was conducted in three 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 (LC-MS/MS) 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 (CV) were <5% and inter-assay CVs were 9% for LIAISON and <5% for LC-MS/MS. The total 25(OH)D for 77 samples run on both platforms showed a mean difference of 0.6 ng/ml (25^{th} to 75^{th} 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/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 one. 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 three 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 three 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 Figure 2).

Fibroid Growth.—The lead sonographer (TC) and one other author (DB) identified fibroids that could be seen across two successive visits using archived images and fibroid locations. The growth analyses used data from 434 participants (n=1357 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; 25^{th}-75^{th} percentiles: 18–21) by calculating daily growth rates and multiplying by 540. To compare growth of fibroids from participants in high versus 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 not to have fibroids at enrollment (N=1246). 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 1232 participants. After excluding observations with insufficient serum for 25(OH)D, the incidence analysis included 1230 participants (Supplemental Figure 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 two successive visits, we excluded intervals including or following a myomectomy, hysterectomy, or uterine artery embolization. When there are numerous fibroids, it can be difficult to accurately count tumors (31), 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 Figure 2). 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 (5), previous work in this cohort (32, 33), and availability of SELF data. We measured height during the first clinic visit, and weight at every visit to calculate body mass index (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 (34) in final models. Parameterization of covariates (categorization or continuous) for modelling is noted in the table footnotes for each fibroid

outcome. Categorical covariates were usually modelled using indicator variables to allow for non-linear 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 (HR) and 95% confidence intervals (95% CI) 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, non-fibroid-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 (RR) 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 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 mid-point of the interval rather than end. For fibroid growth and loss we removed fibroid-number and fibroid-volume covariates 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 1610 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, 25^{th}-75^{th} percentiles: 11.1–20.6). Compared with 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 non-smokers (Table 1). There was no time trend in 25(OH)D concentrations across the study's duration (2010–2016) (Supplemental Figure 3). 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 Endocrine Society (30) cut point for sufficiency (≥30 ng/ml) (Supplemental Figure 4). Very few (1.7%) of the participants had this higher concentration at all three visits. Participants had a median length of study participation of 4.8 years (25^{th}-75^{th} percentiles: 4.7–5.0 years). 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 while the incidence analysis requires participants without fibroids (Supplemental Table 2).

Most fibroids, including undiagnosed fibroids detected at enrollment and incident fibroids that developed during the study, were small. Median volume of incident fibroids at time of first detection was 0.56 cubic centimeters (cm^3) (25^{th}-75^{th} percentiles: 0.23–1.4 cm^3). Median volume for those followed for growth was 2.2 cm^3 (25^{th}-75^{th} percentiles: 0.7–8.6 cm^3). At enrollment, 23% of participants had ≥1 fibroid (median: 1 fibroid, 75^{th} percentile: 2 fibroids), while by the study's end, 32% had ≥1 fibroid (median: 2 fibroids, 75^{th} 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 (HR 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 (RR 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).

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 interval.

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 three 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). While the Institute of Medicine has identified ≥20 ng/ml as needed for skeletal health (29), the Endocrine Society argues that concentrations ≥30 ng/ml are needed (30). Observational and clinical trials for improved reproductive outcomes including the success of fertility treatment (38), fecundability in natural conceptions (39), preterm birth (40), menstrual cycle characteristics (41), and breast cancer risk (42) suggest that concentrations of 25(OH)D ≥40 ng/ml may be required.

Although most fibroids in this cohort were small and might not warrant immediate clinical attention, our study still has important clinical implications. Symptoms (43) and major interventional treatments (44) are more likely with larger fibroids. 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 are consistent with results of small clinical trials that treated participants with vitamin D supplements (19–22), though most of those studies (19, 21, 22) included 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. 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 compared with 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 “non-case” 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 more

than 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 (reviewed in Vergara (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 through changes in cell proliferation, apoptosis, extracellular matrix composition, Wnt/β-catenin and TGFβ3 expression, and down regulation of estrogen and progesterone receptors (12, 13, 16, 18, 49–51). Treatment with $1,25(OH)_2D$ or an analogue in mice and Eker rats with fibroid tumors also results in decreased tumor size through such mechanisms (17, 50, 52).

There have been fewer laboratory studies 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 evidence of DNA damage with upregulation 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 three 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 above 30 ng/ml which limits our power to find associations at the higher vitamin D concentrations recommended by the Endocrine Society.

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 prior 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 in 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 U.S. women in general (47) suggests that further public health messaging about vitamin D is needed. An additional issue for U.S. 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 above 20 ng/ml were associated with reduced fibroid growth. Results for analyses examining 25(OH)D concentrations above 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Management of symptomatic uterine leiomyomas: ACOG practice bulletin, number 228. Obstet Gynecol. 2021;137:e100–e15. doi: 10.1097/aog.0000000000004401. [PubMed: 34011888]
2. Laughlin SK, Baird DD, Savitz DA, Herring AH, Hartmann KE. Prevalence of uterine leiomyomas in the first trimester of pregnancy: an ultrasound-screening study. Obstet Gynecol. 2009;113:630–5. doi: 10.1097/AOG.0b013e318197bbaf. [PubMed: 19300327]
3. Laughlin-Tommaso SK, Jacoby VL, Myers ER. Disparities in fibroid incidence, prognosis, and management. Obstet Gynecol Clin North Am. 2017;44:81–94. doi: 10.1016/j.ogc.2016.11.007. [PubMed: 28160895]
4. Borah BJ, Nicholson WK, Bradley L, Stewart EA. The impact of uterine leiomyomas: a national survey of affected women. Am J Obstet Gynecol. 2013;209:319.e1–.e20. doi: 10.1016/j.ajog.2013.07.017.
5. Wise LA, Laughlin-Tommaso SK. Epidemiology of uterine fibroids: from menarche to menopause. Clin Obstet Gynecol. 2016;59:2–24. doi: 10.1097/grf.0000000000000164. [PubMed: 26744813]
6. Baird DD, Hill MC, Schectman JM, Hollis BW. Vitamin D and the risk of uterine fibroids. Epidemiology. 2013;24:447–53. doi: 10.1097/EDE.0b013e31828acca0. [PubMed: 23493030]
7. Herrick KA, Storandt RJ, Afful J, Pfeiffer CM, Schleicher RL, Gahche JJ, et al. Vitamin D status in the United States, 2011–2014. Am J Clin Nutr. 2019;110:150–7. doi: 10.1093/ajcn/nqz037. [PubMed: 31076739]
8. Brakta S, Diamond JS, Al-Hendy A, Diamond MP, Halder SK. Role of vitamin D in uterine fibroid biology. Fertil Steril. 2015;104:698–706. doi: 10.1016/j.fertnstert.2015.05.031. [PubMed: 26079694]

9. Wise LA, Ruiz-Narváez EA, Haddad SA, Rosenberg L, Palmer JR. Polymorphisms in vitamin D-related genes and risk of uterine leiomyomata. Fertil Steril. 2014;102:503–10.e1. doi: 10.1016/j.fertnstert.2014.04.037. [PubMed: 24890271]
10. Vergara D, Catherino WH, Trojano G, Tinelli A. Vitamin D: mechanism of action and biological effects in uterine fibroids. Nutrients. 2021;13. doi: 10.3390/nu13020597.
11. Mohammadi R, Tabrizi R, Hessami K, Ashari H, Nowrouzi-Sohrabi P, Hosseini-Bensenjan M, et al. Correlation of low serum vitamin-D with uterine leiomyoma: a systematic review and meta-analysis. Reprod Biol Endocrinol. 2020;18:85. doi: 10.1186/s12958-020-00644-6. [PubMed: 32795307]
12. Al-Hendy A, Diamond MP, El-Sohemy A, Halder SK. 1,25-dihydroxyvitamin D3 regulates expression of sex steroid receptors in human uterine fibroid cells. J Clin Endocrinol Metab. 2015;100:E572–82. doi: 10.1210/jc.2014-4011. [PubMed: 25625804]
13. Sharan C, Halder SK, Thota C, Jaleel T, Nair S, Al-Hendy A. Vitamin D inhibits proliferation of human uterine leiomyoma cells via catechol-o-methyltransferase. Fertil Steril. 2011;95:247–53. doi: 10.1016/j.fertnstert.2010.07.1041. [PubMed: 20736132]
14. Ali M, Shahin SM, Sabri NA, Al-Hendy A, Yang Q. Hypovitaminosis D exacerbates the DNA damage load in human uterine fibroids, which is ameliorated by vitamin D3 treatment. Acta Pharmacol Sin. 2019;40:957–70. doi: 10.1038/s41401-018-0184-6. [PubMed: 30478352]
15. Al-Hendy A, Diamond MP, Boyer TG, Halder SK. Vitamin D3 inhibits Wnt/β-catenin and mTOR signaling pathways in human uterine fibroid cells. J Clin Endocrinol Metab. 2016;101:1542–51. doi: 10.1210/jc.2015-3555. [PubMed: 26820714]
16. Halder SK, Osteen KG, Al-Hendy A. 1,25-dihydroxyvitamin D3 reduces extracellular matrix-associated protein expression in human uterine fibroid cells. Biol Reprod. 2013;89:150. doi: 10.1095/biolreprod.113.107714. [PubMed: 24174578]
17. Halder SK, Sharan C, Al-Hendy A. 1,25-dihydroxyvitamin D3 treatment shrinks uterine leiomyoma tumors in the Eker rat model. Biol Reprod. 2012;86:116. doi: 10.1095/biolreprod.111.098145. [PubMed: 22302692]
18. Corachán A, Trejo MG, Carbajo-García MC, Monleón J, Escrig J, Faus A, et al. Vitamin D as an effective treatment in human uterine leiomyomas independent of mediator complex subunit 12 mutation. Fertil Steril. 2021;115:512–21. doi: 10.1016/j.fertnstert.2020.07.049. [PubMed: 33036796]
19. Arjeh S, Darsareh F, Asl ZA, Azizi Kutenaei M. Effect of oral consumption of vitamin D on uterine fibroids: a randomized clinical trial. Complement Ther Clin Pract. 2020;39:101159. doi: 10.1016/j.ctcp.2020.101159. [PubMed: 32379687]
20. Hajhashemi M, Ansari M, Haghollahi F, Eslami B. The effect of vitamin D supplementation on the size of uterine leiomyoma in women with vitamin D deficiency. Caspian J Intern Med. 2019;10:125–31. doi: 10.22088/cjim.10.2.125. [PubMed: 31363390]
21. Ciavattini A, Delli Carpini G, Serri M, Vignini A, Sabbatinelli J, Tozzi A, et al. Hypovitaminosis D and “small burden” uterine fibroids: opportunity for a vitamin D supplementation. Medicine (Baltimore). 2016;95:e5698. doi: 10.1097/md.0000000000005698. [PubMed: 28033263]
22. Davari Tanha F, Feizabad E, Vasheghani Farahani M, Amuzegar H, Moradi B, Samimi Sadeh S. The effect of vitamin D deficiency on overgrowth of uterine fibroids: a blinded randomized clinical trial. Int J Fertil Steril. 2021;15:95–100. doi: 10.22074/ijfs.2020.134567. [PubMed: 33687161]
23. Suneja A, Faridi F, Bhatt S, Guleria K, Mehndiratta M, Sharma R. Effect of vitamin D3 supplementation on symptomatic uterine leiomyoma in women with hypovitaminosis D. J Midlife Health. 2021;12:53–60. doi: 10.4103/jmh.JMH_90_19. [PubMed: 34188427]
24. Baird DD, Harmon QE, Upson K, Moore KR, Barker-Cummings C, Baker S, 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;24:907–15. doi: 10.1089/jwh.2015.5277. [PubMed: 26334691]
25. Wagner D, Hanwell HE, Vieth R. An evaluation of automated methods for measurement of serum 25-hydroxyvitamin D. Clinical biochemistry. 2009;42:1549–56. doi: 10.1016/j.clinbiochem.2009.07.013. [PubMed: 19631201]

26. Ersfeld DL, Rao DS, Body JJ, Sackrison JL Jr., Miller AB, Parikh N, et al. Analytical and clinical validation of the 25 OH vitamin D assay for the liaison automated analyzer. Clinical biochemistry. 2004;37:867–74. doi: 10.1016/j.clinbiochem.2004.06.006. [PubMed: 15369717]
27. Sachs MC, Shoben A, Levin GP, Robinson-Cohen C, Hoofnagle AN, Swords-Jenny N, et al. Estimating mean annual 25-hydroxyvitamin D concentrations from single measurements: the multi-ethnic study of atherosclerosis. Am J Clin Nutr. 2013;97:1243–51. doi: 10.3945/ajcn.112.054502. [PubMed: 23615830]
28. 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;101:3370–7. doi: 10.1210/jc.2016-1658. [PubMed: 27490916]
29. Institute of Medicine Food and Drug Board. Dietary reference intakes for calcium and vitamin D. Washington, DC: National Academy Press; 2010.
30. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2011;96:1911–30. doi: 10.1210/jc.2011-0385. [PubMed: 21646368]
31. Dueholm M, Lundorf E, Hansen ES, Ledertoug S, Olesen F. Accuracy of magnetic resonance imaging and transvaginal ultrasonography in the diagnosis, mapping, and measurement of uterine myomas. Am J Obstet Gynecol. 2002;186:409–15. doi: 10.1067/mob.2002.121725. [PubMed: 11904599]
32. Baird DD, Patchel SA, Saldana TM, Umbach DM, Cooper T, Wegienka G, et al. Uterine fibroid incidence and growth in an ultrasound-based, prospective study of young African Americans. Am J Obstet Gynecol. 2020;223:402.e1–.e18. doi: 10.1016/j.ajog.2020.02.016.
33. Harmon QE, Patchel SA, Zhao S, Umbach DM, Cooper TE, Baird DD. Depot medroxyprogesterone acetate use and the development and progression of uterine leiomyoma. Obstet Gynecol. 2022;139:797–807. doi: 10.1097/aog.0000000000004745. [PubMed: 35576339]
34. Mickey RM, Greenland S. The impact of confounder selection criteria on effect estimation. Am J Epidemiol. 1989;129:125–37. doi: 10.1093/oxfordjournals.aje.a115101. [PubMed: 2910056]
35. Peddada SD, Laughlin SK, Miner K, Guyon JP, Haneke K, Vahdat HL, et al. Growth of uterine leiomyomata among premenopausal Black and White women. Proc Natl Acad Sci U S A. 2008;105:19887–92. doi: 10.1073/pnas.0808188105. [PubMed: 19047643]
36. Laughlin SK, Hartmann KE, Baird DD. Postpartum factors and natural fibroid regression. Am J Obstet Gynecol. 2011;204:496 e1–6. doi: 10.1016/j.ajog.2011.02.018.
37. Giustina A, Adler RA, Binkley N, Bollerslev J, Bouillon R, Dawson-Hughes B, et al. Consensus statement from 2(nd) international conference on controversies in vitamin D. Rev Endocr Metab Disord. 2020;21:89–116. doi: 10.1007/s11154-019-09532-w. [PubMed: 32180081]
38. Pal L, Zhang H, Williams J, Santoro NF, Diamond MP, Schlaff WD, et al. Vitamin D status relates to reproductive outcome in women with polycystic ovary syndrome: secondary analysis of a multicenter randomized controlled trial. J Clin Endocrinol Metab. 2016;101:3027–35. doi: 10.1210/jc.2015-4352. [PubMed: 27186859]
39. Jukic AMZ, Baird DD, Weinberg CR, Wilcox AJ, McConnaughey DR, Steiner AZ. Pre-conception 25-hydroxyvitamin D (25(OH)D) and fecundability. Hum Reprod. 2019;34:2163–72. doi: 10.1093/humrep/dez170. [PubMed: 31665286]
40. McDonnell SL, Baggerly KA, Baggerly CA, Aliano JL, French CB, Baggerly LL, et al. Maternal 25(OH)D concentrations ≥40 ng/ml associated with 60% lower preterm birth risk among general obstetrical patients at an urban medical center. PLoS One. 2017;12:e0180483. doi: 10.1371/journal.pone.0180483. [PubMed: 28738090]
41. Jukic AMZ, Wilcox AJ, McConnaughey DR, Weinberg CR, Steiner AZ. 25-hydroxyvitamin D and long menstrual cycles in a prospective cohort study. Epidemiology. 2018;29:388–96. doi: 10.1097/ede.0000000000000804. [PubMed: 29337846]
42. O'Brien KM, Sandler DP, Taylor JA, Weinberg CR. Serum vitamin D and risk of breast cancer within five years. Environ Health Perspect. 2017;125:077004. doi: 10.1289/ehp943. [PubMed: 28728134]

43. Wegienka G, Baird DD, Hertz-Picciotto I, Harlow SD, Steege JF, Hill MC, et al. Self-reported heavy bleeding associated with uterine leiomyomata. Obstet Gynecol. 2003;101:431–7. doi: 10.1016/s0029-7844(02)03121-6. [PubMed: 12636944]
44. Baird DD, Saldana TM, Shore DL, Hill MC, Schectman JM. A single baseline ultrasound assessment of fibroid presence and size is strongly predictive of future uterine procedure: 8-year follow-up of randomly sampled premenopausal women aged 35–49 years. Hum Reprod. 2015;30:2936–44. doi: 10.1093/humrep/dev235. [PubMed: 26409013]
45. Tunau KA, Garba JA, Panti AA, Shehu CE, Adamu AN, AbdulRahman MB, et al. Low plasma vitamin D as a predictor of uterine fibroids in a Nigerian population. Niger Postgrad Med J. 2021;28:181–6. doi: 10.4103/npmj.npmj_495_21. [PubMed: 34708704]
46. Xu F, Li F, Li L, Lin D, Hu H, Shi Q. Vitamin D as a risk factor for the presence of asymptomatic uterine fibroids in premenopausal Han chinese women. Fertil Steril. 2021;115:1288–93. doi: 10.1016/j.fertnstert.2020.12.001. [PubMed: 33589134]
47. Mitro SD, Zota AR. Vitamin D and uterine leiomyoma among a sample of US women: findings from nhanes, 2001–2006. Reprod Toxicol. 2015;57:81–6. doi: 10.1016/j.reprotox.2015.05.013. [PubMed: 26047529]
48. Sheng B, Song Y, Liu Y, Jiang C, Zhu X. Association between vitamin D and uterine fibroids: a study protocol of an open-label, randomised controlled trial. BMJ Open. 2020;10:e038709. doi: 10.1136/bmjopen-2020-038709.
49. Bläuer M, Rovio PH, Ylikomi T, Heinonen PK. Vitamin D inhibits myometrial and leiomyoma cell proliferation in vitro. Fertil Steril. 2009;91:1919–25. doi: 10.1016/j.fertnstert.2008.02.136. [PubMed: 18423458]
50. Halder SK, Sharan C, Al-Hendy O, Al-Hendy A. Paricalcitol, a vitamin D receptor activator, inhibits tumor formation in a murine model of uterine fibroids. Reprod Sci. 2014;21:1108–19. doi: 10.1177/1933719114537721. [PubMed: 24925855]
51. Corachán A, Ferrero H, Aguilar A, Garcia N, Monleon J, Faus A, et al. Inhibition of tumor cell proliferation in human uterine leiomyomas by vitamin D via Wnt/β-catenin pathway. Fertil Steril. 2019;111:397–407. doi: 10.1016/j.fertnstert.2018.10.008. [PubMed: 30458994]
52. Corachán A, Ferrero H, Escrig J, Monleon J, Faus A, Cervelló I, et al. Long-term vitamin D treatment decreases human uterine leiomyoma size in a xenograft animal model. Fertil Steril. 2020;113:205–16.e4. doi: 10.1016/j.fertnstert.2019.09.018. [PubMed: 31739978]
53. Mehine M, Mäkinen N, Heinonen HR, Aaltonen LA, Vahteristo P. Genomics of uterine leiomyomas: insights from high-throughput sequencing. Fertil Steril. 2014;102:621–9. doi: 10.1016/j.fertnstert.2014.06.050. [PubMed: 25106763]
54. Elkafas H, Ali M, Elmorsy E, Kamel R, Thompson WE, Badary O, et al. Vitamin D3 ameliorates DNA damage caused by developmental exposure to endocrine disruptors in the uterine myometrial stem cells of Eker rats. Cells. 2020;9. doi: 10.3390/cells9061459.
55. Moshesh M, Peddada SD, Cooper T, Baird D. Intraobserver variability in fibroid size measurements: estimated effects on assessing fibroid growth. J Ultrasound Med. 2014;33:1217–24. doi: 10.7863/ultra.33.7.1217. [PubMed: 24958408]
56. Pilz S, Zittermann A, Obeid R, Hahn A, Pludowski P, Trummer C, et al. The role of vitamin D in fertility and during pregnancy and lactation: a review of clinical data. Int J Environ Res Public Health. 2018;15. doi: 10.3390/ijerph15102241.
57. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr. 2000;72:690–3. doi: 10.1093/ajcn/72.3.690. [PubMed: 10966885]
58. Kimball SM, Holick MF. Official recommendations for vitamin D through the life stages in developed countries. Eur J Clin Nutr. 2020;74:1514–8. doi: 10.1038/s41430-020-00706-3. [PubMed: 32820241]

Table 1

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

Characteristic[a]		Overall N=1610	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 (years)	Mean (SD)	29.2 (3.4)	29.1 (3.4)	29.5 (3.4)
Highest educational attainment [d]	High School/GED	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		1002 (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 (years)	<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	No birth	624 (39)	457 (39)	164 (39)
	<3 years	360 (22)	256 (22)	101 (24)
	3–4 years	207 (13)	146 (12)	61 (14)
	5–9 years	291 (18)	226 (19)	64 (15)
	≥10 years	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)

Characteristic[a]		Overall N=1610	25(OH)D <20 ng/ml N=1179[b]	25(OH)D ≥20 ng/ml N=424[b]
	≥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; GED = General Education Diploma; 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).

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]
<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)
<20	918	REF	REF
≥20	439	−8.4 (−16.4, 0.3)	−9.7 (−17.3, −1.3)
<30	1239	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 due to missing data on at least one covariate.

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 Case	Person-Years	Hazard Ratio (95% Confidence Interval)	
			Unadjusted[b]	Fully adjusted[c]
<12	88	1717	REF	REF
12–<20	119	2213	1.06 (0.81, 1.40)	1.03 (0.78, 1.37)
20–<30	71	1075	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)
<20	207	3930	REF	REF
≥20	87	1377	1.11 (0.87, 1.43)	1.05 (0.81, 1.36)
<30	278	5004	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 1230 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 due to missing data on at least one covariate.

Table 4

Serum 25(OH)D and fibroid loss in 539[a] participants in tne 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]
<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)
<20	168/725 (23.2)	REF	REF
≥20	80/294 (27.2)	1.08 (0.86, 1.36)	1.17 (0.92, 1.48)
<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 due to missing data on at least one covariate.