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® 2011;95:1000–4. ©2011 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-dihydroxivitamin 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

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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 Odensen, 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 g = 1 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°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) × 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 (≤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.