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Lower 25-hydroxyvitamin D is associated with long menstrual cycles in a prospective cohort study

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Abstract

Background—Vitamin D insufficiency is associated with subfertility and prolonged estrus cycles in animals, but humans have not been well-studied.

Methods—A prospective time-to-pregnancy study, Time to Conceive (2010–2015), collected up to four months of daily diary data. Participants were healthy, late reproductive-aged women in North Carolina who were attempting pregnancy. We examined menstrual cycle length as a continuous variable, as well as in categories: long (35+ days) and short (< 25 days). Follicular phase length and luteal phase length were categorized as long (18+ days) or short (< 10 days). We estimated associations between those lengths and serum 25-hydroxyvitamin D (25(OH)D) using linear mixed models and marginal models.

Results—There were 1278 menstrual cycles from 446 women of whom 5% were vitamin D deficient (25(OH)D <20ng/ml), 69% were between 20 and 39ng/ml, and 26% were 40ng/ml or higher. There was a dose-response association between vitamin D levels and cycle length. Compared with the highest 25(OH)D level (> 40ng/ml), 25(OH)D deficiency was associated with almost three times the odds of long cycles (adjusted odds ratio (aOR) (95% confidence interval (CI)): 2.8 (1.0, 7.5)). The aOR was 1.9 (1.1, 3.5) for 20–<30ng/ml. The probability of a long follicular phase and the probability of a short luteal phase both increased with decreasing 25(OH)D.

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Conflicts of interest: ClearBlue ovulation predictor kits were generously donated to AMZJ and AJW by Swiss Precision Diagnostics. Swiss Precision Diagnostics had no role in the design, conduct, analysis, interpretation, or presentation of these data.

Conclusions—Lower levels of 25(OH)D are associated with longer follicular phase and an overall longer menstrual cycle. Our results suggest that vitamin D affects the reproductive axis, suggesting broader implications for reproductive success.

Keywords

vitamin D; ovulation; ovary; reproduction; menstrual cycle length; fertility

Introduction

Vitamin D is important for bone health,¹ and may be important for reproduction.^{2–4} Vitamin D receptor is expressed in the ovary, placenta, and the uterus.^{2–4} Diet-induced vitamin D deficiency reduces fertility in both mice and rats, in the latter by 45–70%.⁵ Knock-out mice that cannot convert vitamin D to its active form show estrus cycle disturbances including arrested follicular development, prolonged estrous cycles, and anovulation.^{6,7}

Vitamin D deficiency is a risk factor for polycystic ovarian syndrome.⁸ Among women with this syndrome, supplementation with vitamin D can normalize menstrual cycles and improve ovarian folliculogenesis.^{9,10} However, there are few studies of vitamin D and ovarian function in healthy women. Two previous studies found associations between 25-hydroxyvitamin D (25(OH)D), the accepted biomarker of vitamin D status, and long menstrual cycles¹¹ or irregular cycles.¹² These studies were based on self-reported, retrospectively recalled, menstrual cycle information. This question has not been examined in prospective daily diary data.

Long or irregular menstrual cycles can require hormonal therapy and medical intervention, with their associated costs. Long menstrual cycles (>35 days) are more likely to be anovulatory¹³ and may also be subfecund¹⁴. If vitamin D is associated with menstrual cycle length, it would suggest that the human reproductive axis is responsive to vitamin D, with possible implications for reproductive success. Our primary objective was to examine the association of vitamin D and menstrual cycle length in a prospective cohort study of time to pregnancy. Given the reported effects of vitamin D deficiency on follicular development in animal studies, we also sought to evaluate the association between vitamin D and follicular phase length.

Methods

Study design

Time to Conceive is a prospective, time-to-pregnancy cohort study of biomarkers of infertility (2008–2015).¹⁵ Women who were intending to become pregnant were recruited through mass emails, introductory letters, and web and radio advertising. Eligible women had to be between the ages of 30 and 44 and were trying to conceive naturally, for 3 months or less. This was self-reported as the amount of time they had been “having regular intercourse without doing anything to prevent pregnancy”. Women were excluded if they reported a history of infertility, polycystic ovarian syndrome, endometriosis, a partner with infertility, or current breastfeeding. Women completed a self-administered questionnaire that

included demographic data, reproductive history, contraceptive history, and other behaviors. At the same time, women were asked to begin keeping daily diaries (described below). Women were instructed to schedule a study visit at the beginning of their next menses, on day 2, 3, or 4. If they missed this window, they were asked to come in at the next menstrual cycle.

At the study visit, women gave informed consent, provided a blood sample, and were given pregnancy test kits. In 2010, the study protocol was amended to add the collection of whole blood spots, which were dried and stored frozen. The present analysis is limited to the cohort of women who enrolled in or after 2010. From 2013 on, women were provided ClearBlue digital ovulation tests. Prior to 2013, participants may have voluntarily used ovulation predictor kits. In either case, participants were asked to record ovulation test results in their daily diary (described below).

Women were asked to complete a daily diary for up to four months and monthly diaries thereafter, until either a positive pregnancy test was observed or 12 months had elapsed. Women recorded their menstrual bleeding and results of their ovulation and pregnancy tests. Women were withdrawn from the study if they began fertility treatment or stopped trying to conceive. Women in this analysis were enrolled between February 2010 and July 2015, and daily diary information collected through July 2015 was included in this analysis. In total 587 women recorded at least one cycle in the daily diary, with a total of 1834 cycles (Figure 1). Of these women, 56 did not contribute a blood spot, leaving 1674 cycles to 531 women.

All study activities were approved by the University of North Carolina IRB.

Measurement of 25(OH)D

25(OH)D was extracted from 6mm punches from stored blood spots using previously described methods.¹⁶ 25(OH)D₃ and 25(OH)D₂ were quantified through liquid chromatography-tandem mass spectrometry. 25(OH)D measured in dried blood spots shows good agreement with plasma measures.¹⁷ Blinded samples indistinguishable from test samples were also sent to the lab. Based on these samples, the intra-assay coefficient of variation was 6.3 and the inter-assay coefficient of variation was 7.7.

Estimating cycle-specific 25(OH)D

A single measured value of 25(OH)D may become less relevant over time, with possible changes in vitamin D levels due to season of the year or alterations in supplement use. Because we did not have longitudinal measures of 25(OH)D we used an imputation model to estimate a time-varying cycle-specific 25(OH)D level.¹⁸ Details of the imputation are in eAppendix 1. Eighty-nine cycles and 33 women were missing information for imputing 25(OH)D and were excluded from the analyses.

We examined cycle-specific 25(OH)D both as a linear variable and in categories (<20, 20–40, >40ng/ml). The lowest category (<20 ng/ml) corresponds to increased risk of vitamin D deficiency, based on the Institute of Medicine and Endocrine Society guidelines.^{19,20} In our sample, the approximate 75th percentile of the 25(OH)D distribution was 40ng/ml. Due to

small numbers, restricted cubic splines of 25(OH)D were examined as predictors for the follicular and luteal phase analyses.

Menstrual cycle length

Daily diary information was used to identify each menses.²¹ Menstrual cycle length was defined as the number of days from the first day of menses to the day before onset of the next menses. Conception cycles have an undefined length, and were excluded (N=255 cycles, Figure 1). Participants contributed 1 to 6 menstrual cycles (eTable 1).

Menstrual cycle length was natural log-transformed for analysis as a continuous variable. Two dichotomous variables were also created: “long cycles” of 35 days or more and “short cycles” of 25 days or less.

Follicular and luteal phase length

The estimated day of ovulation was defined as the day after a positive ovulation test in the daily diary (per ClearBlue instructions). Cycles were excluded from the phase length analyses if the day prior to a positive test was missing. Follicular phase length was defined as the number of days from the first day of menses to the day before ovulation.²² Luteal phase length was defined as the number of days from the day after ovulation to the day before onset of subsequent menses.²² Luteal phase length could not be determined in conception cycles or cycles missing total cycle length, as in the cycle length analysis (Figure 1). Thus, of the 1277 cycles in the cycle length analysis, 328 have a documented ovulation day, and a measurable luteal phase length (675 cycles occurred among women enrolled prior to the distribution of ovulation predictor kits). Conception cycles were included in the follicular phase analysis as were cycles in which cycle end was not known (N=1545 eligible cycles), of these, 435 had an identified day of ovulation and therefore a measurable follicular phase. Of the cycles missing a follicular phase, N=797 occurred among women who enrolled prior to the distribution of ovulation kits.

Follicular phase length was analyzed through two dichotomous variables: “long” (≥ 18 days) and “short” (< 10 days). Similarly, “long” luteal phases were defined as at least 18 days and “short” luteal phases were 10 days or less^{23,24}.

Covariates

Variables examined as potential confounders were based on the literature,^{25–28} and included self-reported age, race, body mass index, and education. Participants also reported their monthly number of alcoholic drinks, number of caffeinated drinks per day, and the number of cigarettes smoked per day. Participants were asked about use of other nicotine products, but none were reported. These variables were averaged across months to obtain a single variable for each woman. Participants also reported in each month, in categories, their average amount of vigorous exercise (0 hours, <1, 1–3, 4–7, >7 hours per week) and their level of stress (not at all, mildly, moderately, or very). Each category was assigned an ordinal number and averaged for each woman. This exact average was used in the analysis and rounded down for presentation. We defined the cycle of attempt at the time of the blood draw by subtracting the first recorded last menstrual period date from the date they

discontinued contraception and divided by their usual reported menstrual cycle length. This amount of time may be somewhat different from the woman's self-reported attempt time at screening for two reasons. First, women may vary in how they define the beginning or length of their pregnancy attempt. Second, we are measuring in cycles while the women reported in months. Cycle of attempt at the time of blood draw was included as a covariate because it is plausible that vitamin D is associated with fecundability. If so, women who have high vitamin D and short cycles would have more opportunities to conceive and would have less opportunity to enroll in the study prior to conceiving (even in the absence of a causal effect of vitamin D on cycle length). This could create a spurious association between vitamin D and cycle length that is removed by adjusting for the attempt cycle number at enrollment to the study. We evaluated several parameterizations of age with each endpoint of interest independently. We used Akaike's Information Criterion to choose the best-fitting parameterization of age in each model. All other continuous variables (body mass index (BMI), cigarette smoking, caffeine, alcohol, stress, and exercise) were evaluated in a similar way: several parameterizations were compared after adjustment for age, race, and cycle of study entry. Fifty-three cycles and 6 women were excluded due to missing covariate information leaving 1277 cycles from 446 women. A comparison of cycles included and excluded is shown in eTable 2.

Statistical Analysis

Menstrual cycle length was analyzed with linear mixed models, with a random intercept for each woman. To achieve approximate normality of the model residuals, the linear mixed model was limited to cycles between 22 and 36 days long (11 cycles were less than 22 days, and 119 cycles were longer than 36 days). Dichotomous variables were analyzed using generalized linear models and generalized estimating equations with an exchangeable working correlation matrix. The referent category for each dichotomous variable is "normal" cycle or phase length. For example, "long" cycles were compared with "normal" length cycles and "short" cycles were excluded from that particular analysis. For cycle length analysis we present two models, one fully adjusted and one with a minimal adjustment set (cycle of attempt at the time of the blood draw, age, race, BMI, education, and time since estrogen use). For the follicular phase and luteal phase analyses, only fully adjusted results are presented. We also performed a sensitivity analysis using the measured 25(OH)D value rather than the cycle-specific imputed values described previously.

Ecologic Analysis

To further explore the ecological associations between season, which influences vitamin D status, and menstrual cycle length, we examined a large study of prospectively collected menstrual diaries.²⁹ The Treloar study (1930 – 1976) included 998 women who provided at least 5 years of data resulting in 106,060 menstrual cycles (excluding cycles reported within one year of a delivery, within a month of breastfeeding, or within one month of hormone use). We used mixed effect harmonic models, stratified by age, to examine the association between season and menstrual cycles in this study. Similarly, we also used data from the North Carolina Early Pregnancy Study, a cohort study of women attempting to become pregnant, to examine the association between season and timing of ovulation^{30–32}. We used

these models to identify season-associated variation in cycle length, and to estimate the percent change in average cycle length between the peak and nadir times of year.

Results

Univariable description

The distribution of 25(OH)D among women is shown in eTable 3. Among all cycles (N=1278), the geometric mean 25(OH)D was 34ng/ml and the median was 34ng/ml (interquartile range: 28, 41ng/ml). Menstrual cycle length ranged from 19 to 68 days (Table 1). Ninety percent of the short cycles in this sample were between 23 and 25 days long, and 75% of the long cycles were between 35 and 48 days. Out of 446 women, 119 had at least one long cycle and 142 women had at least one short cycle. Vitamin D deficiency was more likely to occur in women who were in their mid-thirties, African-American, less educated, or obese. Higher 25(OH)D was more likely with high levels of physical activity or alcohol intake.

Multivariable analysis: menstrual cycle length

In the fully adjusted model, a 10 ng/ml decrease in 25(OH)D was associated with a 0.5% increase in cycle length (95% Confidence Interval (CI): -0.24, 1.3) (Table 2). In the fully adjusted model, each 10ng/ml decrease in 25(OH)D was also associated with a 30% increase in the odds of long menstrual cycles (odds ratio (OR)(CI): 1.3 (1.0, 1.6)). 25(OH)D deficiency (<20ng/ml) was associated with almost three times the odds of long menstrual cycles compared with 25(OH)D levels of at least 40 ng/ml (OR(CI): 2.8 (1.0, 7.5)). Insufficiency (20–<30 ng/ml) was associated with almost twice the odds of long menstrual cycles (OR(CI): 1.9 (1.1, 3.5)). Further adjustment for season and supplement use, which would have been inappropriate if they were causal ancestors of 25(OH)D and not independent causes of cycle length, did not alter the associations with long cycles. 25(OH)D was not associated with the occurrence of short menstrual cycles.

We also compared a 25(OH)D of less than 30ng/ml (the Endocrine Society cutoff for insufficiency) to a level of at least 40 ng/ml. For continuous cycle length, a 25(OH)D of less than 30ng/ml was associated with a 1.8% increase in cycle length (CI: -0.40, 3.9). A 25(OH)D of less than 30ng/ml was associated with twice the odds of long cycles (CI: 1.1, 3.6), and 0.75 times the odds of short cycles (CI: 0.46, 1.2) compared with at least 40ng/ml.

Given the late reproductive age of our study population, some women may have been perimenopausal. Although women with known polycystic ovarian syndrome were ineligible for the study, it is also possible that some women were affected, but undiagnosed. To address both of these possibilities, we performed a sensitivity analysis excluding women with either low levels of anti-Müllerian hormone (< 0.6ng/ml, suggesting low ovarian reserve) or high levels (>10ng/ml, suggesting possible polycystic ovarian syndrome). These exclusions did not materially alter our results (eTable 4).

Approximately 190 cycles were only partially observed in the daily diary, either because the participant did not fill in the diary or because it was her first or last cycle in the daily diary (first cycle start date was defined with self-reported last menstrual period date and the last

cycle end date was obtained from the monthly diary). We could therefore determine cycle length for these cycles, because we had the first and last day, but not all of the intervening cycle days were accounted for in the daily diary, which is likely to be the most accurate data source and was where supplement use would have been recorded. If the analysis of long cycles is limited to cycles that were at least 50% observed in the daily diary the associations are stronger, <20ng/ml OR(CI): 4.1 (1.5, 12), 20–<30 ng/ml OR(CI): 2.3 (1.2, 4.8), and 30–<40 ng/ml OR(CI): 1.7 (0.85, 3.5), for a 10ng/ml decrease in 25OHD, OR(CI): 1.5 (1.1, 1.9).

It is possible that higher 25(OH)D improves fertility and shortens time to pregnancy, resulting in a smaller number of observed cycles for these women (i.e. informative cluster size). Mixed models avoid this bias³³. To address this for our long cycle analysis which used generalized estimating equations, we performed a sensitivity analysis by weighting each cycle by the inverse of the number of contributed cycles³⁴. The results were only minimally changed, <20ng/ml: 2.7 (0.93, 7.7), and 20–<30ng/ml: 1.7 (0.87, 3.3), 30–<40 ng/ml: 1.4 (0.75, 2.7).

Follicular phase length

Lower 25(OH)D was associated with a higher probability of a long follicular phase (Figure 2). The probability decreased sharply with increasing vitamin D levels up to 30 ng/ml and then leveled off around 40 ng/ml. 25(OH)D was not associated with the occurrence of short follicular phases.

Luteal phase length

Lower 25(OH)D was also associated with a higher probability of a short (but not long) luteal phase (Figure 3).

Ecologic Analysis

In the Treloar data, we found that November/December cycles were about 0.5% longer than May/June cycles in younger women (aged 20–29) (CI: 0.3, 0.8%), and 0.4% longer in older women (aged 30–39) (CI: 0.1, 0.6%). Among women who were at least age 30 in the North Carolina Early Pregnancy Study (N=89), follicular phase length was 12% longer (CI: 4, 22%) in menstrual cycles that began in early March compared with those that started in early September. Among women under age 30, follicular phase length was 3% longer (CI: –5, 12%) in January compared with July.

Discussion

This is the first study to examine possible effects of vitamin D on menstrual patterns determined from prospectively-recorded data on menses and ovulation. Lower levels of 25(OH)D were associated with both long menstrual cycles and long follicular phases and with a tendency towards short luteal phases. The strongest associations were for vitamin D deficiency (<20 ng/ml), however, cycles with a 25(OH)D of 20–<30ng/ml were still at increased risk relative to cycles where 25(OH)D was at least 40 ng/ml. The associations remained after excluding women with extreme anti-Müllerian hormone values. We did not

see associations between 25(OH)D and short menstrual cycles, short follicular phases, or long luteal phases.

Two previous studies have reported associations between lower levels of 25(OH)D and an increased odds of either irregular¹² or long¹¹ menstrual cycles. Those studies were cross-sectional and relied on self-reported menstrual cycle length. Nevertheless, the current study agrees with those studies in suggesting that lower levels of 25(OH)D may increase menstrual cycle length.

25(OH)D is converted to its active form 1,25(OH)₂D by the enzyme 1 α -hydroxylase, a product of the *CYP27B1* gene. The active form of vitamin D binds to the vitamin D receptor. Mice that lack either *CYP27B1* or the vitamin D receptor exhibit arrested follicular development and prolonged estrous cycles.^{6,7,35,36} The mechanism for these effects is unknown, and may involve suboptimal gonadotropin secretion from the pituitary or hypothalamus, or defects in the ovarian response to gonadotropin.⁶ Active vitamin D may influence ovarian anti-Müllerian hormone expression or signaling (reviewed in⁸), which is known to regulate follicle recruitment.⁴

In humans, long menstrual cycles correlate with long follicular phases, i.e. delayed ovulation.^{37–39} Ovulation might be delayed by diminished ovarian response to gonadotrophin stimulation³⁷ or from extended periods of low follicular estrogen.⁴⁰ The hormonal milieu of long cycles is variable⁴¹. Some long cycles show a delay in the follicular rise of estrogen,^{13,41} while others show typical early follicular increases in estrogen followed by variable patterns of estrogen rise or fall.⁴¹ Anovulation appears to occur in both short and long menstrual cycles.¹³ While anovulation may contribute to an association between 25(OH)D and long cycles it cannot be the sole explanation, since vitamin D was also associated with a long follicular phase, which would not be measurable in an anovulatory cycle.

Short luteal phases are associated with pre-ovulatory hormone disturbances⁴², which might suggest a shared pathology between long follicular phases and short luteal phases. Short luteal phases are often accompanied by low luteal progesterone, and are considered sub-fertile²³.

This study has several limitations. First, most of the women studied had sufficient 25(OH)D levels. While we did detect a strong association of 25(OH)D deficiency with menstrual cycle length, the association was estimated imprecisely. Second, the participants in this study provided only a single blood sample, at the beginning of their pregnancy attempt. 25(OH)D levels may have changed over their participation in the study. We attempted to address this by projecting a cycle-specific adjusted 25(OH)D level that incorporated cycle-specific supplement use and season in her other cycles. These results agreed with the results using only the baseline measured 25(OH)D, suggesting that changes over time are not an important source of error. Third, most of the women in this study were White and well-educated, which may limit the generalizability of our study. Fourth, it is possible that some of the women in our analysis had undiagnosed polycystic ovarian syndrome or were perimenopausal, which could underlie the association between vitamin D and cycle length.

However, we attempted to address this with our sensitivity analysis excluding women with extreme anti-Müllerian hormone values. The results suggest that this may not be an important source of bias in our study. Finally, it is possible that low vitamin D influences the performance of the urinary ovulation test, resulting in a systematically late detection of ovulation. While we know of no mechanism for this, it would cause an apparent association between vitamin D and both long follicular phase and short luteal phase, although such bias could not explain our results for menstrual cycle length.

Our study also has strengths: our cohort was prospective, and included both menstrual cycle length and follicular and luteal phase length. The cohort was recruited from the community and is representative of women in the Chapel Hill area.⁴³ Menstrual cycle information and supplement use information were collected daily. Vitamin D status was assessed with the accepted biomarker, 25(OH)D,⁴⁴ and was adjusted to account for time-varying factors.

Due to changes in ultraviolet light intensity across seasons, season is a determinant of 25(OH)D. Thus, if low 25(OH)D lengthens the follicular phase, and levels of 25(OH)D tend to be low in winter, we might expect to find longer cycles in the winter. We found only one study of this question; in 129 Russian women, summer menstrual cycles were one day shorter than winter cycles, and they were also more likely to be ovulatory and showed larger follicles on ultrasound.⁴⁵ Moreover, both of our ecologic analyses showed an association between winter months and either longer menstrual cycles or later ovulation. While these analyses used an indirect surrogate of vitamin D (season), they add to the plausibility of our findings.

In conclusion, these findings suggest that vitamin D status influences menstrual cycle length through an association with longer follicular phase (delayed ovulation). Vitamin D may play a role in ovarian function, and future studies should investigate the role of vitamin D in follicular recruitment, selection, and growth using longitudinal measures. Prospective randomized trials of vitamin D and cycle length may be justifiable to determine the utility of vitamin D as a treatment for oligomenorrhea.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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A resource sharing plan is in place for accessing Time to Conceive data. Please contact Dr. Steiner (asteiner@med.unc.edu) for additional information.

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Figure 1.
Flow diagram of Time to Conceive participants included in these analyses.

Figure 2. Restructured cubic splines of 25(OH)D and the predicted probability of long (18 days) or short (10 days) follicular phase with confidence interval shading, depicted at the referent level for all covariates.

Figure 3. Restricted cubic splines of 25(OH)D and the predicted probability of long (18 days) or short (10 days) luteal phase with confidence interval shading, depicted at the referent level for all covariates.

Table 1

Distribution of 25(OH)D among menstrual cycles from the Time to Conceive study (2010 – 2015) (N=1278 cycles).

	N	Number of women (N=446) (percent)	25(OH)D (ng/ml)			
			Overall	<20	20 – <40	40
		(%)	N (%)	N (%)	N (%)	N (%)
Menstrual cycle length ^a						
Short (19 – 25 days)	27	(4)	223 (17)	14 (6)	145 (65)	64 (29)
Normal (26 – 34 days)	355	(82)	902 (71)	39 (4)	614 (68)	249 (28)
Long (35 – 68 days)	64	(14)	153 (12)	13 (8)	112 (73)	28 (18)
Follicular phase length ^a						
Short (10)	19	(9)	69 (15)	1 (2)	33 (67)	15 (31)
Normal (11–17)	156	(75)	321 (70)	9 (3)	218 (68)	94 (29)
Long (18)	34	(16)	67 (15)	9 (13)	44 (66)	14 (21)
Luteal phase length ^a						
Short (10)	15	(9)	30 (9)	5 (17)	17 (57)	8 (27)
Normal (11–17)	128	(80)	265 (81)	9 (3)	177 (67)	79 (30)
Long (18)	18	(11)	33 (10)	1 (3)	26 (79)	6 (18)
Age (when started trying)						
29 – 30	89	(20)	227 (18)	1 (0.4)	175 (77)	51 (22)
31 – 32	129	(29)	355 (28)	13 (4)	231 (65)	111 (31)
33 – 35	124	(28)	369 (29)	23 (6)	265 (72)	81 (22)
36 – 40	85	(19)	261 (20)	26 (10)	168 (64)	67 (26)
>40	19	(4)	66 (5)	3 (5)	32 (48)	31 (47)
Race						
African-American	39	(9)	130 (10)	23 (18)	91 (70)	16 (12)
Caucasian	347	(78)	978 (77)	24 (2)	651 (67)	303 (31)
Other ^b	60	(13)	170 (13)	19 (11)	129 (76)	22 (13)
Education						
Some college or less	36	(8)	106 (8)	26 (25)	67 (63)	13 (12)
College graduate	91	(20)	279 (22)	6 (2)	210 (75)	63 (23)

	Number of women (N=446) (percent)	25(OH)D (ng/ml)		
		Overall	<20	20 – <40
	N	N (%)	N (%)	N (%)
Some graduate school or Master's degree	200	568 (44)	11 (2)	374 (66)
Terminal degree (MD, PhD)	119	325 (25)	20 (6)	226 (69)
Body mass index				
<20	52	156 (12)	6 (4)	110 (71)
20 – <25	239	668 (52)	26 (4)	427 (64)
25 – <30	85	249 (19)	7 (3)	192 (77)
30	70	205 (16)	27 (13)	142 (69)
Menstrual cycle of attempt at blood draw				
1	63	147 (12)	7 (5)	88 (60)
2	192	539 (42)	16 (3)	393 (73)
3	98	304 (24)	23 (8)	187 (62)
4	56	173 (14)	18 (10)	126 (73)
5	37	115 (9)	2 (2)	77 (67)
Time since estrogen use				
More than 3 months	377	1104 (86)	65 (6)	757 (69)
One month or less	21	49 (4)	0 (0)	29 (59)
Two months	17	40 (3)	0 (0)	29 (72)
Three months	31	85 (7)	1 (1)	56 (66)
Average hours of vigorous exercise per week during prospective diary collection				
0	58	160 (13)	15 (9)	125 (78)
0 – <1	127	391 (31)	19 (5)	275 (70)
1 – 3	176	494 (39)	26 (5)	341 (69)
4	85	233 (18)	6 (3)	130 (56)
Average number of alcoholic drinks per month during prospective diary collection				
0 – 2.5	145	421 (33)	38 (9)	305 (72)
>2.5 – <10	148	433 (34)	16 (4)	310 (72)
10 – 59	153	424 (33)	12 (3)	256 (60)
Average number of cigarettes per day during prospective diary collection				
None	434	1242 (97)	64 (5)	842 (68)

	Number of women (N=446) (percent)		25(OH)D (ng/ml)			
	N	(%)	Overall N (%)	<20 N (%)	20 – <40 N (%)	40 N (%)
>0 – 10	12	(3)	36 (3)	2 (6)	29 (81)	5 (14)
Average number of caffeinated drinks per day during prospective diary collection						
0 – <1	119	(27)	336 (26)	19 (6)	237 (71)	80 (24)
1 – <1.5	191	(43)	564 (44)	31 (5)	387 (69)	146 (26)
1.5 – 5	136	(30)	378 (30)	16 (4)	247 (65)	115 (30)
Average degree of stress during prospective diary collection						
Not at all	30	(7)	87 (7)	6 (7)	62 (71)	19 (22)
Mildly	234	(53)	669 (52)	34 (5)	448 (67)	187 (28)
Moderately	164	(37)	484 (38)	21 (4)	346 (72)	117 (24)
Very	18	(4)	38 (3)	5 (13)	15 (40)	18 (47)

^aIn the “Number of women” column, each woman is categorized by her geometric mean menstrual cycle or phase length during the study

^b“Other” included American Indian/Alaskan, Asian/Pacific Islander, Hispanic, and mixed or unknown race

Table 2

Associations of continuous 25(OH)D and categories of 25(OH)D with continuous menstrual cycle length, long menstrual cycles, and short menstrual cycles^a.

	Number of cycles ^b	Percent change in cycle length (95% CI) ^c	Percent change in cycle length (95% CI) ^d	Percent change in cycle length (95% CI) ^e
25(OH)D, 10ng/ml decrease	1148	0.56 (−0.20, 1.3)	0.53 (−0.24, 1.3)	0.73 (−0.14, 1.6)
25(OH)D				
< 20 ng/ml ^f	55	1.8 (−2.2, 6.0)	1.5 (−2.4, 5.5)	1.3 (−3.0, 5.7)
20 – <30	296	2.0 (−0.15, 4.2)	1.8 (−0.37, 4.0)	1.8 (−0.53, 4.1)
30 – <40	483	−0.02 (−1.8, 1.8)	0.02 (−1.8, 1.9)	0.36 (−1.7, 2.4)
40	314	0	0	0
Long cycles (≥ 35 days)				
	N cycles (N long)	OR (CI) ^c	OR (CI) ^d	OR (CI) ^e
25(OH)D, 10ng/ml decrease	1055 (153)	1.2 (0.99, 1.6)	1.3 (1.0, 1.6)	1.3 (1.0, 1.6)
25(OH)D				
< 20 ng/ml	52 (13)	2.5 (0.94, 6.4)	2.8 (1.0, 7.5)	3.1 (1.1, 8.8)
20 – <30	289 (49)	1.7 (0.96, 3.2)	1.9 (1.1, 3.5)	2.0 (1.1, 3.7)
30 – <40	437 (63)	1.3 (0.77, 2.3)	1.3 (0.75, 2.3)	1.5 (0.84, 2.8)
40	277 (28)	1	1	1
Short cycles (≤ 25 days)				
	N cycles (N short)	OR (CI) ^c	OR (CI) ^d	
25(OH)D, 10ng/ml decrease	1125 (223)	0.97 (0.83, 1.1)	0.94 (0.81, 1.1)	0.92 (0.77, 1.1)
25(OH)D				
< 20 ng/ml	53 (14)	1.1 (0.45, 2.7)	1.0 (0.44, 2.4)	1.3 (0.50, 3.5)
20 – <30	288 (48)	0.76 (0.47, 1.2)	0.72 (0.44, 1.2)	0.71 (0.42, 1.2)
30 – <40	471 (97)	1.1 (0.75, 1.7)	1.1 (0.72, 1.6)	1.1 (0.68, 1.6)
40	313 (64)	1	1	1

^aCycle length as modeled on the natural log scale. To achieve normality of the model residuals, the continuous analysis was limited to cycles between 22 and 36 days long. Results are displayed as the percentage change in cycle length for a given increase in 25(OH)D.

^bThe analysis of continuous cycle length includes 425 women, the analysis of long cycles includes 435 women and is limited to long or normal cycles, the analysis of short cycles includes 415 women and is limited to short or normal cycles.

^cAdjusted for cycle of blood draw, age, race, BMI, education, and time since estrogen use.

^dAdjusted for cycle of blood draw, age, race, BMI, education, time since estrogen use, exercise, alcohol, stress, cigarette smoking and caffeine intake.

^eUsing the single measured baseline value of 25(OH)D, adjusted as in ^d.

^f<20 ng/ml is the definition of vitamin D deficiency from the Endocrine Society guidelines.