

# Are serum levels of vitamin D associated with semen quality? Results from a cross-sectional study in young healthy men

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**Objective:** To examine the association between low serum vitamin D concentration and estimates of male reproductive function.

**Design:** Cross-sectional study.

**Setting:** University hospital.

**Patient(s):** From a Danish pregnancy cohort established in 1984–1987, 347 sons were selected for a study conducted in 2005–2006.

**Intervention(s):** Semen parameters and reproductive hormones were related to vitamin D concentrations in 307 men.

**Main Outcome Measure(s):** Semen characteristics and reproductive hormones.

**Result(s):** A high vitamin D level was unexpectedly associated with lower crude median total sperm count and percentage of normal morphology sperm and a high level of crude median sex hormone-binding globulin and FSH. After adjustment, the associations attenuated to nonsignificant associations, except for sex hormone-binding globulin. Additionally, adjusted free androgen index was lower at higher vitamin D levels, and men with high vitamin D had 11% (95% confidence interval, 1%–20%) lower free androgen index compared with men with low vitamin D.

**Conclusion(s):** These results do not indicate that low vitamin D is a risk factor for poor semen quality in a population of young healthy men, but we may not have enough men with low vitamin D levels to detect an effect. New studies should include a larger proportion of vitamin D-deficient men. (Fertil Steril® 2010; ■: ■–■. ©2010 by American Society for Reproductive Medicine.)

**Key Words:** Reproductive hormones, risk factor, sperm count, 25-hydroxyvitamin D

Low vitamin D has been associated with increased risk of osteoporosis (1), impaired cognitive performance (2), infectious diseases (3), autoimmune diseases (4), common cancers (5), and all-cause mortality (6). Deleterious mutations in the vitamin D receptor (VDR) gene are linked to the rare monogenetic disease 1,23-dihydroxyvitamin D resistant rickets, and several VDR polymorphisms have been associated with a wide range of diseases (e.g., osteoarthritis, prostate cancer, nephrolithiasis, and diabetes) (7). The main sources of vitamin D are sunlight, food, and supplementation.

Studies in rodents have shown lower fertility rates among vitamin D-deficient males in comparison with vitamin D-replete males (8, 9), and vitamin D supplementation improved testicular function in vitamin D-deficient rats independently of calcium levels, suggesting

a direct and reversible detrimental effect of vitamin D deficiency (10). Moreover, VDR null mutant mice had decreased sperm count and motility, histologic changes of the testis, and elevated levels of LH and FSH (11). Estrogen supplementation increased sperm count and motility, decreased levels of LH and FSH, and reversed the histologic changes of the VDR null mutant mice, suggesting that estrogen deficiency induced by VDR ablation may be the cause of abnormal spermatogenesis in vitamin D receptor null mutant mice (11). Another study found an indirect potential effect of vitamin D deficiency via hypocalcemia in rats (9). A few epidemiologic studies reported only a weak, if any, association between calcium levels and semen quality (12, 13).

Vitamin D receptors are present in human testis (14) and human sperm cells (15, 16). Recently, vitamin D-metabolizing enzymes have been found in human testis, the ejaculatory tract, and mature sperm cells, suggesting that vitamin D is important for spermatogenesis and maturation of sperm cells (17). In one cross-sectional study, vitamin D was positively associated with T and free androgen index (FAI) and inversely associated with sex hormone-binding globulin (SHBG) (18).

Whether low vitamin D is associated with poor semen quality in human males has, to the best of our knowledge, not been reported. If such an association exists it may be added to the potential causes of

Received August 13, 2010; revised October 13, 2010; accepted November 2, 2010.

C.H.R.-H. has nothing to disclose. U.K.M. has nothing to disclose. J.P.B. has nothing to disclose. J.O. has nothing to disclose. A.M.T. has nothing to disclose.

Supported by grant no. 271-07-0051 from the Danish Medical Research Council, Denmark.

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the large differences in semen quality between different populations and secular or seasonal time periods (19). We investigate the association between serum vitamin D concentrations, semen quality, and levels of reproductive hormones in a population-based cross-sectional study of young adult men.

## MATERIALS AND METHODS

### Population

We analyzed data from a study originally designed to examine the association between prenatal tobacco smoke and adult semen quality, and participants were selected according to levels of maternal smoking during pregnancy (20). The population of young men aged 18–21 years comprised sons of mothers recruited to the “Healthy Habits for Two” cohort in Aalborg and Odense, Denmark during their pregnancy from April 1984 to April 1987, and 11,980 women with singleton pregnancies (more than 80% of all invited) participated (21). Sons who were alive and living in Denmark by December 2004 were identified in the Danish Civil Registration System ( $n = 5,109$ ).

The participants received modest economic compensation (Dkr 500) for responding to the follow-up questionnaire and delivering semen and blood samples. Men with severe handicaps, metabolic disease, or psychiatric disorders were not invited. The regional ethics committee approved the study (registration nos. 20040174 and 20090203), and participation was conditional on written informed consent. The authors have no conflict of interest to report.

### Data Collection

Data were collected from February 2005 through January 2006. The young men were instructed to provide a semen sample by masturbating into a plastic container at home after at least 48 hours of sexual abstinence and to keep the container close to the body during transportation to avoid cooling. One trained medical laboratory technician performed all the initial semen analyses. Blood samples were taken between 7:25 AM and 7:15 PM, with no requirement for fasting. Participants completed questionnaires on their reproductive experience, medical and lifestyle factors, time and date of the preceding ejaculation, and spillage during semen sample collection.

### Semen Analysis

Semen analyses were performed blinded to any prenatal conditions. Semen volume was estimated by its weight ( $1 \text{ g} = 1 \text{ mL}$ ). Sperm motility and sperm concentration were assessed as described in the World Health Organization’s *WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction* (22). Examination of 82% of the samples was initiated within 1 hour of ejaculation, and examination of 99.7% of the samples was initiated within 2 hours. Sperm morphology was determined using the Tygerberg strict criteria (23). The laboratory took part in the European Society for Human Reproduction and Embryology external quality control program, and all control tests were in agreement with results obtained by expert examiners within the external quality control program.

### Analysis of Serum Samples

After centrifugation, serum was stored at  $-80^\circ\text{C}$  for a maximum of 16½ months (reproductive hormones) and 3 years (vitamin D) until analyzed. Serum samples for vitamin D were analyzed by isotope dilution liquid chromatography–tandem mass spectrometry by a method adapted from Maunsell et al. (24, 25), and samples for T,  $E_2$ , FSH, and LH were analyzed by Avida Centaur (Bayer Healthcare, Leverkusen, Germany). The SHBG concentrations were determined using IMMULITE (DPC, Koege, Denmark), and inhibin B was measured by a commercially available enzyme-linked immunosorbent assay (Oxford Bio-Innovation, Oxford, United Kingdom) according to the manufacturer’s instructions. The blood samples were analyzed as single measurements in random order.

The inhibin B samples were analyzed at the Laboratory of Reproductive Biology, University Hospital of Copenhagen, Denmark, and all other samples were analyzed at the Department of Clinical Chemistry, Aarhus University Hospital, Denmark.

## Statistical Analysis

Three exposure groups were formed by dividing participants according to serum concentrations of vitamin D: low vitamin D, 8–62 nmol/L ( $n = 103$ ); medium vitamin D, 63–93 nmol/L ( $n = 103$ ); and high vitamin D, 94–227 nmol/L ( $n = 101$ ). Serum vitamin D concentrations below 50 nmol/L are considered to be suboptimal: 25–50 nmol/L constitutes vitamin D insufficiency, and a level below 25 nmol/L indicates regular vitamin D deficiency.

Outcome variables included all measured semen parameters and reproductive hormones and, additionally, the calculated level of FAI [(T/SHBG)  $\times$  100]. For each of the outcome variables we performed multiple linear regressions, using the three strata of vitamin D levels as a categorically coded explanatory variable, with low vitamin D as the referent. When testing for trend, the vitamin D variable was entered as a continuous variable, using low vitamin D as a starting point.

Data on the outcome variables were transformed by use of the natural logarithm before multiple linear regressions were performed, and the estimates were back-transformed to the original scale and presented as relative differences with 95% confidence intervals (CIs) of the adjusted geometric means. To obtain a more symmetric distribution of residuals, we also cubic-root transformed all the outcome variables (with the exception of percentages of motile sperm, which were logit transformed, and inhibin B, which were not transformed). The risk estimates were, however, essentially the same as in analyses based on the natural logarithm. Results were adjusted for season (summer/winter), history of diseases of the reproductive organs (cryptorchidism, hypospadias, varicocele, hydrocele, orchitis, and chlamydia combined into one variable, present or not present), smoking of the young men (yes/no), maternal smoking during pregnancy (0, 1–9, 10+ cigarettes per day), and maternal alcohol during pregnancy (yes, no). The semen outcome variables were additionally adjusted for abstinence time ( $\leq 48$  hours, 49 hours–5 days,  $> 5$  days) and spillage during collection of the sample (yes, no). Data on participants who reported spillage during masturbation ( $n = 80$ ) were excluded from all statistical analyses on semen volume and total sperm count. The results on motility were additionally adjusted for time from ejaculation to analysis (continuous, in minutes). The blood sample outcome variables were adjusted for season, diseases of the reproductive organs, sons’ and maternal smoking, maternal alcohol intake, and time of day of blood sampling (6:00 AM to 8:59 AM, 9:00 AM to 12:00 PM, after 12:00 PM).

## RESULTS

A total of 716 men were invited to take part in the study, and 347 (48.5%) gave consent and participated. Information on vitamin D concentrations was available for 307 men (42.9%), who constitute the study group for this study. Characteristics of the 307 participants according to tertiles of serum concentrations of vitamin D are presented in Table 1. Among the 103 men in the low vitamin D group, respectively, 19 (18%) and 73 (71%) had vitamin D serum concentrations below 25 nmol/L and 50 nmol/L, indicating suboptimal vitamin D levels. Among all 307 participants, 163 (53%) had vitamin D serum concentrations below 80 nmol/L.

There was a trend of lower crude median total sperm count and percentage of normal morphology sperm with higher vitamin D, and men with high vitamin D had approximately 31% lower total sperm count and 23% lower normal morphology compared with men with low vitamin D (Table 2). Additionally, the crude median sperm concentration and semen volume tended to be lower at higher vitamin D levels, but these trends were not statistically significant, as were differences between groups: 31% lower sperm concentration and 21% lower semen volume among men with high vitamin D in comparison with men with low vitamin D. However, after transformation and adjustment for potential confounders, the associations between vitamin D and semen parameters attenuated and were not statistically significant (Table 2). For percentage normal morphology, it was the transformation of data, rather than confounder adjustment, that attenuated the association found in the multivariate analysis relative to the crude analysis on untransformed data.

TABLE 1

Characteristics of 307 Danish young men by tertiles of serum concentrations of 25-hydroxyvitamin D.

Characteristic	Low vitamin D (8–62 nmol/L) (n = 103)	Medium vitamin D (63–93 nmol/L) (n = 103)	High vitamin D (94–227 nmol/L) (n = 101)
Person-related characteristics of sons			
Body mass index (kg/m <sup>2</sup> ), mean (SD)	23.4 (4.1)	24.0 (4.1)	23.1 (2.8)
Birth weight (g), mean (SD)	3,482 (494)	3,440 (487)	3,608 (552)
History of diseases in reproductive organs <sup>a</sup>	16 (16)	16 (16)	22 (22)
Recent fever	13 (13)	14 (14)	23 (23)
Cigarette smoking, daily	39 (38)	43 (42)	31 (31)
Alcohol consumption, weekly	47 (47)	46 (45)	47 (47)
Person-related characteristics of mothers			
Mother smoking during pregnancy	76 (74)	78 (76)	67 (66)
Mother drinking alcohol during pregnancy	88 (85)	82 (80)	82 (81)
Semen- and blood-related characteristics			
Season			
October–March	61 (59)	27 (26)	31 (31)
April–September	42 (41)	76 (74)	70 (69)
Duration of abstinence			
≤ 48 h	29 (28)	35 (34)	38 (38)
49 h–5 d	66 (64)	64 (62)	58 (57)
> 5 d	8 (8)	4 (4)	4 (5)
Minutes from ejaculation to start of analysis, mean (SD)	52 (25)	54 (18)	51 (15)
Spillage at semen sampling	20 (19)	26 (25)	34 (34)
Blood collected between 6:00 AM and 12:00 PM	32 (31)	29 (28)	34 (34)

Note: Values are number (percentage) unless otherwise noted.

<sup>a</sup> History of diseases in the reproductive organs includes cryptorchidism, hypospadias, varicocele, hydrocele, orchiditis, and chlamydial infection combined into one variable (present or not present).

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Crude median SHBG and FSH were higher at higher vitamin D levels (Table 2). For SHBG, this trend remained after transformation and adjustment for potential confounders, and men with high vitamin D had 16% (95% CI, 4%–28%) higher levels than men with low vitamin D. Adjusted FAI was lower at higher vitamin D levels, and men in the high vitamin D group had 11% (95% CI, 1%–20%) lower FAI compared with men in the low vitamin D group. Crude, unadjusted FAI was not associated with vitamin D, and it was primarily the use of transformed data that strengthened the association. Testosterone, E<sub>2</sub>, LH, and inhibin B were not significantly associated with vitamin D levels (Table 2).

We repeated the analyses, also adjusting for the young men's body mass index (<18.5, 18.5–24.9, >24.9 kg/m<sup>2</sup>), but that did not change the results (data not shown). We also omitted the covariate "season" from the multivariate analyses, but that also produced results similar to those presented (data not shown).

## DISCUSSION

Contrary to our hypothesis, higher vitamin D levels were associated with lower crude median total sperm count and percentage of normal-morphology sperm and higher SHBG and FSH. All trends except for SHBG were attenuated after transformation and adjustment for potential confounders. Adjusted FAI was lower at higher vitamin D levels. We found no associations between vitamin D and T, E<sub>2</sub>, LH, or inhibin B. Although we found no support for our hypothesis, it should be taken into consideration that we had only few men with vitamin D deficiency in the study.

It is possible that the reported association observed in rodents does not apply to humans or only reflects severe vitamin D deficiency. Although we used objective biomarkers for both exposure and semen quality, our exposure data were not taken at the time of spermatogenesis, which is up to 72 days before sampling of fertility markers. The participation rate for the original study was 48.5%, which is rather high for semen studies but far from high enough to eliminate the risk of selection bias. To cause bias away from the null in the observed direction (crude results), selection has to be related to both semen quality and vitamin D concentrations, which we consider unlikely. The population of sons was young, most had no reproductive experience, and they were not aware of the study hypothesis, which reduces the risk of selection bias. We compared participants and nonparticipants who completed a small questionnaire on health (n = 82) and found no difference in the proportion of men with diseases of the reproductive organs (including cryptorchidism and hypospadias).

We controlled for season, abstinence time, diseases of the reproductive organs, time of day of blood sampling, maternal smoking during pregnancy, and a number of other potential confounders. Residual confounding or confounding by other unknown factors cannot be ruled out. Adjustment for body mass index in a subanalysis did not change the results.

Vitamin D is mainly synthesized in the skin under the influence of ultraviolet sunlight, resulting in the highest concentrations in the summer (26). The seasonal fluctuation of the semen parameters with the poorest outcome in summer (27–30) may reflect a longer photoperiod in summer rather than heat (29), although not all find an association between season and semen quality (31–34).

TABLE 2

Semen and hormonal characteristics for 307 Danish young men stratified by tertiles of serum concentrations of 25-hydroxyvitamin D.

Parameter	Median (p25, p75)			Adjusted <sup>a</sup> percentage difference (95% CI)		Test for trend <sup>b</sup>	
	Low vitamin D (8–62 nmol/L) (n = 103)	Medium vitamin D (63–93 nmol/L) (n = 103)	High vitamin D (94–227 nmol/L) (n = 101)	Medium vitamin D	High vitamin D	Crude median P value	Adjusted geometric mean P value
Sperm concentration ( $\times 10^6$ /mL)	49 (22, 95)	46 (21, 85)	34 (17, 76)	47 (–10, 140)	6 (–34, 73)	.08	.53
Semen volume (mL)	3.3 (2.3, 4.1)	2.8 (2.2, 3.8)	2.6 (2.0, 3.9)	–5 (–18, 10)	–5 (–19, 11)	.07	.62
Sperm total count ( $\times 10^6$ )	137 (62, 357)	127 (60, 271)	94 (32, 206)	22 (–29, 110)	–21 (–55, 38)	.05	.17
Percent normal morphology sperm	6.5 (3.5, 10.0)	5.0 (3.0, 9.0)	5.0 (2.0, 8.0)	–18 (–47, 27)	–29 (–54, 9)	.05	.22
Percent motile sperm	69 (61, 75)	71 (63, 78)	72 (61, 78)	–9 (–25, 12)	0 (–18, 22)	.06	.89
Testosterone (nmol/L)	16.1 (13.6, 19.5)	16.1 (12.7, 19.7)	17.0 (13.6, 20.6)	–2 (–11, 7)	3 (–6, 13)	.31	.76
FAI	66.0 (53.9, 83.5)	65.7 (49.3, 84.2)	63.2 (50.3, 80.7)	–7 (–17, 3)	–11 (–20, –1)	.25	.006
Estradiol (nmol/L)	0.10 (0.08, 0.13)	0.10 (0.07, 0.12)	0.10 (0.08, 0.13)	–8 (–19, 4)	–1 (–12, 12)	.37	.51
SHBG (nmol/L)	24.4 (20.1, 31.9)	24.7 (17.7, 34.0)	27.0 (20.3, 34.7)	5 (–5, 17)	16 (4, 28)	.02	.003
FSH (IU/L)	2.8 (2.2, 4.0)	2.9 (2.1, 4.2)	3.2 (2.3, 4.4)	–8 (–29, 19)	14 (–12, 47)	.05	.16
LH (IU/L)	4.2 (3.1, 5.6)	4.4 (3.5, 5.3)	4.2 (3.3, 5.3)	7 (–14, 35)	13 (–10, 41)	.93	.44
Inhibin B (ng/mL)	155 (118, 188)	152 (118, 185)	147 (110, 177)	3 (–9, 16)	–1 (–12, 12)	.26	.26

Note: P = percentile.

<sup>a</sup> Back-transformed differences in geometric means from the low vitamin D reference group adjusted for season (summer/winter), history of diseases of the reproductive organs (present, not present), smoking (yes/no), maternal smoking during pregnancy (0, 1–9, 10+ cigarettes per day), and maternal alcohol during pregnancy (yes, no). The semen outcome variables were additionally adjusted for abstinence time ( $\leq 48$  hours, 49 hours–5 days, >5 days), and spillage during collection of the sample (yes, no). Participants who reported spillage during collection (n = 80) were excluded from all statistical analyses of semen volume and total sperm count. The results for motility were also adjusted for minutes from ejaculation to analysis (continuous). The blood sample outcome variables were additionally adjusted for time of day of blood sampling (6:00 AM to 8:59 AM, 9:00 AM to 12:00 PM, or later than 12:00 PM). Sampling between October and March, no history of diseases of the reproductive organs, no smoking, no maternal smoking during pregnancy, no maternal alcohol intake during pregnancy, 48 hours–5 days of abstinence, no spillage, and blood sampling between 9:00 AM and 12:00 PM were chosen as the reference categories.

<sup>b</sup> Trends on vitamin D concentrations (continuous data) were tested by Spearman's rank correlation test (untransformed data) and multiple linear regression (transformed to obtain a normal distribution of residuals), with low vitamin D as the starting point.

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One study found that vitamin D was positively associated with T and FAI and inversely associated with SHBG among 2,299 German men (18). Our results do not support these findings: we found vitamin D to be inversely associated with FAI and positively with SHBG, but two different study populations were used. The German men were in their 60s and referred for coronary angiography, whereas the men in our study were young and mainly healthy. In the German study, only 11% had a vitamin D serum concentration  $\geq 75$  nmol/L, compared with 53% among our participants (i.e., a larger proportion of the participants in our study were vitamin D sufficient). In addition, the measurements of vitamin D in the two studies may not be comparable (35), limiting the ability to compare proportions of vitamin D-sufficient men in different studies. Among

our participants, nearly 40% were sampled between October and March, and 6% had vitamin D serum concentrations below 25 nmol/L (vitamin D deficiency), whereas 18% had levels lower than 50 nmol/L (vitamin D insufficiency). Among 392 Danish blood donors with a mean age of 43 years, of whom 48% were sampled during winter, 9% and 31% had concentrations below 25 nmol/L and 50 nmol/L, respectively (36).

In conclusion, our results do not indicate that low vitamin D is a risk factor for poor semen quality in a population of young healthy men. New studies should provide vitamin D levels for the time period of spermatogenesis and include a larger proportion of vitamin D-deficient men. Case-control studies of oligospermic and normospermic men could also add valuable knowledge.

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