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Association of vitamin D status with serum androgen levels in men

E. Wehr[1], S.Pilz[1], B.O. Boehm[2], W. März[3,4], and B. Obermayer-Pietsch[1]

[1]Department of Internal Medicine, Division of Endocrinology and Nuclear Medicine, Medical University Graz, Graz, Austria; [2]Department of Internal Medicine, Division of Endocrinology and Diabetes, Ulm University, Ulm, Germany; [3]Department of Public Health, Social and Preventive Medicine, Mannheim Medical Faculty, University of Heidelberg, Mannheim, Germany; [4]Synlab Center of Laboratory Diagnostics, Heidelberg, Germany

Short title: Vitamin D and serum androgens in men

Corresponding author
Winfried März, MD
Synlab Center of Laboratory Diagnostics
Postfach 10 41 29
69031 Heidelberg
Germany
Telephone number: +49-6221-793-0
Fax +49-6221-793-111
E-mail: maerz@synlab.de

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The authors have nothing to disclose.

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Abstract

Objective: Studies in rodents indicate a role of vitamin D in male reproduction but the relationship between vitamin D and androgen levels in men is largely unexplored. We aimed to investigate the association of 25-hydroxyvitamin D [25(OH)D] levels with testosterone, FAI (free androgen index), and SHBG. Moreover, we examined whether androgen levels show a similar seasonal variation to 25(OH)D.

Design: In this cross-sectional study, 25(OH)D, testosterone, and SHBG levels were assessed by immunoassay in 2299 men who were routinely referred for coronary angiography (1997-2000).

Measurements: Main outcome measures were associations of 25(OH)D levels with testosterone, SHBG, and FAI. FAI was calculated as testosterone (nmol/l)/SHBG (nmol/l) x 100.

Results: Men with sufficient 25(OH)D levels (≥30 µg/l) had significantly higher levels of testosterone and FAI and significantly lower levels of SHBG when compared to 25(OH)D insufficient (20-29.9 µg/l) and 25(OH)D deficient (<20 µg/l) men ($p<0.05$ for all). In linear regression analyses adjusted for possible confounders, we found significant associations of 25(OH)D levels with testosterone, FAI, and SHBG levels ($p<0.05$ for all). 25(OH)D, testosterone, and FAI levels followed a similar seasonal pattern with a nadir in March (12.2 µg/l, 15.9 nmol/l, and 40.8, respectively) and peak levels in August (23.4 µg/l, 18.7 nmol/l, and 49.7, respectively) ($p<0.05$ for all).

Conclusion: Androgen levels and 25(OH)D levels are associated in men and reveal a concordant seasonal variation. Randomized controlled trials are warranted to evaluate the effect of vitamin D supplementation on androgen levels.

Introduction

Vitamin D status is mainly determined by ultraviolet-B induced vitamin D production in the skin, while vitamin D intake by nutrition and supplements plays only a minor role [1]. Vitamin D from either source is hydroxylated in the liver to 25-hydroxyvitamin D [25(OH)D], which is used to determine a patient`s vitamin D status. 25(OH)D is further hydroxylated to its active form, 1,25-dihydroxyvitamin D [1,25(OH)2D]. Serum levels of 1,25(OH)2D are mainly determined by renal 1,25(OH)2D production, which is catalyzed by 1α-hydroxylase. Extrarenal expression of 1α-hydroxylase has, however, also been observed suggesting local 1,25(OH)2D production in various tissues and organs throughout the body. Biological actions of vitamin D are mediated through the vitamin D receptor (VDR), which regulates about 3% of the human genome [2]. The VDR, is almost ubiquitously expressed in human cells, which underlines the clinical significance of the vitamin D endocrine system [1, 3]. Currently, there is great interest in vitamin D because poor vitamin D status is common and has been associated with an increased risk of various chronic diseases including cancer [4], diabetes [5], hypertension [6], autoimmune diseases, musculoskeletal diseases [1] and cardiovascular diseases [1, 4, 7] as well as all-cause mortality [8].

Data on the association of vitamin D and gonadal function are sparse but VDR expression has been observed in reproductive tissues such as ovary, uterus, prostate, testis, and human sperm [9-11]. VDR knock out mice have significant gonadal insufficiency, decreased sperm count and motility, and histological abnormalities of testis [3]. Moreover, there is a distinct seasonal variation in testosterone levels [12], which appears to resemble the seasonal variation of 25(OH)D levels, which is a consequence of seasonal differences in sunlight induced vitamin D production in the skin. The association between vitamin D status and androgens has, however, not been studied in greater detail. Thus, it remains unclear, whether there are similar seasonal distributions of 25(OH)D and androgen levels within a given study population.

Hence, the aim of this study was to investigate the association of 25(OH)D levels with testosterone, SHBG and FAI (free androgen index) and to examine the seasonal variation of 25(OH)D, testosterone, FAI, and SHBG levels.

Methods

Study population

The LUdwigshafen RIsk and Cardiovascular Health (LURIC) study is a prospective study including 3316 patients (2310 men and 1006 women) who were routinely referred for coronary angiography at baseline between July 1997 and January 2000. Serum concentrations of 25(OH)D, testosterone and SHBG were available in 2299 men with a mean±SD age of 62±11 years. The study was performed at a cardiology unit in a tertiary care medical centre in South-West Germany. Inclusion criteria were the avaibility of a coronary angiogram, clinical stability with the exception of acute coronary syndromes, and Caucasian origin, to limit genetic heterogeneity. Patients with a history of malignancy within the past 5 years, any acute illness other than acute coronary syndrome, and any predominant noncardiac diseases were excluded from the study. Seventy-eight patients (2.4%) reported taking vitamin supplements on a regular basis, which usually contained vitamin D3. Because 25(OH)D levels were only slightly higher in users of vitamin D preparations (mean±SD, 22.1±11.3 µg/l) compared with the remaining cohort (mean±SD, 17.2±9.1 µg/l), we decided to include these patients in the present analyses. All study participants gave written informed consent, and the study was approved by the ethics committee at the "Ärztekammer Rheinland-Pfalz".

Procedures

The baseline examination has been published previously in detail [13]. Venous blood sampling was performed in the morning before coronary angiography, and routine laboratory parameters including 25(OH)D, testosterone, and LH were immediately measured on a daily

to weekly basis as previously published [13]. Remaining blood samples were frozen and stored at -80°C until analysis. Serum levels of SHBG were assayed in 2009. Serum concentrations of 25(OH)D were measured by a RIA (DiaSorin, Antony, France, and Stillwater, MN) with intra- and interassay coefficients of variation (CV) of 8.6 and 9.2%, respectively. In 100 randomly chosen samples, we determined 25(OH)D by liquid chromatography tandem mass spectrometry with isotopic labelled standard and two fragments m/z 401.4/382.2 (quantifier) and 401.4/365.3 (qualifier) and found a highly significant correlation between the 25(OH)D levels obtained by RIA and liquid chromatography tandem mass spectrometry ($r=0.875$; $p<0.001$). Testosterone was measured in serum by a solid-phase chemoluminescence enzyme immunoassay (Testosterone.Immulite, DPC Biermann GmbH, Bad Nauheim, Germany) with an intra- and inter-assay CV of 7.2% and 9.1%, respectively. LH was also determined in serum by a microparticle enzyme immunoassay (LH, Abbott GmbH, Wiesbaden, Germany) with an intra- and inter-assay CV of 4.5 to 6.7% and 3.6 to 3.9%, respectively. SHBG was measured by luminescence immunoassay (Roche, Basel, Switzerland) with an intra- and inter-assay CV of 1.3% and 2.1%, respectively. The free androgen index (FAI) was calculated as testosterone (nmol/l)/SHBG (nmol/l) x 100. Male hypogonadism was defined as testosterone levels below 11.3 nmol/l [14]. Angiographic coronary artery disease was defined as the occurrence of at least one stenosis of at least 20% of at least one of 15 coronary segments, using the maximal luminal narrowing estimated by visual analysis. Diabetes mellitus was diagnosed in patients with a fasting glucose greater than 7.0 mmol/l, a 2h-value of an oral glucose tolerance test of greater than 11.1 mmol/l and in patients already on antidiabetic treatment. Arterial hypertension was diagnosed in patients with a systolic and diastolic blood pressure exceeding 140 and/or 90 mmHg and in patients on antihypertensive treatment. Smoking status and wine consumption were assessed by questionnaires [13].

Statistical analysis

According to widely used cut-offs for vitamin D status classification, subjects were divided into four groups: vitamin D sufficiency (25(OH)D ≥30 µg/l), vitamin D insufficiency (25(OH)D 20.0-29.9 µg/l), moderate vitamin D deficiency (10.0-19.9 µg/l), and severe vitamin D deficiency (<10 µg/l) [1, 15]. To convert serum 25(OH)D levels to nanomoles per liter, multiply by 2.5. Data for continuous variables are presented as means ± standard deviation (SD) unless otherwise stated and data for categorical variables are presented as percentages. Kolmogorov-Smirnov test and descriptive statistics were used to examine for normal distribution. Variables following a skewed distribution were logarithmically transformed for parametric statistical analyses. Differences between groups were calculated by ANOVA for continuous variables and with Chi square test for categorical variables. To test for associations of 25(OH)D levels with testosterone, FAI and SHBG we used simple regression analyses. In addition we also used multiple regression analyses to evaluate whether 25(OH)D levels are an independent determinant of testosterone, FAI and SHBG. These multiple regression analyses were calculated with testosterone, FAI or SHBG as dependent variables and with 25(OH)D, BMI and age as independent variables. Additionally we included wine consumption (never/rarely/sometimes/often), smoking (active smoker: yes/no), use of beta-blockers (yes/no), use of statins (yes/no), and diabetes mellitus (yes/no) as independent variables because these parameters are known to influence testosterone levels [14, 16, 17]. To consider the seasonal variation of 25(OH)D levels we also used z-values of 25(OH)D for these above mentioned regression analyses. These z-values were calculated according to the formula: individual value of 25(OH)D minus the mean divided by the standard deviation of 25(OH)D levels within the respective month of blood sampling. To further test for the association of 25(OH)D levels with hypogonadism we used a binary logistic regression analysis with hypogonadism as the dependent variable and 25(OH)D levels as well as other possible confounders as independent variables. Odds ratios (ORs) for male hypogonadism were calculated using the 25(OH)D sufficient group as the reference. Seasonal variability was

examined by calculating monthly means of 25(OH)D, testosterone, SHBG, and FAI levels and comparing the peak mean value to the other monthly mean values by ANOVA. Post hoc testing with Bonferroni correction was performed to detect monthly means significantly different from the peak value. Analyses of covariance (ANCOVA) were used for group comparisons with adjustment for age. A p-value of <0.05 was considered statistically significant. Statistical analyses were performed by SPSS version 16.0 (SPSS Inc., Chicago, IL).

Results

Characteristics of subjects according to vitamin D status are shown in table 1. Vitamin D sufficiency was present in 262 men (11.4%), 589 men presented with vitamin D insufficiency (25.6%), 991 men had moderate vitamin D deficiency (43.1%), and 457 men were severely vitamin D deficient (19.9%).

Hypogonadism (testosterone levels <11.3 nmol/l) was present in 415 men (18%). Men with hypogonadism presented with significantly lower mean 25(OH)D levels than men with sufficient testosterone levels (16.4 vs. 18.6 µg/l, $p<0.001$).

In binary logistic regression analysis adjusted for age, BMI, wine consumption, smoking, use of beta blockers, and diabetes mellitus, ORs for male hypogonadism were 1.84 (1.16-2.90), 1.48 (0.95-2.29), and 2.47 (1.55-3.93) for men with vitamin D insufficiency, moderate vitamin D deficiency, and severe vitamin D deficiency, respectively, when compared to men with vitamin D sufficiency. When different cut-off values for hypogonadism were used (8.7 nmol/l and 10.4 nmol/l), results did not materially change.

In multiple regression analyses, we found significant associations of 25(OH)D levels with testosterone, FAI, and SHBG after adjustment for age and BMI (table 2). These associations did not materially change after additional adjustments for wine consumption, smoking, beta-

blocker use, statin use, and diabetes and when z-values of 25(OH)D were used instead of absolute 25(OH)D levels for multiple regression analyses.

Seasonal variation

Monthly variations of 25(OH)D, testosterone levels, and FAI levels were statistically significant ($p<0.001$, $p=0.018$, and $p=0.030$, respectively), a pattern that became stronger when adjusting for age ($p<0.001$ for all). No significant monthly variation of SHBG, LH or albumin levels was found. Mean 25(OH)D, testosterone, and FAI levels for each month are shown in figure 1. Levels of 25(OH)D, testosterone, and FAI follow a similar seasonal pattern with a nadir in spring and peak levels during late summer. In detail, peak levels of 25(OH)D, testosterone, and FAI were observed in August (23.4 µg/l , 18.7 nmol/l, and 49.7, respectively). The nadir of 25(OH)D, testosterone, and FAI levels was reached in March (12.2 µg/l, 15.9 nmol/l, and 40.8, respectively). Relative seasonal changes of 25(OH)D, testosterone, and FAI levels are shown in figure 2. 25(OH)D levels showed the largest seasonal variation with a 64% difference between peak and nadir. From the highest to the lowest monthly mean, we found a difference of 16% and 18% for testosterone and FAI levels, respectively.

Discussion

We are the first to present detailed data on the association of 25(OH)D levels with androgens in a large cohort of men. We found independent associations of 25(OH)D levels with testosterone, FAI, and SHBG levels. Moreover, there was a similar seasonal variation of 25(OH)D, testosterone, and FAI, with peaks in late summer and nadirs in spring.

Our results of an association of vitamin D status and androgen levels in men are in line with reports from animal studies. VDR knockout mice present with significant gonadal insufficiency, with decreased sperm count and motility, and histological abnormalities of the

testis [3]. High LH and FSH levels in these male VDR knockout mice indicate the presence of hypergonadotropic hypogonadism [3]. Underlying pathophysiological mechanisms remain to be explored in detail but reduced gonadal aromatase activity was observed in these VDR knockout mice. Given the important role of estrogens for testicular function including steroidogenesis it was speculated that reduced aromatase activity might partially explain the gonadal abnormalities in mice lacking the VDR. Interestingly, male vitamin D-deficient rats, although capable of reproducing, have a remarkably decreased overall fertility [18]. Data on testosterone synthesis are lacking but 1,25(OH)2D treatment upregulates various genes in Sertoli cells, which are relevant for spermatogenesis [19]. In addition, the presence of VDR receptor in human sperm suggest a role of vitamin D for the capacitation and survival of sperm [20]. Hence, experimental data indicate a significant role of vitamin D for reproductive processes but the detailed mechanisms and to what extent they are related to testosterone deserves further investigation.

Our finding of an independent association of 25(OH)D and androgens is supported by the observation that seasonal variations in 25(OH)D levels are paralleled by similar changes in androgen levels. Seasonal variations in testosterone levels have also been reported by others [12, 21]. In line with data from the LURIC study, Andersson et al. described a seasonal pattern of testosterone levels with a nadir in spring and peak levels during late summer [12]. With reference to our results on seasonal variations of 25(OH)D and androgens it is of interest that a peak in conception rate during summer leading to a maximum in birth rate in spring has been observed in northern countries [22]. Athletic performance also peaks in late summer and declines during winter [23]. This might partly be explained by an increased training during summer, but this seasonal variation of athletic performance remains significant after controlling for time spent exercising [23]. We are aware that testosterone is not a main determinant of both fertility and athletic performance and that there exist various testosterone

independent pathways by which vitamin D may influence muscle strength and reproductive processes [24]. We are, however, of the opinion that a possible relationship of 25(OH)D levels with androgens, fertility and athletic performance is a reasonable hypothesis which might explain seasonal changes in these health related issues.

Our results on 25(OH)D and androgens might have important clinical implications because both vitamin D deficiency and hypogonadism have been associated with adverse health consequences including an increased mortality [8, 25]. In this context, a meta-analysis of 18 randomized controlled trials found that vitamin D supplementation significantly reduced mortality [26]. In addition, it is well known that the age-related decline in testicular function, which leads to decreased levels of testosterone and FAI, has detrimental effects including impaired sexual [27] and cognitive function [28], loss of bone mineral density, muscle mass and strength, as well as metabolic disturbances [29]. Testosterone supplementation improves bone mass, body composition, and muscle strength [29] but might also increase the risk for prostate cancer and benign prostate hyperplasia [30]. In view of the clinical significance of testosterone levels we want to stress that further studies are needed to evaluate the impact of vitamin D supplementation on androgens in men.

The data provided are restricted to older men referred for coronary angiography and may therefore not be generalizable to patients at lower cardiovascular risk, population based cohorts and younger age groups. Given that patients with atherosclerosis frequently suffer from osteoporosis which is usually linked to a poor vitamin D status, our study population might be particularly prone to vitamin D deficiency related disorders. Apart from this, we want to stress that the cross-sectional design of our study precludes any conclusions about causality of the observed associations between 25(OH)D levels and androgens. Hence, high 25(OH)D levels might lead to higher testosterone and FAI levels or vice versa. Moreover, the R^2 for the linear models is relatively small. In addition, there was no consistent dose response relationship in all of our analyses on the association of androgen status and 25(OH)D. The missing seasonal variation of LH might argue against functional variations of androgen levels

throughout the year. In addition, albumin levels remained materially unchanged throughout all seasons contradicting a significant influence of varying protein levels on our results. Another drawback of our study is the fact that we did not measure vitamin D binding protein. Finally, we investigated a cohort of Caucasian men living in Germany, and results might not relate to other ethnicities or men living in geographical areas with less extreme seasonal variations in 25(OH)D levels.

In summary, we have presented evidence for an association of 25(OHD levels with androgens in a large cohort of older men. This notion is further supported by similar seasonal variations of 25(OH)D and serum androgens levels. We suggest that these results may provide rationale for randomized controlled trials evaluating the impact of vitamin D supplementation on testosterone levels per se and hypogonadism associated adverse health consequences.

Disclosure

Conflict of interest

The authors declare no conflict of interest.

Funding

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Tables

Table 1: Characteristics of subjects according to vitamin D status.

	Severe vitamin D deficiency (n=457)		Mild vitamin D deficiency (n=991)		Vitamin D insufficiency (n=589)		Vitamin D sufficiency (n=262)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p-value
25(OH)D (µg/l)[a]	7.2	1.9	14.9	2.8	24.2	2.8	36.3	6.3	<0.001
Age	63.1	11.4	62.0	10.6	61.1	10.5	60.2	9.6	0.007
BMI (kg/m^2)	27.6	4.3	27.8	3.7	27.4	3.6	27.3	3.4	0.170
Testosterone (nmol/l)	16.3	7.3	17.3	6.9	17.7	7.3	18.4	6.6	<0.001
FAI	40.9	21.0	44.9	20.5	48.4	30.4	49.3	19.8	<0.001
SHBG (nmol/l)	44.5	21.1	42.7	20.1	40.9	19.3	40.1	18.3	0.021
LH (IU/l)	5.6	4.7	4.9	4.3	4.8	4.2	4.1	2.5	<0.001
Diabetes (%)	38.7		35.2		26.3		19.5		<0.001
Arterial hypertension (%)	72.2		72.7		69.6		67.9		0.112
Active smokers (%)	27.1		20.7		20.2		19.1		0.016
Wine consumption (%)									

Never	42.4		32.7		28.1		23.7		<0.001
Rarely	31.9		36.4		37.1		36.6		0.277
Sometimes	24.8		30.8		34.5		39.7		<0.001
Often	0.9		0.2		0.0		0.0		0.029
CAD (%)	88.5		83.0		82.0		85.0		0.024
Beta-blocker use (%)	65.4		64.1		64.9		64.9		0.978

BMI=body mass index, FAI=free androgen index, IU=international unit, CAD=coronary artery disease.

[a]To convert serum 25(OH)D levels to nanomoles per liter, multiply by 2.5.

Table 2: Linear regression analyses using 25(OH)D as explanatory variable and testosterone, SHBG, and FAI as dependent variables.

	Beta	p-value	R^2
FAI	0.163	<0.001	0.026
FAI[a]	0.136	<0.001	0.217
FAI[b]	0.129	<0.001	0.226
Testosterone	0.114	<0.001	0.014
Testosterone[a]	0.100	<0.001	0.082
Testosterone[b]	0.105	<0.001	0.091
SHBG	-0.071	0.001	0.005
SHBG[a]	-0.058	0.005	0.152
SHBG[b]	-0.046	0.020	0.176

FAI=free androgen index, SHBG

[a] Adjusted for age and BMI

[b] Adjusted for age, BMI, wine consumption, smoking, beta blocker use, statin use, and diabetes

Figure legends

Figure 1: Seasonal variation of mean 25(OH)D, testosterone, and FAI levels and p-value for the distribution. Significant differences from the peak value (↓); §, $p<0.05$; #, $p<0.001$.

Figure 1a: Mean 25(OH)D levels (**µg/l**) by month ($p<0.001$). To convert serum 25(OH)D levels in nanomoles per litre, multiply by 2.5.

Figure 1b: Mean testosterone levels (nmol/l) by month ($p=0.018$).

Figure 1c: Mean FAI (free androgen index) levels by month ($p=0.003$).

Figure 2: Relative seasonal variation of 25(OH)D, testosterone, and FAI levels

Figure 2a: Seasonal variation of testosterone, FAI, and 25(OH)D levels. Changes are shown relative to the mean values (17.3 nmol/l, 45.3, and 17.6 µg/l for testosterone, FAI, and 25(OH)D, respectively).

Figure 2b: Seasonal variation of testosterone, FAI, and 25(OH)D levels. Changes are shown relative to the mean values normalized to the standard deviation.

Figures

Figure 1a

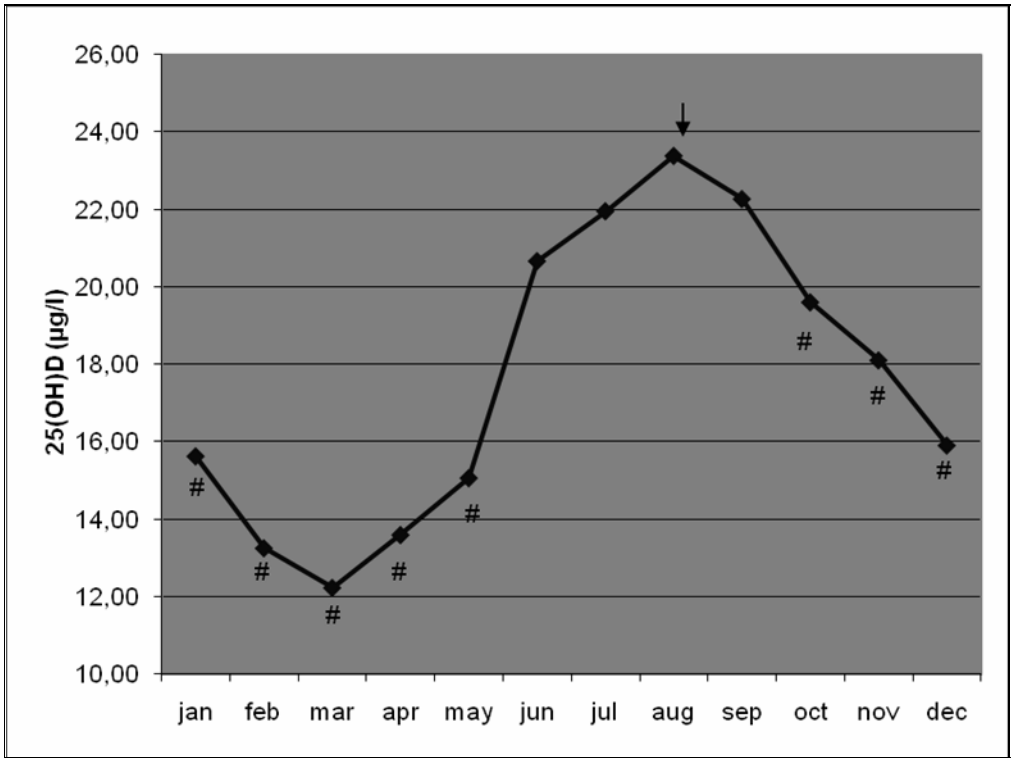

Figure 1b

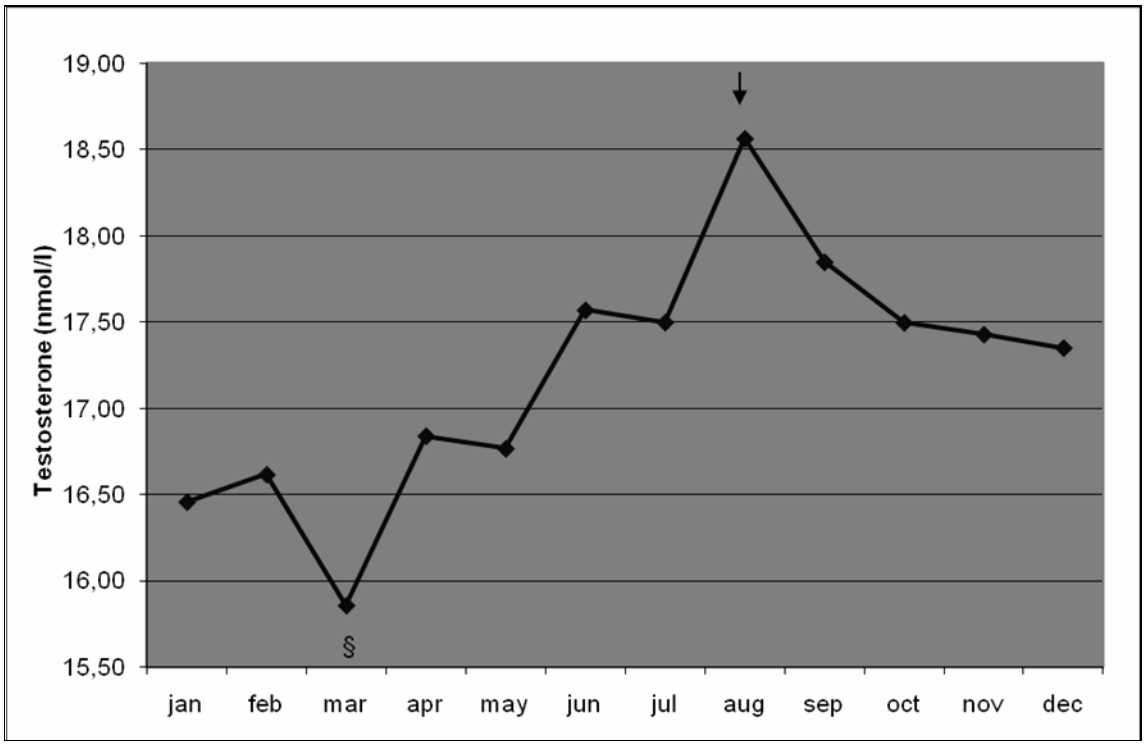
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Figure 1c

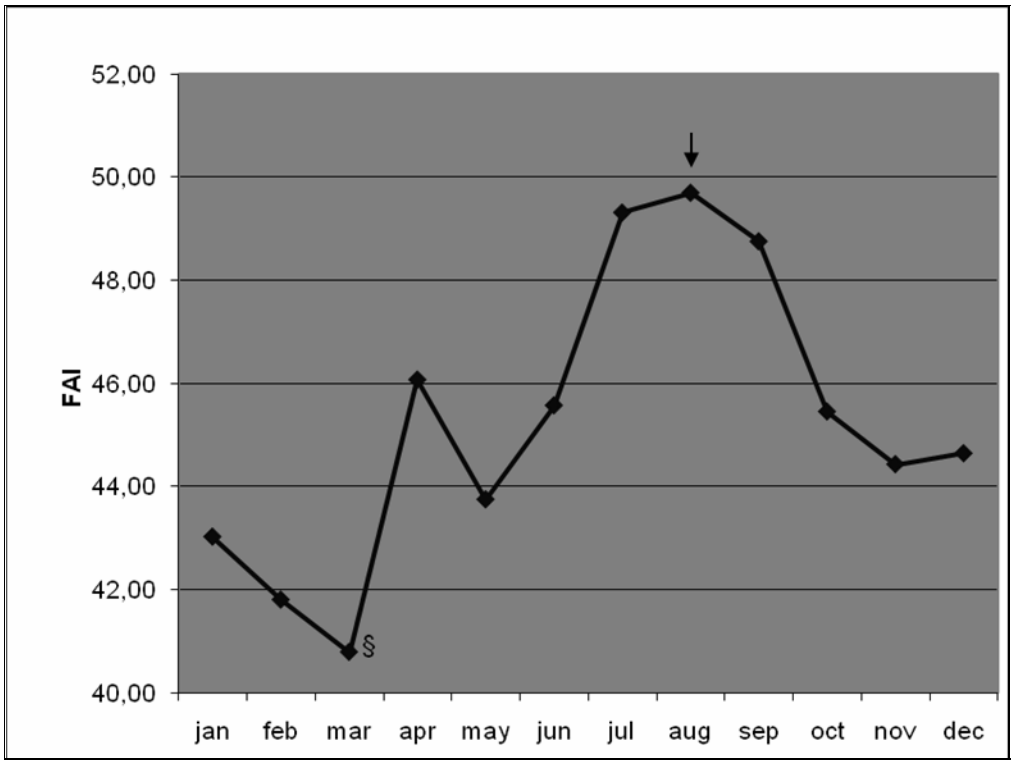
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Figure 2a

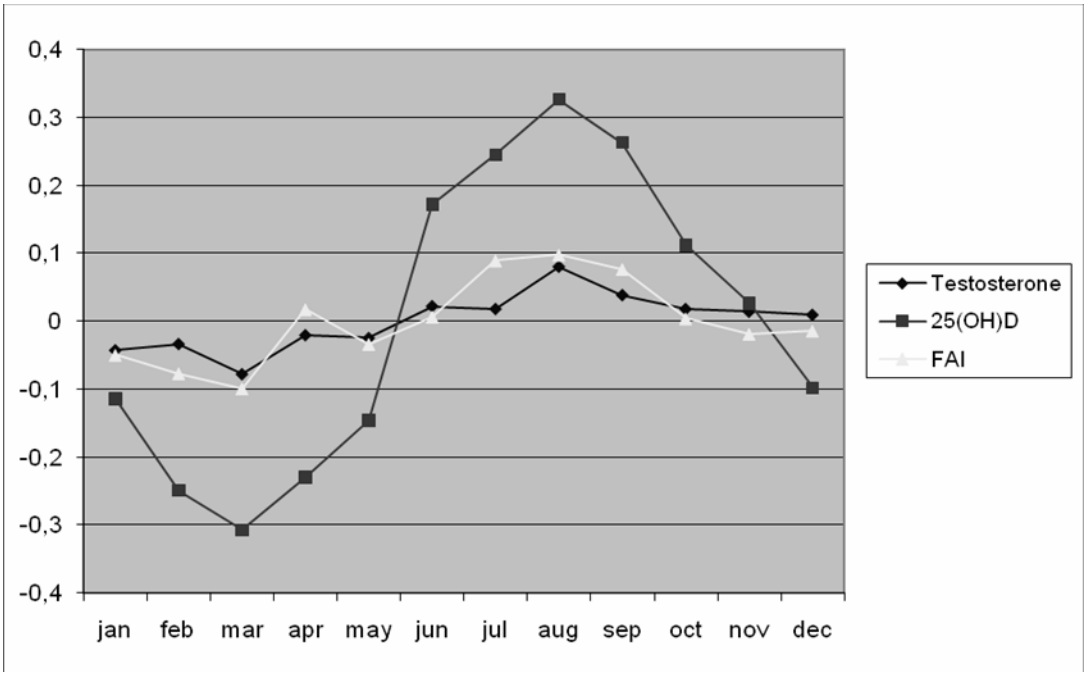
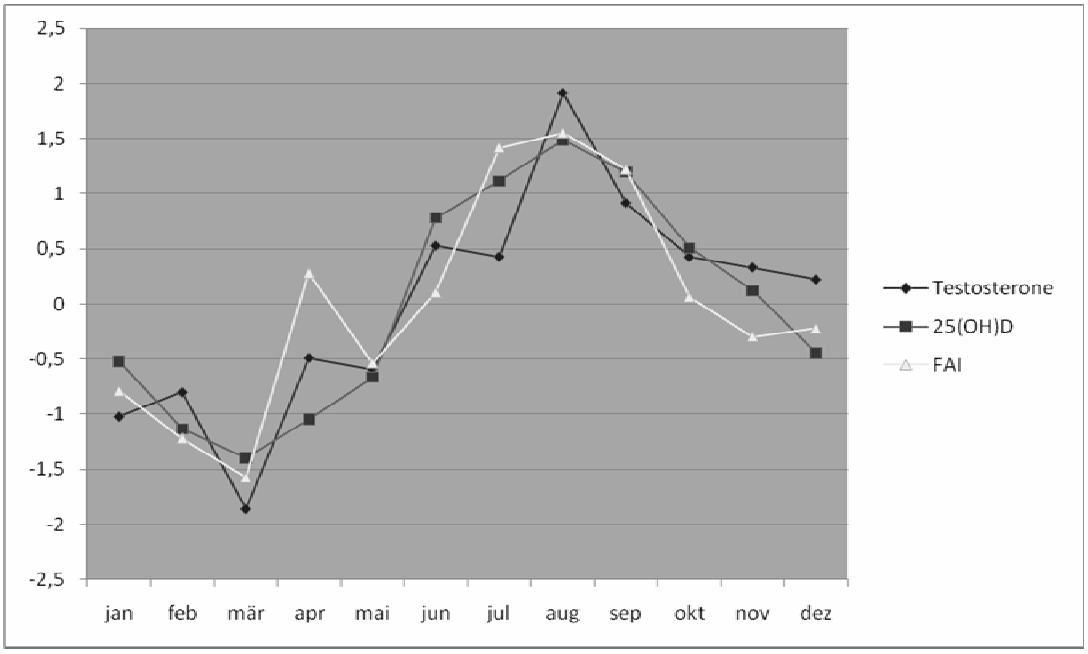


Figure 2b



References

1 Holick MF. (2007) Vitamin D deficiency. *N Engl J Med*, **357**, 266-281.

2 Bouillon R, Carmeliet G, Verlinden L, et al. (2008) Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr Rev*, **29**, 726-776.

3 Kinuta K, Tanaka H, Moriwake T, et al. (2000) Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. *Endocrinology*, **141**, 1317-1324.

4 Pilz S, Dobnig H, Winklhofer-Roob B, et al. (2008) Low serum levels of 25-hydroxyvitamin D predict fatal cancer in patients referred to coronary angiography. *Cancer Epidemiol Biomarkers Prev*, **17**, 1228-1233.

5 Wehr E, Pilz S, Schweighofer N, et al. (2009) Association of hypovitaminosis D with metabolic disturbances in polycystic ovary syndrome. *Eur J Endocrinol*, **161**, 575-582.

6 Pilz S, Tomaschitz A, Ritz E, et al. (2009) Vitamin D status and arterial hypertension: a systematic review. *Nat Rev Cardiol*, **6**, 621-630.

7 Pilz S, März W, Wellnitz B, et al. (2008) Association of vitamin D deficiency with heart failure and sudden cardiac death in a large cross-sectional study of patients referred for coronary angiography. *J Clin Endocrinol Metab*, **93**, 3927-3935.

8 Pilz S, Dobnig H, Nijpels G, et al. (2009) Vitamin D and mortality in older men and women. *Clin Endocrinol (Oxf)*, **71**, 666-672.

9 Aquila S, Guido C, Perrotta I, et al. (2008) Human sperm anatomy: ultrastructural localization of 1alpha,25-dihydroxyvitamin D receptor and its possible role in the human male gamete. *J Anat*, **213**, 555-564.

10 Habib FK, Maddy SQ, Gelly KJ. (1990) Characterisation of receptors for 1,25-dihydroxyvitamin D3 in the human testis. *J Steroid Biochem*, **35**, 195-199.

11 Johnson JA, Grande JP, Roche PC, et al. (1996) Immunohistochemical detection and distribution of the 1,25-dihydroxyvitamin D3 receptor in rat reproductive tissues. *Histochem Cell Biol*, **105**, 7-15.

12 Andersson AM, Carlsen E, Petersen JH, et al. (2003) Variation in levels of serum inhibin B, testosterone, estradiol, luteinizing hormone, follicle-stimulating hormone, and sex hormone-binding globulin in monthly samples from healthy men during a 17-month period: possible effects of seasons. *J Clin Endocrinol Metab*, **88**, 932-937.

13 Winkelmann BR, März W, Boehm BO, et al. (2001) Rationale and design of the LURIC study--a resource for functional genomics, pharmacogenomics and long-term prognosis of cardiovascular disease. *Pharmacogenomics*, **2**, S1-73.

14 Harman SM, Metter EJ, Tobin JD, et al. (2001) Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. *J Clin Endocrinol Metab*, **86**, 724-731.

15 Mosekilde L. (2005) Vitamin D and the elderly. *Clin Endocrinol (Oxf)*, **62**, 265-281.

16 Travison TG, Araujo AB, Kupelian V, et al. (2007) The relative contributions of aging, health, and lifestyle factors to serum testosterone decline in men. *J Clin Endocrinol Metab*, **92**, 549-555.

17 Dobs AS, Schrott H, Davidson MH, et al. (2000) Effects of high-dose simvastatin on adrenal and gonadal steroidogenesis in men with hypercholesterolemia. *Metabolism*, **49**, 1234-1238.

18 Kwiecinski GG, Petrie GI, DeLuca HF. (1989) Vitamin D is necessary for reproductive functions of the male rat. *J Nutr*, **119**, 741-744.

19 Hirai T, Tsujimura A, Ueda T, et al. (2009) Effect of 1,25-dihydroxyvitamin d on testicular morphology and gene expression in experimental cryptorchid mouse: testis specific cDNA microarray analysis and potential implication in male infertility. *J Urol*, **181**, 1487-1492.

20 Corbett ST, Hill O, Nangia AK. (2006) Vitamin D receptor found in human sperm. *Urology*, **68**, 1345-1349.

21 Svartberg J, Jorde R, Sundsfjord J, et al. (2003) Seasonal variation of testosterone and waist to hip ratio in men: the Tromsø study. *J Clin Endocrinol Metab*, **88**, 3099-3104.

22 Rojansky N, Brzezinski A, Schenker JG (1992) Seasonality in human reproduction: an update. *Hum Reprod*, **7**, 735-745.

23 Cannell JJ, Hollis BW, Sorenson MB, et al. (2009) Athletic performance and vitamin D. *Med Sci Sports Exerc*, **41**, 1102-1110.

24 Visser M, Deeg DJ, Lips P; Longitudinal Aging Study Amsterdam. (2003) Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. *J Clin Endocrinol Metab*, **88**, 5766-5772.

25 Laughlin GA, Barrett-Connor E, Bergstrom J. (2008) Low serum testosterone and mortality in older men. *J Clin Endocrinol Metab*, **93**, 68-75.

26 Autier P, Gandini S. (2007) Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. *Arch Intern Med*, **167**, 1730-1737.

27 Kwan M, Greenleaf WJ, Mann J, et al. (1983) The nature of androgen action on male sexuality: a combined laboratory-self-report study on hypogonadal men. *J Clin Endocrinol Metab*, **57**, 557-562.

28 Moffat SD, Zonderman AB, Metter EJ, et al. (2002) Longitudinal assessment of serum free testosterone concentration predicts memory performance and cognitive status in elderly men. *J Clin Endocrinol Metab*, **87**, 5001-5007.

29 Mauras N, Hayes V, Welch S, et al. (1998) Testosterone deficiency in young men: marked alterations in whole body protein kinetics, strength, and adiposity. *J Clin Endocrinol Metab*, **83**, 1886-1892.

30 Gormley GJ, Stoner E, Bruskewitz RC, et al. (1992) The effect of finasteride in men with benign prostatic hyperplasia. The Finasteride Study Group. *N Engl J Med*, **327**, 1185-1191.