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Association between Vitamin D Deficiency, Obesity, and Menstrual Irregularities among Women of Reproductive Age: A Cross-Sectional Analysis of NHANES Data

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ABSTRACT:

Background: Menstrual irregularities can reflect metabolic and endocrine dysfunction, yet the independent roles of vitamin D deficiency and obesity remain uncertain. This study examined their separate and joint associations with a severe menstrual-irregularity phenotype among U.S. women of reproductive age.

Methods: A cross-sectional analysis was conducted using August 2021-August 2023 National Health and Nutrition Examination Survey data. Women aged 18-49 years with valid reproductive-health, serum 25-hydroxyvitamin D [25(OH)D], body mass index (BMI), and survey-design data were included. The outcome identified women reporting no menstrual period during the previous 12 months for a non-pregnancy, non-lactation, nonsurgical, and non-menopausal reason. Vitamin D deficiency was defined as 25(OH)D <50 nmol/L and obesity as BMI >=30 kg/m². Survey-weighted logistic regression estimated crude and adjusted odds ratios (ORs).

Results: Among 980 women, 69 met the outcome definition; weighted prevalence was 6.1% (95% CI, 4.3%-8.0%). Obesity was associated with higher crude odds (OR, 1.61; 95% CI, 0.97-2.67), but the adjusted association was attenuated and imprecise (OR, 1.34; 95% CI, 0.79-2.27). Vitamin D deficiency was not associated with higher odds after adjustment (OR, 0.67; 95% CI, 0.26-1.73). Joint exposure to obesity and vitamin D deficiency did not increase odds, and no multiplicative interaction was detected. Women with obesity nevertheless had substantially lower mean 25(OH)D concentrations.

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Conclusion: Vitamin D deficiency was not independently associated with this severe menstrual phenotype. Obesity showed a directionally positive but statistically inconclusive association, while the coexistence of obesity and vitamin D deficiency did not confer additional odds. Prospective studies using detailed menstrual-cycle phenotyping are needed.

INTRODUCTION

Clinically informative indicators of reproductive and metabolic health are menstrual patterns. Longitudinaldata are associatedwith persistent irregular cycles and later type 2 diabetes and premature mortality (Solomon et al., 2001; Wang et al., 2020) and irregular or prolonged cycles may accompany irregularities in ovulation, insulin sensitivity and androgen activity and hypothalamic-pituitary-ovarian signaling. A recent systematic review and meta-analysis also found a correlation between the occurrence of menstrual irregularity and poor cardiometabolic outcomes (Afzal et al., 2026). Together these observations point towards menstruation being a tangible signal of a healthy or unhealthy reproductive physiology throughout the reproductive period.

Two potentially modifiable exposures, obesity and vitamin D status, are possible overlaps with menstrual function. Obesity has been linked to irregular or prolonged periods several times in the past and may be due to insulin resistance, hyperandrogenism,a change in sex hormone-binding globulin (SHBG), as well as disruption of ovulatory signaling by adipokines (Seif et al., 2015; Wei et al., 2009; Zhou & Yang, 2020). Further, vitamin D is biologically important as vitamin D receptors and metabolizing enzymes are found in reproductive tissues, where they might play a role in steroidogenesis and follicular function (Irani & Merhi, 2014; Shahrokhi et al., 2016).However, there are conflicting epidemiologic dataon the association of 25(OH)D with menstrual characteristics in different populations and definitions of the outcomes (Jukic et al., 2015, 2016, 2018; Singh et al., 2021).

The lower levels of circulating 25(OH)D are even more difficult to interpret as obesity is known to show a strong inverse association with circulating 25(OH)D, and there is a proposedbody-size-relatedvolumetric dilution effect (Drincic et al., 2012; Saneei et al., 2013). Therefore, obesity could affect the menstrual physiology and the actual vitamin D status. Studies examining each exposure separately may thus miss the true picture of the relationship of vitamin D to adiposity or the combination of two exposures may reveal a more dangerous sub-group.

This study fills this gap by leveraging serum 25(OH)D directly measured by NHANES and measured anthropometry, along with reproductive-health responses, and the survey's complex sampling design, in August 2021-August 2023. The main objective is to study the isolated and combinedrelationship between vitamin D deficiency and obesity with the occurrenceof menstrual irregularities in women in the reproductive age group 18-49 years. Specifically, the study investigates if vitamin D deficiency is associated with increased risk of menstrual irregularity, if obesity alone is associated with increased risk of menstrual irregularity and if the combination of vitamin D deficiency with obesity is associated with increased risk of menstrual irregularity compared to women with neither of the risk factors. The hypothesis is that vitamin D deficiency is positively associated with menstrual irregularity (H1), obesity is positively associated with menstrual irregularity (H2) and concurrently vitamin D deficiency and obesity are associated with the highest odds after appropriate demographic, socioeconomic and seasonal adjustments (H3).

LITERATURE REVIEW

Function of the menstrual cycle relies on theproperly co-ordinated messagesfrom hypothalamus, pituitary, the ovary and the endometrium. Any disturbance at any level could affect the timing of any of the cycles, ovulation orfrequency of the menstrual cycle. Menstrual regularity and menstrual cycle length are thus observable indicators of reproductive-endocrine function that have been used in epidemiologic studies, and the definitions are quite variable between studies (Seif et al., 2015). Menstrual irregularity can be manifested as a length fluctuation of cycles, oligomenorrhea, long cycles, anovulation and amenorrhea and each of these phenotypes is not equivalent on a biological level. These are heterogeneous and will further hinder comparisons between populations and are relevant when assessing metabolic exposures. However, long (or highly irregular) cycles have been linked in prospective cohort studies with higher risk of type 2 diabetes and early death, suggesting that menstrual irregularities may signal a woman's overall metabolic risk (Solomon et al., 2001; Wang et al., 2020).

Of all metabolic exposures, adiposity is one of the most uniform ones related to menstrual disturbance. Wei et al. (2009) reported at least two-fold higher risk in women with obesity in Australia for irregular cycles, irrespective of obesity defined by BMI, waist circumference or WHR. However, adjustment for metabolic and hormonal measures (insulin, sex hormone-binding globulin, and androgen-related measures) markedly reduced the associations, indicating that these processes partially mediate the associations. Similarly, Zhou and Yang (2020) found thatChinese womenwith BMI >=30 kg/m² had increased risk of oligomenorrhea and irregular menstruation and central adiposity had a strong association. Several plausible hypotheses have been proposed such as hyperinsulinemia, increased production of ovarian androgens, decreased sex hormone-binding globulin, altered peripheral estrogen metabolism and the effects of adipokines on ovulatory signaling (Seif et al., 2015).These data, combined with others, helpto support obesity as an important exposure and possible confounder in menstrual health studies.

The expression of vitamin D receptors and vitamin D metabolizing enzymes in ovarian and endometrialtissues has raisedinterest in vitamin D in reproductive epidemiology. Based on experimental and clinical studies, the possibleinvolvement in follicular development, anti-Müllerian hormone signaling, follicle-stimulating hormone sensitivity, progesterone production and steroidogenesis has been suggested (Irani & Merhi, 2014; Shahrokhi et al., 2016). These pathways offer biological plausibility that an association exists between vitamin D and menstrual function, and human observational findings are heterogeneous. The biological plausibility of the association between low serum 25(OH)D and menstrual irregularity cannot thus determine if this association would be independent in population-based settings.

There have been severalobservational studiesthat have reported a relationship between decreased 25(OH)D and negative menstrual characteristics. Jukic et al. (2015) conducted a study of 636women, aged 35 to 44 years, and discovered that women with a 10-ng/mL lower level of 25(OH)D had about 1.9 times the odds of having cycles that were too irregular to estimate. In African American women, a higher seasonally adjusted 25(OH)D level was associated with decreased likelihood of having along menstrual cycle, but was not clearly associated with irregular periods, indicating the differential relationship between 25(OH)D level and menstrual period phenotype (Jukic et al., 2016). Jukic et al. (2018) also found prospective evidence for an association between vitamin D deficiencyand a higher likelihood of long cyclesand longer follicular phase. In another, smaller clinical cross-sectional study, Singh et al. (2021) found vitamin D levels were significantly lower in women with menstrual irregularities and the risk of vitamin D deficiency was significantly higher. Harmon et al. (2020) on the other hand reported minimal intra-menstrual fluctuation in 25(OH)Dand associations with select reproductive hormones, but not with a menstrual-irregularity end-point. Thus, the age, ethnicity, clinical context, vitamin D distribution and definition of outcome in each study may account for some of the variation among studies.

Interpretation is key, as there is a strong relationship between vitamin D status and adiposity. A systematic review and meta-analysis of 34 studies revealed a significant but poor inverse association between serum 25(OH)D and BMI in adults (including women) (Saneei et al., 2013). Drincic et al. (2012) showed that much of the decreased 25(OH)D seen in obesity might be due to volumetric dilution. Thus at least part of the association between low 25(OH)D and menstrual dysfunction may be attributed to body size differences and obesity per se is known to be directly linked to endocrine and metabolic disturbances of menstrual dysfunction. Obesity studies in women with polycystic ovary syndrome (PCOS) also indicate that there is a link between vitamin D status, insulin resistance, adiposity and menstrual function; however, the results of vitamin D supplementation are inconsistent and have not been directly translated to unselected women of reproductive age (Fang et al., 2017; Zhang et al., 2023).

Thus, population-basedanalyses that comparevitamin D and obesity are necessary. NHANES offers the opportunity to assess both exposures (25(OH)D and anthropometry) in a probability sample in the same design. The reproductive-health questionnaire for the cycle August 2021-August 2023, however, does not capture month-to-month variability of the cycle.The available item asksif a participant had any period in the past year. Therefore, the current study employs a conservative definition of severe menstrual-irregularity, and excludes amenorrhea due to pregnancy, breastfeeding, hysterectomy or menopause. With this operationalization the analysis will determine whether the association between vitamin D deficiency and obesity is linked to a clinically meaningful type of menstrual disturbance or if it is associated with any kind of menstrual disturbance.

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METHODOLOGY

Study Design and Data Source

This secondary cross-sectional analysis used the NHANES August 2021-August 2023 cycle. NHANES is carried out by the National Center for Health Statistics (NCHS) and is a complex, multistage probability sample of the civilian noninstitutionalized population of the United States and includes household interviews, standardized examinations, and laboratory testing (NCHS, 2024a). The respondent identifier SEQN was used to link the four participant-level public-use files: Demographic Variables and Sample Weights (DEMO_L), Body Measures (BMX_L), Reproductive Health (RHQ_L), and Vitamin D (VID_L) (NCHS, 2024a, 2024b, 2024c, 2024d). The Strengthening the Reporting of Observational Studies in Epidemiology recommendations for cross-sectional studies (von Elm et al., 2007) were followed when reporting.

Study Population and Eligibility

There were 11,933 participants in the source demographic file. To define an adult reproductive-age population,women aged 18 to 49 yearswere selected (n = 1,931). All participants had to have a classifiable menstrual outcome, a measured serum 25(OH)D, a measured BMI and a valid phlebotomy survey weight. By applying RIDEXPRG or RHD143, current pregnancy was excluded. Women whohad a hysterectomy (RHD280 = 1) or bilateral oophorectomy (RHQ305 = 1) were not included as this interferes withthe biology of amenorrhea. Women who reported no period in the past year were not considered cases if they reported pregnancy, lactation, hysterectomy, menopause/change of life, refusal or unknown. After applying these criteria, the final descriptive analytic sample included 980 women, depending on the availability of covariates in the analysis models.

Outcome Definition

The main binary phenotype was basedon RHQ031 and RHD043 (menstrual-irregularity phenotype).RHQ031 asksif the respondent had one or more periods in the last 12 months other than those that were due to surgery, hormone therapy or medical conditions (NCHS, 2024c). The reference group consisted of women who answered yes. Women with a response of "no" were considered cases only if RHD043 provided a reason of "other". This definition is used deliberately because it does not reflect the full range of irregular menstruation (e.g. short periods, long periods occurring, periods occurring month-to-month, but not menstruating for prolonged periods of time). Thus the interpretation will have a strong emphasis on a severe menstrual-irregularity/amenorrhea phenotype rather than all menstrual irregularity.

Primary Exposures

Total serum 25(OH)D (LBXVIDMS) was collected in nmol/L in the VID_L file via high-performance liquid chromatography-tandem mass spectrometry (NCHS, 2024d). Vitamin D deficiency was a priori defined as 25(OH)D <50 nmol/L (<20 ng/mL) and continuous 25(OH)D was assessed in sensitivity analysis. While there are ongoing debates around universal cutpoints for extraskeletal outcomes, this threshold is clearly a threshold of low vitamin D status and has been widely adopted for use in clinical and epidemiologic research (Giustina et al., 2024; Holick et al., 2011). Obesity was defined as BMI >= 30 kg/m2 and BMI <30 kg/m2 was used as the reference category from measured BMI (BMXBMI). BMI was alsoevaluated continuouslyandwaist circumference(BMXWAIST) was assessed as a substitute measure of central adiposity because of the association between abdominal adiposity and menstrual disturbance found in earlier studies (Wei et al., 2009; Zhou & Yang, 2020).

Covariates

Covariates were selected based on biological plausibility, prior evidence, and availability in NHANES and were specified a priori. The main model was adjusted for age (RIDAGEYR), race/ethnicity (RIDRETH3), family poverty-to-income ratio (INDFMPIR) and examination season (RIDEXMON). Season was considered due to variations incirculating 25(OH)D levelswith ultraviolet exposure. Vitamin D deficiency and obesitywere included simultaneously to allow mutual adjustment of their estimates.In a secondary model, education (DMDEDUC2)was added. Automated stepwise selection was not used.

Statistical Analysis

All estimates accounted for the complex NHANES design using the 2-year phlebotomy weight (WTPH2YR), masked variance pseudo-stratum (SDMVSTRA), and pseudo-primary sampling unit (SDMVPSU), consistent with the laboratory-specific

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weighting structure for vitamin D (NCHS, 2024d). Descriptive characteristics were summarized with survey-weighted means and standard errors or weighted percentages. Variances were estimated by Taylor linearization from PSU-level linearized contributions within strata. With 30 masked PSUs across 15 strata, hypothesis tests and confidence intervals used 15 design degrees of freedom.

Statistical Analysis - Regression and Sensitivity Analyses

Logistic regression with survey weights was used to estimate ORs and 95% CIs. The crude models analysed vitamin D deficiency and obesity independently. The main model also adjusted for age, race/ethnicity, poverty-to-income ratio and examination season. A four-level joint-exposure variable was used to compare vitamin D deficiency, obesity, both exposures, and women who were not vitamin D deficient or obese. The vitamin D deficiency x obesity product term was used to test multiplicative interaction. Prespecified sensitivity analyses were performed for 25(OH)D per 10-nmol/L increment, BMI per 5-kg/m2 increment, waist circumference per 10-cm increment, excluded socioeconomic factors in a model similar to the primary model to maximize analytic sample size, and included education in an additional model with complete adjustment. A supplementary weighted linear model was used to estimate the mean difference in 25(OH)D concentrations between obesity groups. Two-sided $P < .05$ was considered statistically significant; interpretation was based on effect sizes and CIs. All statistical analyses were performed in Python with the use ofthe packages pandas, patsy, statsmodels, and SciPywith the complex survey design of NHANES taken into account when estimating variance.Exposure and outcome were measured cross-sectionallyso no causal interpretation was made.

Ethical Considerations

NHANES protocols are reviewed and approved by the NCHS Research Ethics Review Board, and participants provide informed consent under NHANES procedures. This study used de-identified public-use data and involved no direct participant contact; therefore, the analysis did not require access to identifiable information or intervention with participants.

RESULTS

Participant Characteristics

Figure 1 summarizes participant selection. Of 11,933 NHANES participants, 1,931 were women aged 18-49 years. Sequential application of menstrual-outcome, pregnancy, reproductive-surgery, anthropometric, vitamin D, and survey-weight eligibility criteria yielded 980 women for descriptive analysis, including 69 women with the severe menstrual-irregularity phenotype. The primary adjusted model included 861 women and 65 outcome cases after excluding participants with missing poverty-to-income ratio.

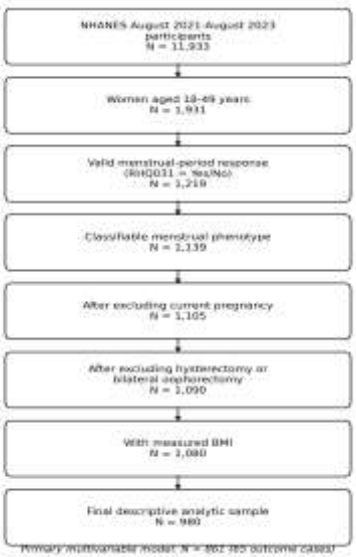

Figure 1. Participant selection for the analytic sample.

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The survey-weighted prevalence of the menstrual-irregularity phenotype was 6.1% (95% CI, 4.3%-8.0%). Women with the phenotype were older on average than those without it (35.7 vs 32.5 years) and had somewhat higher mean BMI (30.5 vs 29.4 kg/m²) and waist circumference (98.0 vs 93.9 cm). Obesity prevalence was 48.2% among women with the phenotype compared with 36.6% among women without it. In contrast, vitamin D deficiency was less frequent among outcome cases (20.4%) than among noncases (26.8%), and mean serum 25(OH)D was higher among cases (76.3 vs 70.2 nmol/L). Table 1 presents the weighted characteristics.

Primary Associations

In the crude survey-weighted model, obesity was associated with 61% higher odds of the menstrual-irregularity phenotype, although the CI included the null (OR, 1.61; 95% CI, 0.97-2.67; P = 0.064). After adjustment for vitamin D deficiency, age, race/ethnicity, poverty-to-income ratio, and examination season, the estimate attenuated to an OR of 1.34 (95% CI, 0.79-2.27; P = 0.255). Vitamin D deficiency was not associated with higher odds in either the crude model (OR, 0.70; 95% CI, 0.36-1.37) or the primary adjusted model (OR, 0.67; 95% CI, 0.26-1.73; P = 0.379) (Table 2).

Table 2 - Survey-weighted logistic regression associations with the menstrual-irregularity phenotype

Model/exposure	N	OR	95% CI	P
Crude: vitamin D deficiency	980	0.70	0.36-1.37	0.274
Crude: obesity	980	1.61	0.97-2.67	0.064
Adjusted: vitamin D deficiency	861	0.67	0.26-1.73	0.379
Adjusted: obesity	861	1.34	0.79-2.27	0.255

Note. Adjusted models include vitamin D deficiency, obesity, age, race/ethnicity, poverty-to-income ratio, and examination season.

Joint Exposure and Sensitivity Analyses

Weighted outcome prevalence was 5.5% among women with neither exposure, 3.4% with vitamin D deficiency only, 8.9% with obesity only, and 6.1% with both conditions (Figure 2). Relative to women with neither exposure, adjusted odds were not significantly different for vitamin D deficiency only (OR, 0.75; 95% CI, 0.18-3.18), obesity only (OR, 1.39; 95% CI, 0.82-2.35), or both exposures (OR, 0.85; 95% CI, 0.25-2.89). The vitamin D deficiency x obesity interaction was not significant (P = 0.788).

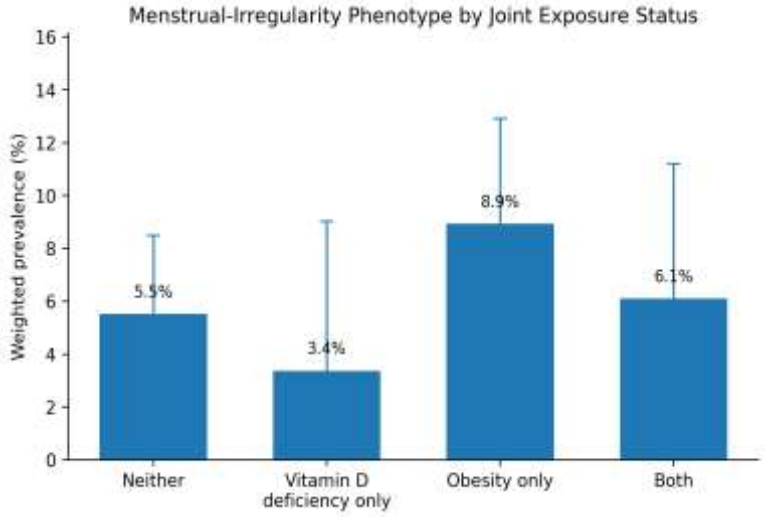

Figure 2. Survey-weighted prevalence of the menstrual-irregularity phenotype according to joint vitamin D deficiency and obesity status. Error bars represent 95% confidence intervals.

Sensitivity analyses were consistent with the primary findings (Table 3; Figure 3). Each 10-nmol/L higher 25(OH)D concentration was associated with an adjusted OR of 1.05 (95% CI, 0.95-1.16), and each 5-kg/m² higher BMI with an OR of 1.04 (95% CI, 0.88-1.23). Replacing BMI with waist circumference also produced a null estimate. In the model that omitted poverty-to-income ratio, the obesity estimate was somewhat larger (OR, 1.52; 95% CI, 0.94-2.48), whereas additional adjustment for education produced a similar direction of effect. Finally, obesity was strongly related to lower circulating vitamin D: the weighted mean 25(OH)D concentration was 75.4 nmol/L among women without obesity and 62.5 nmol/L among women with obesity, a difference of -12.9 nmol/L (95% CI, -17.6 to -8.2; P < .001).

Table 3- Joint-exposure and sensitivity analyses

Analysis/exposure	N	OR	95% CI	P
Joint: vitamin D deficiency only	861	0.75	0.18-3.18	0.676
Joint: obesity only	861	1.39	0.82-2.35	0.201
Joint: both exposures	861	0.85	0.25-2.89	0.779
25(OH)D per 10 nmol/L	861	1.05	0.95-1.16	0.333
BMI per 5 kg/m²	861	1.04	0.88-1.23	0.597
Waist circumference per 10 cm	847	1.04	0.89-1.23	0.574
Obesity, model without socioeconomic covariates	980	1.52	0.94-2.48	0.085
Obesity, model additionally adjusted for education	783	1.30	0.69-2.43	0.391

Note. Joint and continuous models are adjusted for age, race/ethnicity, poverty-to-income ratio, and examination season; the waist model additionally includes vitamin D deficiency.

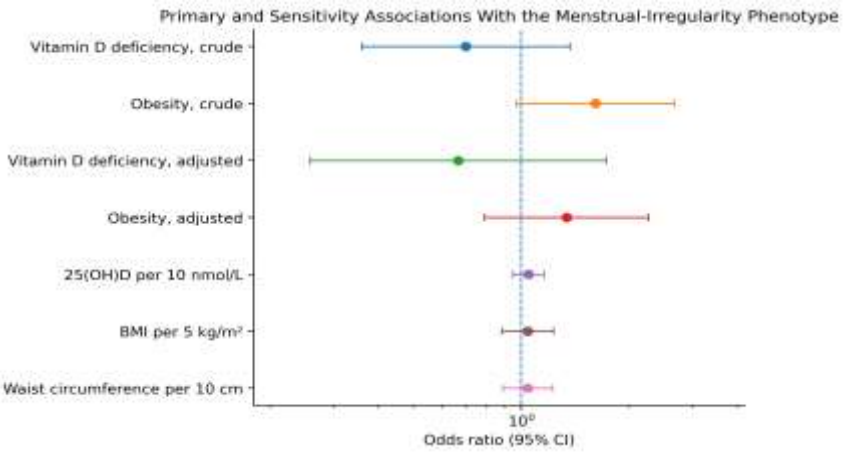

Figure 3. Survey-weighted odds ratios from primary and sensitivity models. The dashed vertical line denotes the null value (OR = 1.0).

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DISCUSSION

This nationally representative cross-sectional analysis hasthree principal findings.First, there was no relationshipbetween vitamin D deficiency (serum 25(OH)D <50 nmol/L) and increased odds of the severe menstrual-irregularity phenotype, even after adjusting for obesity, age, race/ethnicity, socioeconomic status and examination season. Second, obesity was positively associated in the crude model butthe estimate attenuated after adjustmentand was not statistically precise. Third, the odds were not increased in women who were obese and had vitamin D deficiency, and there was no multiplicative interaction. The sensitivity analyses based on continuous 25(OH)D, continuous BMI, continuous WC anddifferent covariate specificationswere consistent with the primary models. So H1 and H3 were not supported butH2 was directionally supported; however, the estimate was too imprecise to conclude an independent associationin this sample.

The obesity finding is consistent with the direction of previous findingsalthough the magnitude was smaller thanthat reported for less severe menstrual phenotypes.More than twice the odds of irregular cycles were reported in individuals with obesityas defined by BMI, waist circumference or waist-to-hip ratio (Wei et al., 2009). The association was significantly blunted after adjustment forfasting insulin, sex hormone-binding globulin and androgen-related measures, suggesting that metabolic and hormonal pathways play a significant role in the relationship. Zhou and Yang (2020) also found increased risk for both oligomenorrhea and irregular menstruation in Chinese women withBMI ≥ 30kg/m2, and central adiposity was also strongly associated with irregular menstruation, with waist circumference being a better predictor of irregular menstruation than BMI.Almost all anthropometric measuresin the current study were higher among women who met the definition of the outcome, including mean BMI and WC, and weighted prevalence of obesity. But the adjusted OR for obesity was 1.34 (95% CI, 0.79-2.27) and there was no significant change in the association when BMI was replaced with waist circumference. The differences are probably more a reflection of the limits of statistical precision and the definition of phenotype than that adiposity is not important in the physiology of menstruation.

Comparing the current estimate with that of Wei et al. (2009) and Zhou and Yang (2020), it is important to draw upon the definition of the outcome. The NHANES item used in this study identified the absence of any menstrual period in the past year, while those studies focused on irregularity, oligomenorrhea or variability in cycle. Once we remove the common causes of amenorrhea (pregnancy, breastfeeding, hysterectomy, and absence of menses due to menopause), then the phenotype is quite rare and rather severe. Women who have long, irregular or less frequent cycles,but have had at least one period in the yearare kept in the reference group. This more restrictive definition probablymakes the definition less sensitiveto the full range of ovulatory dysfunction related to obesity, and may account for some of the differences in the strength of the association between the two studies that used such a method. The low number of outcome cases also resulted in a wide confidence interval and allowed the data to be consistent with a small association and a clinically important increase in odds.

The null resultfor vitamin Dcontrasts witha number of previous observational studies, but does not necessarily contradict the overall literature. In their study of 636women aged 35 to 44 years, Jukic et al. (2015) found that women with 10-ng/mL lower 25(OH)D levels had ~1.9 times the odds of cycles too irregular to estimate. In a subsequent study in African American women, increased seasonally adjusted 25(OH)D was shown to be associated with reduced odds of long menstrual cycles but the association with irregular cycles was less clear (Jukic et al., 2016). Evidence for a cycle-length relationship was supported by prospective data from Jukic et al. (2018) showing that vitamin D deficiency was linked to almost three times the risk of long cycles and lower 25(OH)D was associated with longer follicular phases. A smaller clinical sample of Singh et al. (2021) also foundsignificantly lower levels of vitamin Din women with menstrual irregularities and a significant association between vitamin D deficiency and menstrual irregularities. In contrast, the current adjusted OR for vitamin D deficiency was 0.67 (95% CI, 0.26-1.73) and continuous 25(OH)D was also not associated with the outcome. These estimates do not show an adverse relationship with vitamin D,and are too imprecise to support or refute an association withthe specific severe phenotype captured by NHANES.

There are a fewmethodological differences that could account for the discrepancywith previous vitamin D studies. Jukic et al. (2015) classified women as regular if they had 28–32 days betweenthe first days of successive periods, Jukic et al. (2018) recorded their cycle length on the day of their visit, and Singh et al. (2021) invited women to attend who were complaining of their periods but whose menstrual disorders were not attributable to obvious causes. The current NHANES itemfails to distinguish abnormalities thatthese approaches detect. There were also differences between the populations in terms of age distribution,

race/ethnicity of the participants, clinical selection, prevalence of vitamin D deficiency and the structure of potential confounders. It is important to note the additional findings reported by Harmon et al. (2020), which also included the results of vitamin D status in the menstrual cycle of intensively followed women: 25(OH)D did not change significantly throughout the menstrual cycle but there were reproductive-hormone differences associated with lower vitamin D status that did not necessarily indicate an irregularity endpoint. From these studies, it can be concluded that vitamin D might beassociated more with certain aspectsof reproductive physiology or the length of the cycle than with all menstrual outcomes in general. Biological plausibility of ovarian vitamin D signaling is therefore still relevant (Irani & Merhi, 2014; Shahrokhi et al., 2016) but the current data do not support the concept of considering deficiency (< 50 nmol/L) as an independent indicator of prolonged absence of menstruation.

The analysis also revealed the association between the two main exposures. There was a mean difference of about 13 nmol/L in 25(OH)D levels between women with and without obesity, with women with obesity being more likely to have a 25(OH)D deficiency. This is in line with the findings of Saneei et al. (2013) who found a significant inverse relationship between BMI and the vitamin D concentration in 34 studies and Drincic et al. (2012) who concluded thatbody-size-related volumetric dilutionmay account for much of the lower vitamin D concentrations found in obesity. This overlap wasimportant to considerbecause a simple vitamin D-menstrual relationship would have, in part, been due to differences in adiposity. However, no positive association was found between vitamin D deficiency and the menstrual phenotypeafter mutual adjustmentand the joint exposure group of obesity and deficiency wasnot associated with higher oddsin the present analysis. The results suggest that the obesity-menstrual association is not simply multiplicative, or synergistic, with lower vitamin D levels, but that the broad confidence intervals indicate that a small interaction cannot be ruled out.

More evidence in the form ofpolycystic ovary syndrome (PCOS)also shows the lack of interchangeability between vitamin D and obesity.Some women with PCOShave been shown to benefit from vitamin D supplementation with positive changes in selected metabolic or reproductive outcomes; however, findings from trials have been variable, and potential benefits to menstrual regularity have been reported in a few meta-analyses of supplementation trials (Zhang et al., 2023; Fang et al., 2017). The results of these studies come from a disorder with a high prevalence of insulin resistance and poor ovulatory function, and thus may not be readily applied to an unselected population. However, the lack of an independent vitamin D association in thecurrent nationally representative samplesuggests that the association with vitamin D found indisease-specific cohortscould be related to the endocrine-metabolic phenotype. It also warns that the cross-sectional associations do not imply that vitamin D supplementation could help to regularize menstruation in the general population.

There are a number of strengths to the study. It was based on recent NHANES data usingobjectively measured serum25(OH)D, standardized anthropometry, and survey-weighted analyses based on the laboratory-specific weight, strata and primary sampling units. The primary interpretation was explored with varying covariate specifications, continuous exposures, waist circumference, joint-exposure categories and interactions. These features minimize a number of potential biases in the measurement and analysis of smaller convenience samples. Important limitations remain.Because this is a cross-sectional design, it is not possible to determineany temporal ordering or causality. RHQ031 is only able to capture a small severe phenotype, asit does not captureusual cycle length, month-to-month variability, oligomenorrhea, or ovulation.This outcome was rare, occurring in69 participants in the descriptive sampleand65 in the primary adjusted model, and therefore it was not possible to achievehigh precision, particularly in the joint and interaction analyses. There may be residual confounding, sincethe variables used in the public-use files do not fully describe hormonal contraceptive use, polycystic ovary syndrome, insulin resistance, levels of reproductive hormones, physical activity, diet, psychosocial stress or other potential causes of menstrual disturbances. Also, a single 25(OH)D level is unlikely to be a perfect reflection of long-term status, though there does not seem to be much variation within cycles (Harmon et al., 2020).

Overall, the findings complement, butdo not challenge, earlier findings. Previous research is consistentwith obesity being metabolically associated with menstrual dysfunctionand is suggestive of a vitamin D association with some menstrual cycle characteristics such as long cycles and irregular cycles. These relationships do not necessarily apply to a rather conservatively defined absence of menses phenotype over 12 months inmodern-day U.S. womenas demonstrated by the present analysis.Vitamin D deficiency alone should not be considered an indicator of this clinical outcome.Although the obesity estimate was directionally positive,the confidence interval precludes concluding an independent association, although obesity remains relevant. Future studies should include multiple 25(OH)D evaluations and objective measures of adiposity, along with

prospective evaluation of menstrual cycles that includes the evaluation of cycle length/regularity, oligomenorrhea, anovulation, and amenorrhea, measurement of insulin resistance and androgens, and exposure to contraceptive medications, and assessment of polycystic ovary syndrome (PCOS) characteristics. These designs would be more appropriate in evaluating vitamin D's independent effects, effects on metabolic risk,or simply effects secondary to adiposity-related physiology.

CONCLUSION

Vitamin D deficiency was not independentlyassociated with a severe menstrual-irregularity phenotypeusing a conservative definition of the phenotype and after adjusting for obesity and for demographic, socioeconomic, and seasonal factors in this nationally representative sample ofU.S. women aged 18-49 years.Crude odds and weighted prevalence of the phenotype were higher among women with obesity, butthe adjusted estimate was attenuated and imprecise.No additional increase in odds was found with concurrent obesity and vitamin D deficiencyand there was no multiplicative interaction. During the same period, there was a strong association between obesity and lower 25(OH)D, thus confirming extensive metabolic overlap of the two exposures.

Based on the current NHANES data, in the absence of any other risk factors, it would be inappropriate to consider vitamin D deficiency as an independent marker of chronic amenorrhea at the population level.These findings also emphasizethe need to differentiate the serious amenorrhea-like manifestation from the wide spectrum of irregular, long or scanty periods which were studied earlier. Future studies that include more frequent vitamin D measurements, detailed menstrual history, reproductive hormone assessment and characterization of insulin resistance and polycystic ovary syndrome are warranted to determine if vitamin D is a factor inany specific type of ovulatory dysfunctionand if the effects of vitamin D are altered by adiposity.

REFERENCES

[1] Afzal, A., Abdelkader, O. K. S., Abd-ElGawad, M., Dean, Y. E., Aboeldahb, M., Popoola-Samuel, H. A. O., Hamdy, A., Elmezayen, R. W., Elalem, A., Aziz, N. Z., Thakur, A., Jain, A., Al Dhneem, H. N., Elkasaby, H. M. H., Atta, R., Anghel, I., Donaldy, W., Toraih, E., & Aiash, H. (2026). Menstrual irregularities and cardiometabolic risk: A systematic review and meta-analysis. European Journal of Obstetrics & Gynecology and Reproductive Biology, 316, 114796. https://doi.org/10.1016/j.ejogrb.2025.114796

[2] Drincic, A. T., Armas, L. A. G., Van Diest, E. E., & Heaney, R. P. (2012). Volumetric dilution, rather than sequestration best explains the low vitamin D status of obesity. Obesity, 20(7), 1444–1448. https://doi.org/10.1038/oby.2011.404

[3] Fang, F., Ni, K., Cai, Y., Shang, J., Zhang, X., & Xiong, C. (2017). Effect of vitamin D supplementation on polycystic ovary syndrome: A systematic review and meta-analysis of randomized controlled trials. Complementary Therapies in Clinical Practice, 26, 53–60. https://doi.org/10.1016/j.ctcp.2016.11.008

[4] Giustina, A., Bilezikian, J. P., Adler, R. A., Banfi, G., Bikle, D. D., Binkley, N. C., Bollerslev, J., Bouillon, R., Brandi, M. L., Casanueva, F. F., di Filippo, L., Donini, L. M., Ebeling, P. R., El-Hajj Fuleihan, G., Fassio, A., Frara, S., Jones, G., Marcocci, C., Martineau, A. R., ... Virtanen, J. K. (2024). Consensus statement on vitamin D status assessment and supplementation: Whys, whens, and hows. Endocrine Reviews, 45(5), 625–654. https://doi.org/10.1210/endrev/bnae009

[5] Harmon, Q. E., Kissell, K., Jukic, A. M. Z., Kim, K., Sjaarda, L., Perkins, N. J., Umbach, D. M., Schisterman, E. F., Baird, D. D., & Mumford, S. L. (2020). Vitamin D and reproductive hormones across the menstrual cycle. Human Reproduction, 35(2), 413–423. https://doi.org/10.1093/humrep/dez283

[6] Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., Murad, M. H., & Weaver, C. M. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. The Journal of Clinical Endocrinology & Metabolism, 96(7), 1911–1930. https://doi.org/10.1210/jc.2011-0385

[7] Irani, M., & Merhi, Z. (2014). Role of vitamin D in ovarian physiology and its implication in reproduction: A systematic review. Fertility and Sterility, 102(2), 460–468.e3. https://doi.org/10.1016/j.fertnstert.2014.04.046

[8] Jukic, A. M. Z., Steiner, A. Z., & Baird, D. D. (2015). Lower plasma 25-hydroxyvitamin D is associated with irregular menstrual cycles in a cross-sectional study. Reproductive Biology and Endocrinology, 13, 20. https://doi.org/10.1186/s12958-015-0012-5

[9] Jukic, A. M. Z., Upson, K., Harmon, Q. E., & Baird, D. D. (2016). Increasing serum 25-hydroxyvitamin D is associated with reduced odds of long menstrual cycles in a cross-sectional study of African American women. Fertility and Sterility, 106(1), 172–179.e2. https://doi.org/10.1016/j.fertnstert.2016.03.004

[10] Jukic, A. M. Z., Wilcox, A. J., McConnaughey, D. R., Weinberg, C. R., & Steiner, A. Z. (2018). 25-Hydroxyvitamin D and long menstrual cycles in a prospective cohort study. Epidemiology, 29(3), 388–396. https://doi.org/10.1097/EDE.0000000000000804

[11] National Center for Health Statistics. (2024a). National Health and Nutrition Examination Survey: August 2021–August 2023 data documentation, codebook, and frequencies: Demographic variables and sample weights (DEMO_L). Centers for Disease Control and Prevention. https://wwwn.cdc.gov/Nchs/Data/Nhanes/Public/2021/DataFiles/DEMO_L.htm

[12] National Center for Health Statistics. (2024b). National Health and Nutrition Examination Survey: August 2021–August 2023 data documentation, codebook, and frequencies: Body measures (BMX_L). Centers for Disease Control and Prevention. https://wwwn.cdc.gov/Nchs/Data/Nhanes/Public/2021/DataFiles/BMX_L.htm

[13] National Center for Health Statistics. (2024c). National Health and Nutrition Examination Survey: August 2021–August 2023 data documentation, codebook, and frequencies: Reproductive health (RHQ_L). Centers for Disease Control and Prevention. https://wwwn.cdc.gov/Nchs/Data/Nhanes/Public/2021/DataFiles/RHQ_L.htm

[14] National Center for Health Statistics. (2024d). National Health and Nutrition Examination Survey: August 2021–August 2023 data documentation, codebook, and frequencies: Vitamin D (VID_L). Centers for Disease Control and Prevention. https://wwwn.cdc.gov/Nchs/Data/Nhanes/Public/2021/DataFiles/VID_L.htm

[15] Saneei, P., Salehi-Abargouei, A., & Esmaillzadeh, A. (2013). Serum 25-hydroxy vitamin D levels in relation to body mass index: A systematic review and meta-analysis. Obesity Reviews, 14(5), 393–404. https://doi.org/10.1111/obr.12016

[16] Seif, M. W., Diamond, K., & Nickkho-Amiry, M. (2015). Obesity and menstrual disorders. Best Practice & Research Clinical Obstetrics & Gynaecology, 29(4), 516–527. https://doi.org/10.1016/j.bpobgyn.2014.10.010

[17] Shahrokhi, S. Z., Ghaffari, F., & Kazerouni, F. (2016). Role of vitamin D in female reproduction. Clinica Chimica Acta, 455, 33–38. https://doi.org/10.1016/j.cca.2015.12.040

[18] Singh, V., Tamar, N., Lone, Z., Das, E., Sahu, R., & Majumdar, S. (2021). Association between serum 25-hydroxy vitamin D level and menstrual cycle length and regularity: A cross-sectional observational study. International Journal of Reproductive BioMedicine, 19(11), 979–986. https://doi.org/10.18502/ijrm.v19i11.9913

[19] Solomon, C. G., Hu, F. B., Dunaif, A., Rich-Edwards, J., Willett, W. C., Hunter, D. J., Colditz, G. A., Speizer, F. E., & Manson, J. E. (2001). Long or highly irregular menstrual cycles as a marker for risk of type 2 diabetes mellitus. JAMA, 286(19), 2421–2426. https://doi.org/10.1001/jama.286.19.2421

[20] von Elm, E., Altman, D. G., Egger, M., Pocock, S. J., Gøtzsche, P. C., & Vandenbroucke, J. P. (2007). The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: Guidelines for reporting observational studies. The Lancet, 370(9596), 1453–1457. https://doi.org/10.1016/S0140-6736(07)61602-X

[21] Wang, Y.-X., Arvizu, M., Rich-Edwards, J. W., Stuart, J. J., Manson, J. E., Missmer, S. A., Pan, A., & Chavarro, J. E. (2020). Menstrual cycle regularity and length across the reproductive lifespan and risk of premature mortality: Prospective cohort study. BMJ, 371, m3464. https://doi.org/10.1136/bmj.m3464

[22] Wei, S., Schmidt, M. D., Dwyer, T., Norman, R. J., & Venn, A. J. (2009). Obesity and menstrual irregularity: Associations with SHBG, testosterone, and insulin. Obesity, 17(5), 1070–1076. https://doi.org/10.1038/oby.2008.641

[23] Zhang, B., Yao, X., Zhong, X., Hu, Y., & Xu, J. (2023). Vitamin D supplementation in the treatment of polycystic ovary syndrome: A meta-analysis of randomized controlled trials. Heliyon, 9(3), e14291. https://doi.org/10.1016/j.heliyon.2023.e14291

[24] Zhou, X., & Yang, X. (2020). Association between obesity and oligomenorrhea or irregular menstruation in Chinese women of childbearing age: A cross-sectional study. Gynecological Endocrinology, 36(12), 1101–1105. https://doi.org/10.1080/09513590.2020.1803823