Published in final edited form as:

Am J Obstet Gynecol. 2018 June ; 218(6): 608.e1–608.e6. doi:10.1016/j.ajog.2018.03.012.

Vitamin D is associated with bioavailability of androgens in eumenorrheic women with prior pregnancy loss

Mr Daniel L. Kuhr, BS, Dr Lindsey A. Sjaarda, PhD, Ms Zeina Alkhalaf, BS, Ms Ukpebo R. Omosigho, BS, Dr Matthew T. Connell, DO, Dr Robert M. Silver, MD, Dr Keewan Kim, PhD, Dr Neil J. Perkins, PhD, Ms Tiffany L. Holland, BA, Dr Torie C. Plowden, MD, Dr Enrique F. Schisterman, PhD, and Dr Sunni L. Mumford, PhD

Epidemiology Branch, Division of Intramural Population Health Research (Drs Sjaarda, Connell, Kim, Perkins, Schisterman, and Mumford, Mr Kuhr, Ms Alkhalaf, Ms Omosigho, and Ms Holland), and Program in Reproductive and Adult Endocrinology (Dr Plowden), Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD; and Department of Obstetrics and Gynecology, University of Utah and Intermountain Healthcare, Salt Lake City, UT (Dr Silver).

Abstract

BACKGROUND: Prior studies have reported mixed results regarding relationships between vitamin D, androgens, and sex hormone—binding globulin in patients with polycystic ovary syndrome. However, less is known regarding these associations in eumenorrheic, premenopausal women.

OBJECTIVE: Our objective was to study the relationships between serum vitamin D and androgen biomarkers in eumenorrheic women with a history of pregnancy loss who were attempting pregnancy.

STUDY DESIGN: This was an analysis of a cohort of 1191 participants from the Effects of Aspirin in Gestation and Reproduction trial (2006e2012). Participants were attempting to conceive, aged 18–40 years, with 1–2 documented prior pregnancy losses and no history of infertility, and recruited from 4 academic medical centers in the United States. Serum vitamin D (25-hydroxyvitamin D) and hormone concentrations were measured at baseline.

RESULTS: Vitamin D concentration was negatively associated with free androgen index (percentage change [95% confidence interval, –5% (–8% to –2%)] per 10 ng/mL increase) and positively associated with sex hormone—binding globulin (95% confidence interval, 4% [2–7%]), although not with total testosterone, free testosterone, or dehydroepiandrosterone sulfate after adjusting for age, body mass index, smoking status, race, income, education, physical activity, and season of blood draw.

CONCLUSION: Overall, vitamin D was associated with sex hormone—binding globulin and free androgen index in eumenorrheic women with prior pregnancy loss, suggesting that vitamin D may

play a role in the bioavailability of androgens in eumenorrheic women. We are limited in making assessments regarding directionality, given the cross-sectional nature of our study.

Keywords

androgens; premenopausal women; sex hormone—binding globulin; vitamin D

Although vitamin D is well known for its effects on bone health and metabolism, it is also associated with a host of reproductive outcomes^{1–3} and has receptors in the ovary, uterus, placenta, hypothalamus, and pituitary gland.^{4–6} Additionally, the vitamin has been implicated in a number of pathologies affecting women,^{7,8} including polycystic ovary syndrome (PCOS),^{9–11} yet little is known about how it affects androgen homeostasis in premenopausal, eumenorrheic women.

Studies have linked serum vitamin D to serum testosterone and sex hormone—binding globulin (SHBG) concentrations in populations of postmenopausal women and premenopausal women with PCOS,^{12–14} but none have investigated this relationship in premenopausal, regularly cycling women. The SHBG data gap is especially important, given its role in binding freely circulating steroid hormones, including androgens, and thereby limiting their biological availability.¹⁵

Exploring the normal physiological relationship between vitamin D, SHBG, and androgens in eumenorrheic, premenopausal women may help us better understand the role vitamin D plays in the pathophysiology of androgen excess and shed light on future strategies to address it. Therefore, we aimed to evaluate the relationship between vitamin D and androgen biomarkers in a large cohort of eumenorrheic, premenopausal women with a prior pregnancy loss.

Materials and Methods

This is a cohort from the Effects of Aspirin in Gestation and Reproduction (EAGeR) trial, which was a multicenter, block-randomized, double-blind, placebo-controlled trial of daily low-dose aspirin (81 mg) in 1228 women recruited from 4 US medical centers from 2007 to 2011. Institutional review board approval was obtained at each study site and the data coordinating center. All participants provided written informed consent. The trial was registered with ClinicalTrials.gov, number NCT00467363. Full details of the study design, methods, and participant characteristics have been published elsewhere.¹⁶

Study design and population

Women attempting conception, aged 18–40 years, with regular menstrual cycles of 21–42 days in length, with documented confirmation of 1 or 2 prior pregnancy losses, and up to 2 prior live births were eligible for the EAGeR trial.¹⁶

Exclusion criteria for all women included clinical indication for use of anticoagulant therapy or chronic use of nonsteroidal anti-inflammatory drugs; major medical disorders (eg, diabetes, hypertension, etc); and any prior diagnosis of infertility or subfertility including related conditions such as PCOS, endometriosis, or pelvic inflammatory disease.

Furthermore, all women must have been off long-acting hormonal contraceptive medication (eg, Depo-Provera, Norplant, intrauterine device) for at least 12 months or off oral contraceptive pills or other exogenous hormones (eg, patch, ring) 3 months prior to enrollment.

Study procedures

Participants attended a prerandomization, baseline study visit timed to occur around day 2e4 of their menstrual cycle. During this baseline visit, participants completed questionnaires of reproductive health status, demographic, and lifestyle factors. In addition, anthropometric measures and blood samples were collected. Samples were centrifuged and serum and plasma were aliquoted and frozen within 90 minutes. Samples were stored at e80C until analysis.

Biochemical analysis

Primary outcomes for the present analysis were baseline serum concentrations of total testosterone, free testosterone, free androgen index, SHBG, and dehydroepiandrosterone sulfate (DHEAS). Total testosterone concentration (TT; nanograms per deciliter) was determined by liquid chromatography and tandem mass spectrometry using a Shimadzu Prominence liquid chromatogram (Shimadzu Scientific Instruments, Inc, Columbia, MD) with an ABSceix 5500 tandem mass spectrometer (AB SCIEX, Framingham, MA). Interassay coefficients of variance (CVs) were 2.0% at 189.81 and 1.4% at 809.54 ng/mL. Free testosterone (fT) was calculated as $24.00314 \times TT / \log_{10} S - 0.0499 \times TT^2$ and free androgen index (FAI) as $100 \times (TT / SHBG)$, where TT was measured in nanomoles per liter and SHBG in nanomoles per liter.¹⁷

SHBG concentration was determined by SHBG reagent/sandwich immunoassay method/ electrochemiluminescence (Roche Diagnostics, Indianapolis, IN) utilizing a Roche COBAS 6000 chemistry analyzer (Roche Diagnostics).

Interassay CVs were 3.0% at 55.64 nmol/L and 3.8% at 19.74 nmol/L. DHEAS was determined by DHEA sulfate reagent/competitive immunoassay method/ electrochemiluminescence using a Roche COBAS 6000 chemistry analyzer (Roche Diagnostics). Interassay CVs were 4.6% at 5.43 μ mol/L and 4.9% at 13.01 μ mol/L. Insulin was measured by sandwich immunoassay method using a Roche COBAS 6000 chemistry analyzer (Roche Diagnostics). Interassay CVs were 3.1% at 121.2 pmol/L and 3.1% at 377.9 pmol/L.

Urinary estrone-3-glucuronide (E1G) was determined by E1G/PdG Multiplex competitive chemiluminescence assay (Quansys Biosciences, Logan, UT) from daily first-morning urine samples collected at home during the first cycle. The interassay CVs for E1G were 16.9% and 20.2% at mean concentrations of 36.3 ng/mL and 1.9 ng/mL, respectively, for lyophilized manufacturer's controls and were 14.2% and 15.2% for in-house pooled urine controls at mean concentrations of 23.7 ng/mL and 10.2 ng/mL, respectively.

Preconception 25-hydroxyvitamin D (25[OH]D) concentrations were measured in serum using the 25(OH)D ELISA solid-phase sandwich enzyme immunoassay (BioVendor R&D,

Ashville, NC). The interassay laboratory CVs were 15.8% and 13.1% at mean concentrations of 15.5 and 41.6 ng/mL, respectively, for lyophilized manufacturer's controls and 17% for an in-house pooled serum control. The lower limit of detection was 1.6 ng/mL, and all values were above this limit.

Statistical analysis

Participants were determined to be vitamin D sufficient (25[OH]D $\geq$ 30.0 ng/mL), vitamin D insufficient (20.0 $\leq$ 25[OH]D < 30.0 ng/mL), or vitamin D deficient (<20.0 ng/mL) using baseline 25(OH)D serum concentrations.¹⁸ Demographic and reproductive histories at baseline were compared by vitamin D status using Student's *t* tests and χ^2 tests where appropriate.

Both unadjusted and adjusted geometric mean androgen concentrations by vitamin D concentration are presented. Linear regression was used to estimate the associations between vitamin D and TT, fT, FAI, DHEAS, and SHBG. All hormonal markers were natural log transformed for normality, and results are reported as percentage change in the hormone measures per unit change in vitamin D concentrations with 95% confidence intervals.¹⁹

All models were adjusted for age, body mass index (BMI), smoking status, race, income, education, physical activity, and season of blood draw. Models for SHBG were additionally adjusted for TT, mean follicular-phase E1G, and insulin because they are known to affect SHBG concentrations.²⁰ Models were not adjusted for treatment arm because blood samples were collected before treatment.

All analyses were run using both categorical vitamin D status (sufficient vs insufficient vs deficient) and continuous vitamin D concentrations. Assumptions of linearity were tested using restricted cubic splines and sensitivity analyses, excluding patients with vitamin D above the 99th percentile, and controlling for vitamin supplement intake were evaluated. SAS version 9.4 (SAS Institute, Cary, NC) was used for all statistical analysis.

Role of the funding source

This study was funded by the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health. The funding source had no role in the study design, data gathering, analysis and interpretation, writing of the report, or the decision to submit the report for publication. All authors had full access to the data, and the corresponding author had the final responsibility to submit the report for publication.

Results

Vitamin D data were available for 1191 patients. The median vitamin D concentration was 29.1 ng/mL (interquartile range, 23.1–36.0) and concentrations ranged overall from 5.0 to 143.6 ng/mL. With regard to race, a higher proportion of vitamin D—sufficient women were white than vitamin D—insufficient and —deficient women (Table 1). Vitamin D—sufficient participants also had lower BMI and smaller waist circumference, sum of skinfolds, and

central-to-peripheral skinfold ratio than their vitamin D—insufficient and —deficient counterparts.

Vitamin D was associated with neither TT nor DHEAS (Table 2). However, vitamin D was negatively associated with fT in the unadjusted model (percentage change [95% confidence interval], —4% [—6% to —2%]), but this relationship was attenuated after adjustment for potential confounders (—2% [—4%, 0.4%]). Vitamin D concentrations were negatively associated with the FAI (—5% [—7%, —2%]) and positively associated with SHBG concentrations (4% [2—7%]). These findings remained consistent after additional adjustment for TT, E1G, and insulin (7% [4—10%]).

Results using vitamin D status (sufficient, insufficient, deficient) yielded similar results. The restricted cubic spline models yielded no evidence of nonlinearity, and the sensitivity analyses yielded nearly identical results (data not shown). Based on our results in this population, an average woman in this population with insufficient (compared with sufficient) vitamin D concentration would exhibit a 10% higher FAI (eg, 1.18 vs 1.07). Likewise, for women with SHBG values near the average for this population, a 9% lower SHBG attributable to vitamin D insufficiency would equate to approximately 59.5 nmol/L compared with 65.5 nmol/L.

Comment

Our results show increasing vitamin D concentrations were negatively associated with FAI and positively associated with SHBG in this cohort of eumenorrheic women with history of prior pregnancy loss who were attempting pregnancy. Overall, our results help elucidate the role vitamin D may play in the bioavailability of androgens, a potential factor in the pathophysiology of hyperandrogenism and its related complications for women of reproductive age. The magnitude of the changes in our population of eumenorrheic women was approximately 10%, although the long-term effects of these changes in hormone concentrations on other reproductive outcomes is unknown.

To our knowledge, this is the first comprehensive analysis of the relationship between vitamin D and androgens among eumenorrheic, regularly cycling women. Studies of other populations have shown a positive relationship between vitamin D and TT in normal-BMI patients with PCOS¹² and a negative relationship among postmenopausal women.¹³ A systematic review and meta-analysis of vitamin D supplementation among a total of 183 women with PCOS found that supplementation with vitamin D significantly reduces TT.²¹ Our null results may differ from these studies because our population was, respectively, eumenorrheic, premenopausal, and not being supplemented with vitamin D.

With regard to the bioavailability of testosterone, the largest study to date demonstrated a negative relationship between vitamin D and fT among postmenopausal women,¹⁴ which is consistent with our findings. With regard to SHBG, the positive relationship with vitamin D concentration that we observed is consistent with results observed previously among both women with PCOS^{22,23} and postmenopausal women.^{14,24} However, our positive finding conflicts with results from the aforementioned meta-analysis;²¹ our study's larger cohort

(and therefore greater power) may also explain why we found a relationship between vitamin D and SHBG while the meta-analysis did not.

In addition, the present study included a predominantly nonobese population without diagnosed PCOS; it is plausible that differences in obesity prevalence, and accordingly insulin resistance, could perhaps have an impact on the relationship between vitamin D and SHBG.

Prior research supports a strong biological rationale for the association between vitamin D and hyperandrogenic states, mediated through vitamin D receptor (VDR) polymorphisms. Different VDR polymorphisms have been linked to an increased risk of having PCOS,²⁵ increased severity of PCOS,²⁶ and higher testosterone and androstenedione concentrations than patients with other VDR polymorphisms.²⁷ VDR polymorphism Bsm-I is specifically associated with lower SHBG concentrations,²⁸ providing a potential mechanism for vitamin D to affect the bioavailability of androgens as we see in this study. If vitamin D does in fact influence SHBG homeostasis, then TT could remain unchanged while bioavailability would increase, therefore raising the potential for women to suffer from the physical effects of elevated androgens while having clinically normal total concentrations.

Our study is unique in that it evaluated androgen outcomes in eumenorrheic premenopausal women with prior pregnancy loss, whereas prior studies assessed the relationship of vitamin D with either women diagnosed with PCOS or postmenopausal women. Because 92.4% of participants in the trial were taking some sort of vitamin supplement and we do not have data on dietary intake or vitamin contents, it is possible that some patients were consuming supplemental vitamin D for which we were unable to account. However, serum concentrations have been shown to be an accurate biological reflection of vitamin D status,^{29,30} regardless of source, so we believe these markers should be relevant markers of vitamin D status, given that their intake of vitamins was somewhat consistent over the study. Moreover, we adjusted for the use of any vitamin supplements in a sensitivity analysis and found similar results.

It is important to note that our findings are applicable only to vitamin D concentrations with the vitamin D range observed in our population. The cutoffs we used as designated by the Endocrine Society were developed with regard to bonehealth,¹⁸ so they may not be the most appropriate for reproductive outcomes; however, our use of continuous vitamin D concentration models with similar results suggest the relationships are robust, regardless of vitamin D status cutoff.

Because our data are cross-sectional in nature, we cannot comment on how changes in vitamin D may affect the bioavailability of androgens over time; prospective studies should be done to further delineate the nature of the relationship between androgen homeostasis and vitamin D. Finally, we do not have information on phenotypic features related to androgen activity such as hirsutism or acne; thus, we can evaluate androgens only biochemically and not clinically in this population.

To our knowledge, this is the first study to elucidate the positive relationship of vitamin D with SHBG and negative relationship with the FAI in eumenorrheic, premenopausal women

with prior pregnancy loss. Because vitamin D and SHBG interact in other diseases, it is plausible that vitamin D status may affect SHBG concentrations and subsequently the bioavailability of androgens, although we were limited in assessing the directionality of the associations, given the cross-sectional nature of our study. Overall, these findings shed light on a potential pathological process and spurs the need for prospective studies regarding vitamin D and its potential role in addressing androgen excess.

Acknowledgment

This study had a clinical trial registration number of NCT00467363 (ClinicalTrials.gov).

This work was supported by the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health (contracts HHSN267200603423, HHSN267200603424, and HHSN267200603426). Mr Kuhr and Ms Omosigbo have been supported by the National Institutes of Health (NIH) Medical Research Scholars Program, a public-private partnership jointly supported by the NIH and generous contributions to the Foundation for the NIH by the Doris Duke Charitable Foundation (grant 2014194), the American Association for Dental Research, the Colgate Palmolive Company, Genentech, and other private donors. For a complete list, visit the foundation web site (<http://www.fnih.org>).

References

1. Johnson LE, DeLuca HF. Reproductive defects are corrected in vitamin d-deficient female rats fed a high calcium, phosphorus and lactose diet. *J Nutr* 2002;132:2270–3. [PubMed: 12163674]
2. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. *Am J Clin Nutr* 2008;88:491S–9S. [PubMed: 18689389]
3. Luk J, Torrealday S, Neal Perry G, Pal L. Relevance of vitamin D in reproduction. *Hum Reprod* 2012;27:3015–27. [PubMed: 22824625]
4. Kinuta K, Tanaka H, Moriwake T, Aya K, Kato S, Seino Y. Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. *Endocrinology* 2000;141: 1317–24. [PubMed: 10746634]
5. Halhali A, Acker GM, Garabedian M. 1,25-Dihydroxyvitamin D3 induces in vivo the decidualization of rat endometrial cells. *J Reprod Fertil* 1991;91:59–64. [PubMed: 1995862]
6. Hurley WL, Doane RM. Recent developments in the roles of vitamins and minerals in reproduction. *J Dairy Sci* 1989;72:784–804. [PubMed: 2654228]
7. Skowronska P, Pastuszek E, Kuczynski W, et al. The role of vitamin D in reproductive dysfunction in women—a systematic review. *Ann Agric Environ Med* 2016;23:671–6. [PubMed: 28030942]
8. Akyol A, Simsek M, Ilhan R, et al. Efficacies of vitamin D and omega-3 polyunsaturated fatty acids on experimental endometriosis. *Taiwan J Obstet Gynecol* 2016;55:835–9. [PubMed: 28040129]
9. Jafari-Sfidvajani S, Ahangari R, Hozoori M, Mozaffari-Khosravi H, Fallahzadeh H, Nadjarzadeh A. The effect of vitamin D supplementation in combination with low-calorie diet on anthropometric indices and androgen hormones in women with polycystic ovary syndrome: a double-blind, randomized, placebo-controlled trial. *J Endocrinol Invest* 2017 11 6 10.1007/s40618-017-0785-9. [Epub ahead of print]
10. Karadag C, Yoldemir T, Yavuz DG. Effects of vitamin D supplementation on insulin sensitivity and androgen levels in vitamin-D-deficient polycystic ovary syndrome patients. *J Obstet Gynaecol Res* 2018;44:270–7. [PubMed: 29094433]
11. Bacopoulou F, Kolas E, Efthymiou V, Antonopoulos CN, Charmandari E. Vitamin D predictors in polycystic ovary syndrome: a metaanalysis. *Eur J Clin Invest* 2017;47:746–55. [PubMed: 28795765]
12. Ganie MA, Marwaha RK, Nisar S, et al. Impact of hypovitaminosis D on clinical, hormonal and insulin sensitivity parameters in normal body mass index polycystic ovary syndrome women. *J Obstet Gynaecol* 2016;36:508–12. [PubMed: 26772667]
13. Mason C, Tapsoba JD, Duggan C, et al. Effects of vitamin D supplementation during weight loss on sex hormones in postmenopausal women. *Menopause* 2016;23:645–52. [PubMed: 26859343]

14. Zhao D, Ouyang P, de Boer IH, et al. Serum vitamin D and sex hormones levels in men and women: the Multi-Ethnic Study of Atherosclerosis (MESA). *Maturitas* 2017;96:95–102. [PubMed: 28041602]
15. Hammond GL. Diverse roles for sex hormone-binding globulin in reproduction. *Biol Reprod* 2011;85:431–41. [PubMed: 21613632]
16. Schisterman EF, Silver RM, Perkins NJ, et al. A randomised trial to evaluate the effects of lowdose aspirin in gestation and reproduction: design and baseline characteristics. *Paediatr Perinat Epidemiol* 2013;27:598–609. [PubMed: 24118062]
17. Sartorius G, Ly LP, Sikaris K, McLachlan R, Handelsman DJ. Predictive accuracy and sources of variability in calculated free testosterone estimates. *Annals of Clinical Biochemistry* 2009;46:137–43. [PubMed: 19225026]
18. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011;96:1911–30. [PubMed: 21646368]
19. Statistical Consulting Group. How can I interpret log transformed variables in terms of percent change in linear regression SAS FAQ UCLA: Statistical Consulting Group (vol 2017).
20. Carmina E, Loba RA. Principles and practice of endocrinology and metabolism. Philadelphia, PA: Lippincott Williams & Wilkins; 2001.
21. Azadi-Yazdi M, Nadjarzadeh A, Khosravi-Boroujeni H, Salehi-Abargouei A. The effect of vitamin D supplementation on the androgenic profile in patients with polycystic ovary syndrome: a systematic review and meta-analysis of clinical trials. *Horm Metab Res* 2017;49:174–9. [PubMed: 28351084]
22. Zgliczy ski J Associations of vitamin D concentration with metabolic and hormonal indices in women with polycystic ovary syndrome presenting abdominal and gynoidal type of obesity. *Ginekol Pol* 2014;85:765–70. [PubMed: 25546928]
23. Wehr E, Pilz S, Schweighofer N, et al. Association of hypovitaminosis D with metabolic disturbances in polycystic ovary syndrome. *Eur J Endocrinol* 2009;161:575–82. [PubMed: 19628650]
24. El Maataoui A, El Maghraoui A, Biaz A, et al. Relationships between vertebral fractures, sex hormones and vitamin D in Moroccan postmenopausal women: a crosssectional study. *BMC Womens Health* 2015;15:41. [PubMed: 25968889]
25. Mahmoudi T Genetic variation in the vitamin D receptor and polycystic ovary syndrome risk. *Fertil Steril* 2009;92:1381–3. [PubMed: 19501823]
26. Zadeh-Vakili A, Ramezani Tehrani F, Daneshpour MS, Zarkesh M, Saadat N, Azizi F. Genetic polymorphism of vitamin D receptor gene affects the phenotype of PCOS. *Gene* 2013;515:193–6. [PubMed: 23246977]
27. Wehr E, Trummer O, Giuliani A, Gruber H-J, Pieber TR, Obermayer-Pietsch B. Vitamin Deassociated polymorphisms are related to insulin resistance and vitamin D deficiency in polycystic ovary syndrome. *Eur J Endocrinol* 2011;164:741–9. [PubMed: 21389086]
28. Ranjzad F, Mahban A, Irani Shemirani A, et al. Influence of gene variants related to calcium homeostasis on biochemical parameters of women with polycystic ovary syndrome. *J Assist Reprod Genet* 2011;28:225–32. [PubMed: 21082232]
29. Takeuchi A, Okano T, Ishida Y, Kobayashi T. Effects of dietary vitamin D intake on plasma levels of parathyroid hormone and vitamin D metabolites in healthy Japanese. *Miner Electrolyte Metab* 1995;21:217–22. [PubMed: 7565453]
30. Thomas MK, Lloyd-Jones DM, Thadhani RI, et al. Hypovitaminosis D in medical inpatients. *N Engl J Med* 1998;338:777–83. [PubMed: 9504937]

AJOG at a Glance**Why was this study conducted?**

To study the relationships between serum vitamin D and androgen biomarkers in eumenorrheic women with a history of pregnancy loss who were attempting pregnancy.

What are the key findings?

Vitamin D is negatively associated with the bioavailability of androgens and positively associated with sex hormone—binding globulin concentrations.

What does this study add to what is already known?

This study extends previous work in women with polycystic ovary syndrome and postmenopausal women to evaluate the role of vitamin D and androgen biomarkers in a population of eumenorrheic women with a history of pregnancy.

TABLE 1

Characteristics of women in the EAGeR trial by baseline vitamin D status

Characteristics	Vitamin D deficient (<20 ng/mL) (n = 163)	Vitamin D insufficient (20–30 ng/mL) (n = 473)	Vitamin D sufficient (≥ 30 ng/mL) (n = 555)	P value
Age, mean ± SD, y	28.5 ± 5.3	28.7 ± 4.6	28.9 ± 4.8	.61
BMI, mean ± SD, kg/m ²	30.7 ± 8.9	26.9 ± 6.3	24.5 ± 5.1	< .0001
Waist circumference, mean ± SD, cm	96.9 ± 19.5	88.3 ± 14.9	82.7 ± 12.3	< .0001
Sum of skinfolds, mean ± SD, mm	118.2 ± 36.5	104.8 ± 34.0	92.0 ± 27.6	< .0001
Central to peripheral skinfold ratio, mean ± SD	0.9 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	< .0001
Race, n, %				< .0001
White	132 (81%)	454 (96%)	542 (98%)	
Nonwhite	31 (19%)	19(4%)	13 (2%)	
Married or living with partner, n, %	155 (95%)	465 (98%)	542 (98%)	.09
Education >high school, n, %	126(77%)	416(89%)	491 (89%)	.001
Income, n, %				< .0001
\$100,000	55 (34%)	206 (44%)	209 (38%)	
\$75,000–99,999	9 (6%)	54 (11%)	84 (15%)	
\$40,000–74,999	21 (13%)	54 (11%)	99 (18%)	
\$20,000–39,999	58 (36%)	121 (26%)	128 (23%)	
\$19,999	20 (12%)	37 (8%)	35 (6%)	
Current smokers, n, %	28 (18%)	51 (11%)	69 (13%)	.10
Parity, n, %				.05
Nulliparous	62 (38%)	217(46%)	271 (49%)	
Parous (1 or 2 prior live births)	101 (62%)	256 (54%)	284 (51%)	
Number of previous pregnancy losses, n, %				.83
1	106(65%)	319(67%)	374 (67%)	
2	57 (35%)	154 (33%)	181 (33%)	
Ever used hormonal contraception/meds, any reason, n, %	110(74%)	362 (80%)	435 (82)	.11
Years for periods to become regular, mean ± SD	2.0 ± 4.4	1.6 ± 4.2	1.5 ± 4.2	.53
Usual menstrual bleeding, mean ± SD, d	5.0 ± 1.4	5.1 ± 2.2	5.0 ± 1.3	.72

Characteristics	Vitamin D deficient (<20 ng/mL) (n = 163)	Vitamin D insufficient (20–30 ng/mL) (n = 473)	Vitamin D sufficient (>30 ng/mL) (n = 555)	P value
Period in past 6 months, mean $\pm$ SD, n	4.7 $\pm$ 1.6	4.5 $\pm$ 1.6	4.6 $\pm$ 1.6	.28
Periods being regular, n, %				.20
Yes	129 (86%)	371 (83%)	439 (82%)	
No	14 (9%)	47 (11%)	77 (14%)	
Do not know	7 (5%)	27 (6%)	22 (4%)	
Age at menarche, mean $\pm$ SD, y	12.4 $\pm$ 1.6	12.7 $\pm$ 1.5	12.8 $\pm$ 1.5	.07
Aspirin treatment assignment, n, %	80 (49%)	233 (49%)	285 (51%)	.76

BMI, body mass index.

Kuhr et al. Vitamin D and bioavailability of androgens. *Am J Obstet Gynecol* 2018.

TABLE 2

Associations between vitamin D and androaen concentrations amona women in the EAGeR trial

Serum measurements	Vitamin D sufficient (≥ 30 ng/mL)	Vitamin D insufficient (20–30 ng/mL)	Vitamin D deficient (<20 ng/mL)	Vitamin D (per 10 ng/mL)
Total testosterone				
Unadjusted geometric mean ± SD, ng/dL	20.1 ± 1.0	20.5 ± 1.0	21.4 ± 1.0	
Adjusted geometric mean ± SD, ng/dL	21.1 ± 1.0	21.1 ± 1.0	21.2 ± 1.0	
Unadjusted percentage difference, 95% CI	Reference	2% (–3% to 7%)	6% (–1% to 14%)	–2% (–4% to 0.2%)
Adjusted percentage difference, 95% CI	Reference	–0.1% (–5% to 5%)	1% (–7% to 9%)	–0.4% (–2% to 2%)
Free testosterone				
Unadjusted geometric mean ± SD, ng/dL	0.26 ± 1.02 ^a	0.28 ± 1.02 ^a	0.31 ± 1.03 ^a	
Adjusted geometric mean ± SD, ng/dL	0.29 ± 1.04	0.30 ± 1.04	0.30 ± 1.04	
Unadjusted percentage difference, 95% CI	Reference	7% (2–12%) ^a	16% (8–24%) ^a	–4% (–6% to –2%) ^a
Adjusted percentage difference, 95% CI	Reference	2% (–3% to 8%)	3% (–5% to 11%)	–2% (–4% to 0.4%)
Free androgen index				
Unadjusted geometric mean ± SD	1.0 ± 1.0 ^a	1.3 ± 1.0 ^a	1.5 ± 1.0 ^a	
Adjusted geometric mean ± SD	1.3 ± 1.0 ^a	1.4 ± 1.0 ^a	1.4 ± 1.1 ^a	
Unadjusted percentage difference, 95% CI	Reference	21% (13–31%) ^a	49% (34–65%) ^a	–10% (–13% to –8%) ^a
Adjusted percentage difference, 95% CI	Reference	10% (3–17%) ^a	11% (–0.04% to 22%)	–5% (–7% to –2%) ^a
DHEAS				
Unadjusted geometric mean ± SD, μmol/L	4.3 ± 1.0	4.5 ± 1.0	4.6 ± 1.0	
Adjusted geometric mean ± SD, μmol/L	4.7 ± 1.0	4.8 ± 1.0	4.6 ± 1.0	
Unadjusted percentage difference, 95% CI	Reference	3% (–3% to 10%)	6% (–3% to 15%)	–2% (–4% to 0.5%)
Adjusted percentage difference, 95% CI	Reference	1% (–5% to 8%)	–1% (–10% to 8%)	–1% (–3% to 2%)
SHBG				
Unadjusted geometric mean ± SD, nmol/L	67.0 ± 1.0 ^a	56.4 ± 1.0 ^a	47.8 ± 1.0 ^a	
Adjusted geometric mean ± SD, nmol/L	57.5 ± 1.0 ^a	52.4 ± 1.0 ^a	52.3 ± 1.0 ^a	
Unadjusted percent difference, 95% CI	Reference	–16% (–21% to –10%) ^a	–29% (–35% to –22%) ^a	9% (7%, 12%) ^a

Serum measurements	Vitamin D sufficient (≥ 30 ng/mL)	Vitamin D insufficient (20–30 ng/mL)	Vitamin D deficient (<20 ng/mL)	Vitamin D (per 10 ng/mL)
Adjusted percent difference, 95% CI	Reference	−9% (−14% to −4%) ^a	−9% (−17% to −1%) ^a	4% (2%, 7%) ^a
Additional adjustment for insulin, TT, and E1G	Reference	−9% (−15% to −3%) ^a	−11% (−20% to −2%) ^a	7% (4%, 10%) ^a

^aStatistically significant at $\alpha = .05$.

Adjusted models control for age, body mass index, smoking status, race, income, education, physical activity, and season of blood draw. R^2 for the adjusted models is as follows: TT, 0.08; fT, 0.15; FAI, 0.29; DHEAS, 0.08; and SHBG, 0.26.

CI confidence interval; DHEAS, dehydroepiandrosterone sulfate; E1G, estrone-3-glucuronide; FAI, free androgen index; fT, free testosterone; SHBG, sex hormone-binding globulin; TT, total testosterone.

Kuhr et al. Vitamin D and bioavailability of androgens. Am J Obstet Gynecol 2018.