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Seasonal Variations in Sex Steroids in a Young Male Population and Their Relationship with Plasma Levels of Vitamin D

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Purpose: Vitamin D (VD) acts on sperm motility, capacitation and survival but its role in steroidogenesis is less clear. Aims: To analyze seasonal variations in sex steroids and VD in a healthy male population.

Materials and Methods: Twenty-nine healthy males, 34.0±4.8 years were included. Blood collection in winter (W) and summer (S) was performed to measure: 25OHD, total testosterone (TT), free testosterone (FT), estradiol (E2), luteinizing hormone (LH), and sex hormone binding globulin (SHBG). Testosterone/estradiol (T/E2) ratio was calculated.

Results: In W, lower levels of 25OHD: 18.8±7.2 ng/mL *vs*. 38.8±11.9 ng/mL (p<0.0001) and LH: 3.5±1.2 mU/mL *vs*. 3.9±1.5 mU/mL (p=0.05), and higher levels of TT: 501.9±157.7 ng/dL *vs*. 405.0±128.0 ng/dL (p=0.0003), FT: 11.8±4.1 ng/dL *vs*. 10.2±3.7 ng/dL (p=0.017), SHBG: 28.5±10.9 nmol/L *vs*. 23.6±7.9 nmol/L (p=0.002) and T/E2 ratio: 30.7±19.7 ng/dL/pg/mL *vs*. 17.3±3.6 ng/dL/pg/mL (p=0.0015) with no variation in E2 levels were observed. A positive correlation between 25OHD and E2 (r=0.28, p=0.04) and negative correlations between 25OHD and TT (r=-0.27, p=0.049), 25OHD and FT (r=-0.32, p=0.01), and 25OHD and T/E2 (r=-0.44, p=0.0008) were found.

Conclusions: In healthy young male population, seasonal variations were observed in 25OHD and LH levels (higher in S) and in TT, FT, SHBG levels, and T/E2 (higher in W). Lower values of TT and FT in S are accompanied by higher levels of LH, which rules out a central mechanism for lowering testosterone. 25OHD negatively correlated with TT, FT, and T/E2 and positively correlated with E2, suggesting a relationship between VD status and changes in gonadal steroids.

Keywords: Seasonal variations; Sex hormones; Testosterone; Vitamin D

INTRODUCTION

Vitamin D (VD) is one of the main hormones regulating bone mineralization, and calcium, phosphorus and magnesium homeostasis. However, in addition to the classic actions of VD on bone and muscle, in recent years, nonclassic actions have been detected in immunomodulation, regulation of cell proliferation, insulin secretion, and regulation of the renin-angiotensin system, among others.

In the male reproductive tract, a wide distribution of VD receptor (VDR) and of the enzymes involved in its

activation and metabolism has been found [1,2]. At the reproductive level, actions on spermatozoa are related to sperm motility, capacitation and survival [3,4]. The role of VD in testicular steroidogenesis, if any, is less clear. Some cross-sectional and interventional studies show a direct relationship between testosterone and VD levels. However, this relationship has not been observed in other studies, and the population enrolled has confounding factors mainly age, obesity, and general diseases that may influence the measurement of both hormones.

In studies previously conducted in Argentina, a large seasonal variation in 25-hydroxyvitamin D (25OHD) levels has been observed in adults, with such variation mostly depending on seasonal differences in exposure to ultraviolet radiation (UVR). Fifty percent of the healthy young adult population has 25OHD levels <20 ng/mL in winter and only 5% in summer, and this is observed both in males and females [5].

The aims of this study were: 1) to evaluate seasonal variations in sex steroids and VD in a healthy young male population and 2) to analyze the relationship between 25OHD levels and sex steroids.

MATERIALS AND METHODS

This is a prospective study of 29 healthy subjects aged 18 to 40 years old with no diseases and/or intake of any medication that may alter the calcium and phosphate metabolism and who had been living in Buenos Aires (latitude 34.5° South) for at least 2 years and stayed in this city for the one-year study period.

The exclusion criteria were: body mass index (BMI) ≥30 kg/m^2; presence of severe heart, liver or kidney disease; history of infertility, hypogonadism, cryptorchidism, orchiectomy; use of medication that interferes with the synthesis, action or breakdown of sex steroids (antiandrogens, glucocorticoids, ketoconazole, 5α-reductase inhibitors, opioids); use of medications that interfere with the synthesis, action or breakdown of 25OHD (glucocorticoids, anticonvulsants, antiretroviral drugs, ketoconazole, cholestyramine, anticoagulants); use of supplements containing VD; use of anabolic steroids; alcoholism; extended working hours that may hinder exposure to the sun and being absent from Buenos Aires for over 3 weeks during the one-year study period. These exclusion criteria had to be met both before and throughout the study.

The following data were collected from physical examination: weight and height; BMI (18.5–25 kg/m^2 was considered normal weight; ≥25 kg/m^2 and <30 kg/m^2 was considered overweight and ≥30 kg/m^2 was considered obesity). Testicular examination was performed by palpation of both testes and their volume was measured by Prader orchidometer (©Andrology, Melbourne, Australia, 2007).

Blood samples were collected between 08:00 a.m. and 09:00 a.m. after an 8-hour fast in winter (August 2015) and summer (March 2016) for the following hormonal measurements:

- 25OHD: chemiluminescence immunoassay on the Architect (Abbott, Green Oaks, IL, USA) analyzer; laboratory reference ranges, desirable level: higher than 30 ng/mL; intra-assay coefficient of variation was 2.8% and inter-assay coefficient of variation was 4.1%.

- Total testosterone (TT): chemiluminescence on the Immulite 2000 (Siemens, Flanders, NJ, USA) analyzer; laboratory reference ranges: 300–880 ng/dL for adult males; intra-assay coefficient of variation was 7.5% and inter-assay coefficient of variation was 8.1%.

- Free testosterone (FT): Vermeulen equation; normal: 2.6–17.0 ng/dL.

- Estradiol (E2): chemiluminescence immunoassay on the Architect (Abbott) analyzer; laboratory reference ranges: 10–44 pg/mL; intra-assay coefficient of variation was 7.4% and inter-assay coefficient of variation was 4.5%.

- Luteinizing hormone (LH): chemiluminescence immunoassay on the Architect (Abbott) analyzer; laboratory reference ranges: 2–12 mIU/mL; intra-assay coefficient of variation was 4.1% and inter-assay coefficient of variation was 4.3%.

- Sex hormone binding globulin (SHBG): chemiluminescence on the Immulite 2000 (Siemens) analyzer; laboratory reference ranges: 13–71 nmol/L for adult males; intra-assay coefficient of variation was 5.3% and inter-assay coefficient of variation was 6.6%.

The testosterone/estradiol (T/E2) ratio was calculated.

Of 29 subjects enrolled in the study, 26 completed blood sample collections in winter and summer and 3 had blood samples drawn only in winter. For the analysis of seasonal variation in 25OHD and sex steroids levels, data from the 26 males who had both samples collected were used.

Based on plasma levels of 25OHD, VD deficiency was defined as a 25OHD ≤20 ng/mL, insufficiency as a

25OHD of 20.1–29.9 ng/mL, and 25OHD levels ≥30 ng/mL were considered optimal.

1. Ethics statements

The present study protocol was reviewed and approved by the institutional review board of Hospital Italiano de Buenos Aires (Reg. No. 1547). Informed consent was submitted by all subjects when they were enrolled.

2. Statistical analysis

Data were analyzed using the Instat Statistical Software (version 3.01; GraphPad, San Diego, CA, USA). Differences in the hormonal parameters between winter and summer were compared with a two-sample t-test (parametric) or Wilcoxon Signed-Ranks Test (nonparametric) for continuous variables (n=26); categorical variables were compared using Chi–square. Pearson's correlation coefficients were used to assess the relationship between 25OHD and sex steroids. Data are expressed as the mean±standard deviation. All p-values quoted are two-sided and considered statistically significant when the values are below 0.05.

RESULTS

Twenty-six healthy young males with a mean age of 34.0±4.8 years and BMI of 25.2±2.9 kg/m^2 were included. When comparing seasonal variations in the biochemical parameters evaluated, lower levels of 25OHD and LH were observed in winter as compared to summer: 25OHD, 18.8±7.2 ng/mL $vs.$ 38.8±11.9 ng/mL (p<0.0001); and LH, 3.5±1.2 mU/mL $vs.$ 3.9±1.5 mU/mL (p=0.05), respectively.

In addition, in winter, levels of TT, FT, SHBG, and T/E2 were higher than in summer: TT, 501.9±157.7 ng/dL $vs.$ 405.0±128.0 ng/dL (p=0.0003); FT, 11.8±4.1 ng/dL $vs.$ 10.2±3.7 ng/dL (p=0.017); SHBG, 28.5±10.9 nmol/L $vs.$ 23.6±7.9 nmol/L (p=0.002); and T/E2 ratio, 30.7±19.7 ng/dL/pg/mL $vs.$ 17.3±3.6 ng/dL/pg/mL (p=0.0015), respectively. No significant seasonal variations were observed in E2 levels: 22.2±12.7 pg/mL in winter $vs.$ 24.2±8.2 pg/mL in summer (p=0.52).

Seasonal variations in the hormones evaluated are shown in Table 1. It should be noted that, although with significant seasonal variations, all results were within the reference ranges. When comparing seasonal levels of TT in each subject, 80.8% (n=21) had higher values in winter (n=21), 3.8% (n=1) had equal values in winter and summer, and only 15.4% (n=4) had higher values in summer. The percentage change of TT and E2 was evaluated by comparing the summer value $vs.$ the winter value of each subject (summer value-winter value/winter value×100). It was observed that in summer, most of the subjects presented a positive variation of E2 (Fig. 1) while the majority presented a negative percentage variation of TT (Fig. 2).

With regards to the levels of 25OHD in winter, 65.4% (n=17) had deficiency, 30.8% (n=8) had insufficiency and only 3.8% (n=1) had optimal levels. In summer, no subject had deficiency, 26.9% (n=7) had insufficiency and 73.1% (n=19) had optimal levels. A significant difference was found between winter and summer in subjects with VD deficiency (25OHD ≤20 ng/dL, p<0.0001).

A negative correlation was found between 25OHD and TT (r=-0.27, p=0.049) (Fig. 3); 25OHD and FT (r=-0.32, p=0.01) (Fig. 4) and between 25OHD and T/E2 (r=-0.44, p=0.0008) (Fig. 5). A positive correlation was found between 25OHD and E2 (r=0.28, p=0.04) (Fig. 6).

When we evaluate the correlations between 25OHD

Table 1. Seasonal variation in hormonal measurements (n=26)

Variable	Winter	Summer	p-value
25OHD (ng/mL)	18.8±7.2	38.8±11.9	<0.0001
TT (ng/dL)	501.9±157.7	405.0±128.0	0.0003
FT (ng/dL)	11.8±4.1	10.2±3.7	0.017
T/E2 ratio (ng/dL/pg/mL)	30.7±19.7	17.3±3.6	0.0015
E2 (pg/mL)	22.2±12.7	24.2±8.2	0.52
SHBG (nmol/L)	28.5±10.9	23.6±7.9	0.002
LH (mUI/mL)	3.5±1.2	3.9±1.5	0.05

25OHD: 25-hydroxyvitamin D, TT: total testosterone, FT: free testosterone, T/E2: testosterone/estradiol, SHBG: sex hormone binding globulin, LH: luteinizing hormone.
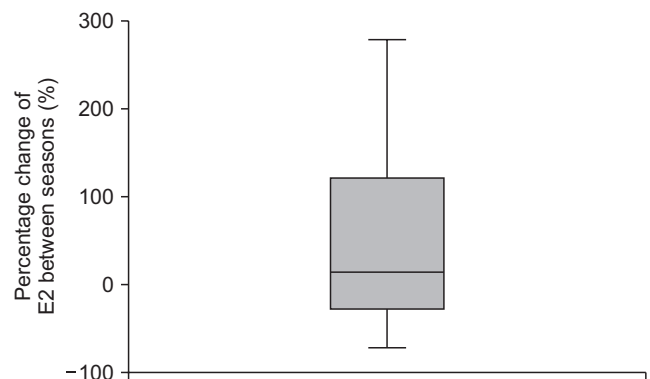

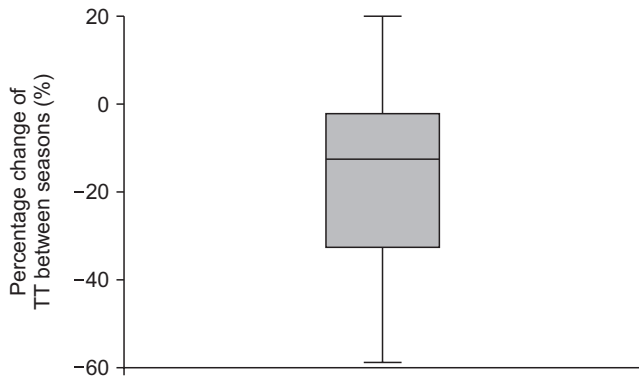**Fig. 1.** Percentage change of estradiol (E2) between summer and winter.

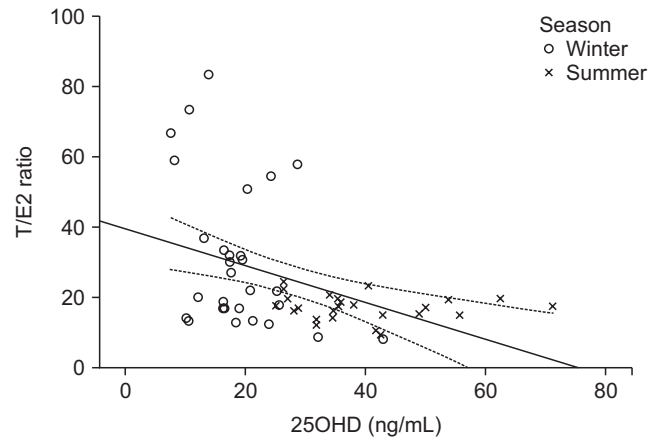**Fig. 2.** Percentage change of total testosterone (TT) between summer and winter.
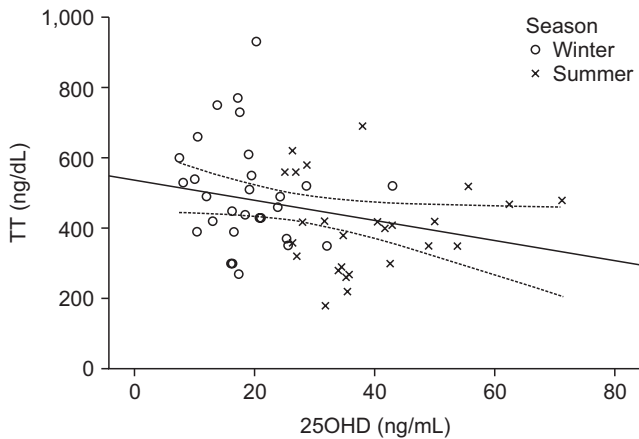

Fig. 3. Correlation between 25-hydroxyvitamin D (25OHD) and total testosterone (TT) (r=-0.27, p=0.049).

Fig. 4. Correlation between 25-hydroxyvitamin D (25OHD) and free testosterone (r=-0.32, p=0.01).

Fig. 5. Correlation between 25-hydroxyvitamin D (25OHD) and testosterone/estradiol (T/E2) ratio (r=-0.44, p=0.0008).

Fig. 6. Correlation between 25-hydroxyvitamin D (25OHD) and estradiol (E2) (r=0.28, p=0.04).

DISCUSSION
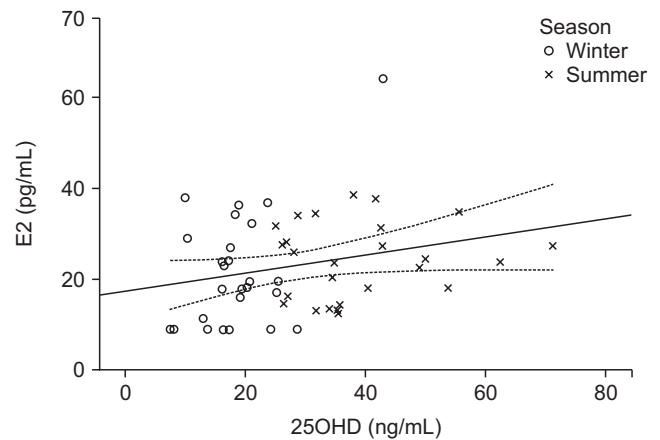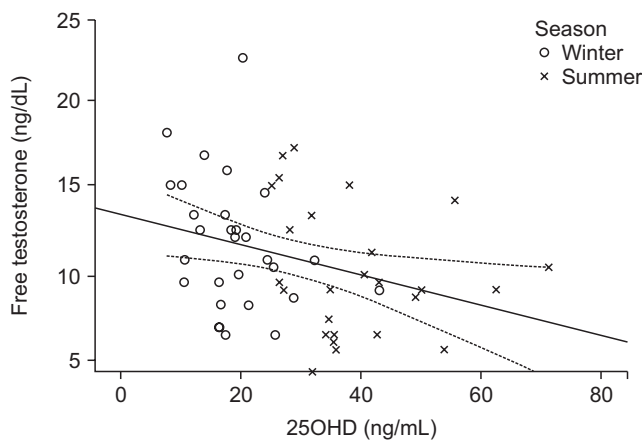
The high prevalence of hypovitaminosis D worldwide across all age groups, including young individuals of childbearing age, is well known. Even if the prevalence of hypovitaminosis is global, there are differences in the geographical areas evaluated depending on the latitude, the weather, the habits, clothing styles and race. In our population of healthy young males, we observed a large number of subjects with VD deficiency in winter (65.4%), a similar proportion of that found in previous study in Buenos Aires [5].

The presence of VDR in the male reproductive tract has been reported as early as 35 years ago, with VDR being widely distributed in the male reproductive system. Furthermore, the genes and activity of VD metabolizing and activating enzymes are expressed in

and sexual steroids in winter and summer separately we observe only a positive correlation between 25OHD and E2 (r=0.46, p=0.017) in winter.

the same tissues suggesting a VD local action [1]. Studies performing functional assessments of spermatozoa, demonstrated that incubation with $1,25(OH)_2D3$ induced important changes, that are important for sperm capacitation, which allows the spermatozoon to enter the oocyte interior [2-4]. In a recent review of observational studies evaluating the relationship between VD levels and seminal parameters, it was concluded that most of the studies agree on the possibility that VD might have a positive effect on semen analysis parameters, mainly on sperm motility [6].

In addition to the seasonal variation in 25OHD values, we found significant seasonal differences in sex steroid levels. In winter, higher values of TT, FT, and SHBG were found as compared to summer; conversely, LH levels were lower in winter than in summer, a behavior that is consistent with the normal functioning of the hypothalamic-pituitary axis, with higher testosterone levels (either from increased synthesis or peripheral conversion) exerting a negative feedback centrally. No differences were found in seasonal levels of E2; however, levels of bioavailable E2 could not be evaluated and the method used lacks high sensitivity to detect low E2 concentrations in males [7]. We did observe a significant positive correlation between E2 and 25OHD and a difference in the T/E2 ratio that was significantly higher in winter.

It is important to highlight that despite the seasonal differences found in most hormones evaluated, results were within reference ranges for the laboratory method used. However, when evaluating paired TT values for each subject, over 80% had higher TT levels in winter than in summer, while the opposite was observed in only 15%.

Several studies have evaluated seasonal variations in sex steroids with contradictory results. Some authors have not observed significant seasonal variations in sex steroids. Brambilla et al [8] have not found variations in androgens in 121 men in Boston. In one of the studies with a large number of subjects enrolled (n=11,623) from the Southwest United States, no circannual variation was observed in testosterone levels, but lower levels of E2 and of the T/E2 ratio were indeed observed in fall-winter [9]. Tancredi et al [10] have not found seasonal variations in FT in community-dwelling males aged 50 to 70 years (n=5,028).

Andersson et al [11] measured monthly variations in sex steroids in 27 healthy males in Copenhagen and found higher LH and testosterone levels in summer, with lower levels in winter. Svartberg et al [12] found seasonal variations in TT levels (up to 19%) and in FT levels (up to 31%) in 1,548 men with a mean age of 60 years living in Tromsø (Norway), with higher values in the late fall and lower values in summer. Variations continued to be significant after adjusting for age, BMI, and waist to hip ratio. The authors observed that the hours of daylight and air temperature were inversely related to TT [12].

One of the hypotheses used to explain seasonal variation in sex steroids is the relationship between sex steroids and 25OHD, a hormone that varies mainly with the exposure to UVR in the different seasons. Wehr et al [13] found an independent association of 25OHD levels with TT, SHBG and the calculated free androgen index in 2,299 males with a mean age of 62±11 years. These authors also found similar seasonal variation patterns for 25OHD and TT with peaks in summer [13]. Males enrolled in the study conducted by Wehr et al [13] were patients with high cardiovascular risk referred for coronary angiography. A high percentage of the population enrolled had comorbidities such as arterial hypertension (80%), coronary artery disease and diabetes (30%). Nimptsch et al [14] did not find seasonal variations in testosterone and E2 levels in 1,362 males with a mean age of 60 years. These authors observed a positive association of 25OHD levels with TT, FT, and E2 [14]. A similar result with no seasonal variation in sex steroids and a positive association between 25OHD and testosterone levels has been obtained in 1,633 adult males in the National Health and Nutrition Examination Surveys (NHANES) [15]. Given the cross-sectional design of these studies, no conclusions can be drawn on the causality or directionality of this association.

Lee et al [16] evaluated the relationship between 25OHD and sex steroids, and their seasonal variation in 3,051 men aged 40 to 79 years. Subjects with VD deficiency had a higher BMI, a higher prevalence of cardiovascular disease, diabetes and poorer physical function and depression scores. After adjusting for age and confounders, no association was found between 25OHD levels and sex steroids [16]. Studies evaluating the relationship between 25OHD and androgens in healthy young populations have found no association, not even negative, of 25OHD levels with testosterone and the free androgen index [17,18]. The lack of an association between these hormones has also been observed in

middle-aged males (35 years) [19].

The interventional studies evaluating the effect of VD supplementation on androgens in males also show inconsistent results. In studies performed in obese men, an increase in TT and FT was observed in the population that received VD [20,21]. These studies have important weaknesses, such as no placebo control, advanced age, the presence of obesity, and other comorbidities. Nevertheless, studies evaluating the effect of VD treatment on testosterone levels in healthy young men have not found any effect [22].

It is difficult to compare studies evaluating the relationship between VD levels and testosterone due to different factors: differences in 25OHD levels, inclusion of population from different geographic areas, times of blood sampling, sample size, wide heterogeneity in the study population, advanced age, and presence of comorbidities that may influence plasma levels of both, sex steroids and 25OHD.

One of these factors is age: the decrease in testosterone levels after the age of 40 has been clearly documented, and the synthesis of VD also declines with age [23]. Most studies evaluating and reporting a direct association between sex steroids and 25OHD include a population over 40 years old [13-16]. This also applies to the studies reporting increased testosterone levels with VD treatment [20,21]. Studies conducted in subjects younger than 40 years have not found any association between these hormones [17-19] or effects of VD treatment on sex steroids [22]. Other factors associated with lower testosterone and 25OHD levels in these studies are the presence of comorbidities, such as obesity [24], diabetes [25], and coronary artery disease. Many of the studies reporting an association between VD and testosterone have these confounders [13,14,16,20,21] and this association is no longer observed when results are adjusted for these confounders [16].

In order to avoid these confounders, we selected a young, healthy population in a region with large seasonal variation in 25OHD levels, and prospectively evaluated the association between sex steroids and 25OHD in the same subjects. 25OHD was negatively correlated with TT, FT, and the T/E2 ratio. We consider it relevant to analyze the correlation of the concentrations of 25OHD and sex steroids in both seasons together, because our interest was to observe the possible relationship in the full range of variation of VD and sex steroids.

The interplay between testosterone and E2 as assessed by T/E2 ratio was suggested to be more informative on the normal physiological balance. In males, a major source of E2 comes from the conversion of testosterone to E2 by the aromatase enzyme. Thus, lower testosterone levels in summer should also imply lower E2 levels, unless any other factor stimulates aromatase action. VD regulates aromatase activity. In VDR null mice, the aromatase activity in the testes was 58% lower than in wild-type mice, and the expression of the aromatase gene was also lower; calcium supplementation increased the aromatase activity, but not to 100% [26]. $1,25(OH)_2D3$ increased aromatase expression in rat Sertoli cells *in vitro* [27].

In a study using a human testicular cell culture model, an increase in testosterone production and in mRNA expression of enzymes involved in androgen production was observed *in vitro* after the addition of 100 nM $1,25(OH)_2D3$ [28]. Testosterone produced in the testes can be converted to E2 by the aromatase (regulated by VD) or to dihydrotestosterone by the 5α-reductase enzyme. Therefore, an increase in intratesticular production of testosterone stimulated by VD might not be reflected in serum levels. In our study in the summer, with higher levels of 25OHD, lower testosterone levels and lower T/E2 ratio were observed. In addition, we observed a significant correlation between 25OHD and E2, and an inverse correlation with testosterone levels.

Our study has several strengths: first, our study population was young, non-obese, and healthy, to avoid the effects of confounding factors on 25OHD and sex steroid levels. Second, the prospective study design with paired comparison in the same population is different from most cross-sectional studies evaluating variations in sex steroids that have been published in the literature. The time of blood sampling for hormonal measurements allowed ruling out circadian variations that might interfere with results. The weakness in our study are that hormonal measurements were not performed by the gold standard method, high-performance liquid chromatography, we were unable to measure bioavailable E2 because this assay is not available in our setting and the small population included. In this study questionnaires about exercise, diet or outdoor activities were not performed, which can vary with the seasons and influence hormonal levels.

CONCLUSIONS

Our study shows that in a healthy young male population, seasonal variations were observed in 25OHD and LH levels (higher in summer) and in TT, FT, SHBG levels, and T/E2 (higher in winter). Lower values of TT and FT in summer are accompanied by higher levels of LH in this period, which rules out a central mechanism for lowering testosterone. 25OHD negatively correlated with TT, FT, and T/E2 and positively correlated with E2, suggesting a relationship between VD status and changes in gonadal steroids.

ACKNOWLEDGEMENTS

Conflict of Interest

The authors have nothing to disclose.

Author Contribution

Conceptualization: all authors. Data curation: PRC, PK. Formal analysis: PRC, PK. Investigation: PRC, SMS. Methodology: AEK. Project administration: PRC. Supervision: SMS. Validation: SMS, PK. Writing-original draft: PK. Writing-review & editing: PK, AEK.

Data Sharing Statement

The data analyzed for this study have been deposited in HARVARD Dataverse and are available at https://doi.org/10.7910/DVN/SBSQO5.

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