

REVIEW

Vitamin D, PCOS and androgens in men: a systematic review

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Abstract

Background: Accumulating evidence from animal and human studies suggests that vitamin D is involved in many functions of the reproductive system in both genders.

Aim: The aim of this review was to provide an overview on the effects of vitamin D on polycystic ovary syndrome (PCOS) in women and androgen metabolism in men.

Methods: We performed a systematic literature search in PubMed for relevant English language publications published from January 2012 until September 2017.

Results and discussion: The vitamin D receptor and vitamin D-metabolizing enzymes are found in reproductive tissues of women and men. In women, vitamin D status has been associated with several features of PCOS. In detail, cross-sectional data suggest a regulatory role of vitamin D in PCOS-related aspects such as ovulatory dysfunction, insulin resistance as well as hyperandrogenism. Moreover, results from randomized controlled trials (RCTs) suggest that vitamin D supplementation may be beneficial for metabolic, endocrine and fertility aspects in PCOS. In men, vitamin D status has been associated with androgen levels and hypogonadism. Further, there is some evidence for a favorable effect of vitamin D supplementation on testosterone concentrations, although others failed to show a significant effect on testosterone levels.

Conclusion: In summary, vitamin D deficiency is associated with adverse fertility outcomes including PCOS and hypogonadism, but the evidence is insufficient to establish causality. High-quality RCTs are needed to further evaluate the effects of vitamin D supplementation in PCOS women as well as on androgen levels in men.

Key Words

- ▶ vitamin D
- ▶ polycystic ovary syndrome
- ▶ testosterone
- ▶ randomized controlled trial
- ▶ systematic review

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Introduction

Considering the high prevalence of an insufficient vitamin D status in many populations as well as the potential link between low vitamin D status and adverse health outcomes (1), vitamin D deficiency is classified as an important public health problem (2). Vitamin D has been well known for its role in maintaining calcium homeostasis and promoting bone mineralization (2). Beyond the established relationship between vitamin D deficiency and musculoskeletal diseases, evidence is accumulating that vitamin D deficiency is also a risk marker for insulin resistance (3), cardiovascular disease (4), infectious and autoimmune diseases (1),

cancer (5), increased mortality (2) as well as decreased fertility (6).

Infertility is a complex disorder with significant medical, psychosocial and economic aspects (7), which affects about 15% of couples (8). One major cause of female infertility is polycystic ovary syndrome (PCOS). Women affected by PCOS frequently suffer from oligo- or anovulation as well as from obesity and insulin resistance (9, 10). Thus, PCOS women are considered to be at high risk for metabolic diseases such as type 2 diabetes mellitus (DM) and metabolic syndrome as well as for cardiovascular diseases (11). Interestingly, there is

accumulating evidence showing an association of vitamin D status with pathogenesis, signs and symptoms of PCOS (3, 12).

Besides the above mentioned important causes of female infertility, population-based studies found that in 30–40% of infertile couples, the underlying cause is the male factor (13). One major aspect regarding male fertility is the complex interaction between pituitary gland and testis. Of note, testosterone is not only an important regulator of spermatogenesis, but decreasing testosterone levels might also contribute to many adverse aspects of male aging (14). It is therefore worth mentioning that similar to the above stated association of low vitamin D status with adverse health outcomes, several lines of evidence suggest that low testosterone levels are associated with adverse events including higher cardiovascular and all-cause mortality (15, 16, 17, 18). In this context, it should be noted that androgen and vitamin D metabolism seem to be associated (19, 20).

Considering the potential link between vitamin D and human fertility, we performed this systematic review summarizing current literature on vitamin D, PCOS and androgen metabolism in men.

Methods

We performed a systematic literature search in PubMed for relevant English language publications published from January 2012 until September 2017. We used the following search terms: ‘vitamin D’ and ‘polycystic ovary syndrome’, ‘vitamin D’ and ‘PCOS’, ‘vitamin D’ and ‘testosterone’, ‘vitamin D’ and ‘hypogonadism’. In addition, we also used the search terms ‘25-hydroxyvitamin D (25(OH) D)’, ‘1,25-dihydroxyvitamin D’ and ‘calcitriol’ instead of vitamin D. We also used listed references from selected articles to expand the search.

We start with a short overview on the background and potential mechanisms explaining the relationship of vitamin D and PCOS. Next, we will discuss observational studies on vitamin D and PCOS, and we will then highlight results of intervention studies investigating vitamin D effects on PCOS. In the second part of the review, we will provide some information on possible mechanisms explaining the link of vitamin D with androgen metabolism in men. Further, we give an overview on observational studies on vitamin D and androgen levels. Finally, we will again focus on intervention studies analyzing vitamin D effects on androgen levels in men. The role of vitamin D in spermatogenesis and other aspects of female fertility

(such as endometriosis or *in vitro* fertilization) as well as earlier articles on vitamin D and fertility have been comprehensively reviewed elsewhere (6, 21, 22) and are not included here.

Vitamin D and PCOS

Background and mechanisms

The vitamin D receptor (VDR) can be found across several tissues within the female reproductive system. VDR mRNA is expressed in ovarian, deciduae, placenta and endometrium cells (6, 23, 24). Furthermore, calcitriol (1,25[OH]₂ vitamin D) directly leads to the production of estrogen and progesterone in cultured human ovary and placenta cells (24, 25), thus possibly potentiating granulosa cell luteinization and leading to an improved endometrial environment (26). Further, there exists a relationship between vitamin D and markers of ovarian reserve, especially anti-Müllerian hormone (AMH). AMH is a glycoprotein that plays a fundamental role in the regression of Müllerian ducts in male embryos, while in its absence, Müllerian ducts develop into female inner reproductive organs. In females, it is secreted by granulosa cells of small follicles in the ovary and is considered an ideal tool for the assessment of the ovarian follicular reserve during a woman’s reproductive life (27). Interestingly, AMH shows seasonal variations correlating with seasonal changes in serum 25(OH)D (28). Moreover, it has been shown that cholecalciferol (vitamin D3) supplementation might prevent seasonal AMH changes (28).

In PCOS, ovarian physiology is closely connected to the commonly observed metabolic disturbances of the syndrome (3, 26). Furthermore, vitamin D deficiency appears to be related to several of these metabolic features of the PCOS phenotype (3, 26, 29). Several studies showed a higher prevalence of insufficient vitamin D status (defined as 25[OH]D concentrations <75 nmol/L) in PCOS women when compared to control women matched for age and body mass index (BMI) (29, 30, 31).

Vitamin D status may play an important role in the development of insulin resistance in PCOS, while vitamin D supplementation might improve insulin sensitivity as well as serum androgen concentrations (32, 33, 34). In a cross-sectional study by Wehr and coworkers (3), 25(OH)D concentrations were an independent predictor for homeostasis model assessment-insulin resistance (HOMA-IR) and BMI. Serum concentrations of 25(OH)D were negatively correlated with adiposity markers, including BMI and central obesity (35, 36). In this context,

it should be noted that results derived from a Mendelian Randomization study suggest that low 25(OH)D levels are a consequence of obesity, but it is unlikely that vitamin D deficiency causes obesity (37). Interestingly, the observed association between vitamin D deficiency and insulin resistance does not seem to be confounded by obesity, since insulin resistance appears to be related to severe vitamin D deficiency regardless of BMI or waist-to-hip ratio in PCOS women (38). Therefore, alternative mechanisms to explain the association between vitamin D deficiency and insulin resistance were proposed: As the transcription of the insulin gene is activated by 1,25(OH)₂D and the vitamin D-responsive element is present in the promoter region of the human insulin gene (39, 40), vitamin D may stimulate the expression of insulin receptors, thus improving insulin responsiveness for glucose transport (6). Furthermore, vitamin D is involved in calcium

homeostasis, which is important for intracellular insulin-mediated procedures in insulin-responsive tissues (41).

Polymorphisms of the VDR could be involved in the pathogenesis of PCOS or increase the susceptibility for its development (42, 43). This mechanism is supported by the fact that 1,25[OH]₂D directly or indirectly controls several hundred genes, including some with relevance for glucose homeostasis (2, 41). Some VDR-related polymorphisms, i.e. iApa-I, Taq-I, Cdx2, Bsm-I and Fok-I, have been related to metabolic features in PCOS (29, 42). Another single-nucleotide polymorphism (rs757343) was associated with the severity of PCOS symptoms, but not with the risk to develop the syndrome itself (44).

Considering the proposed beneficial effects of vitamin D on several metabolic disturbances as well as the above mentioned link between vitamin D, granulosa cell luteinization and improved endometrial

Table 1 Summary of clinical observational studies investigating the association of vitamin D and clinical as well as biochemical parameters in PCOS women.

Author	Subjects	Age	Metabolic parameters	Endocrine parameters	Clinical parameters	Adjustment
Ott <i>et al.</i> (52)	91 PCOS women	29 (19–38) years			25(OH)D deficiency significant predictive parameter for follicle development and pregnancy	Age, BMI, LH:FSH ratio
Kim <i>et al.</i> (49)	38 PCOS women	34.1 ± 4.6 years	No correlation	No correlation	No correlation	
Sahin <i>et al.</i> (51)	50 PCOS women	27.3 ± 0.5 years	No correlation with HOMA-IR			
Pal <i>et al.</i> (47)	540 PCOS women	28.1 ± 4.0 years	Inverse association with BMI, fasting insulin, HOMA-IR Positive association with fasting glucose-insulin ratio	Positive association with SHBG	OR for live birth 0.58 (0.35–0.92) if 25(OH)D level was <75 nmol/L, 25(OH)D status independent predictor of live birth and ovulation after ovulation induction	Age, BMI, ovulatory dysfunction, ethnicity, baseline FAI, cycles to ovulation, hirsutism, creatinine, history of smoking, use of clomiphene citrate and metformin (to predict live birth)
Joham <i>et al.</i> (48)	42 PCOS women	28.6 ± 5.0	Association with IR			Body fat percentage
Mishra <i>et al.</i> (46)	44 PCOS women	Data not available	Negative correlation with HOMA-IR and insulin, positive correlation with HDL-cholesterol	No correlation with testosterone		
Kumar <i>et al.</i> (50)	100 PCOS women	28.6 ± 6.3 years	No correlation	No correlation	No correlation	

Data are given as mean ± s.d. or median (IQR) unless otherwise stated.

25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; FAI, free androgen index; FSH, follicle-stimulating hormone; HDL cholesterol, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; IR, insulin resistance; LH, luteinizing hormone; OR, odds ratio; PCOS, polycystic ovary syndrome; SHBG, sex hormone-binding globulin.

Table 2 Summary of clinical intervention studies investigating the effects of vitamin D treatment on clinical and biochemical parameters in PCOS women.

Author	Study design	Subjects	Age	25(OH)D levels (baseline)	Primary outcome	Study duration	Study medication	25(OH)D levels (study end)	Effects on primary endpoints
Raza-Khan <i>et al.</i> (56)	RCT	28 PCOS women	28.2 ± 5.2 years (vitamin D group) vs 28.7 ± 5.6 years (placebo group)	49.80 ± 23.64 nmol/L (vitamin D group) vs 55.41 ± 17.12 nmol/L (placebo group)	QUICKI	12 weeks	12,000 IU cholecalciferol daily vs placebo	168.13 ± 71.44 nmol/L (vitamin D group) vs 56.04 ± 17.52 nmol/L (placebo group)	No significant effect
Irani <i>et al.</i> (58)	Uncontrolled intervention study	22 PCOS women, 45 control women	27.0 ± 0.9 years (PCOS vitamin D group) vs 31.3 ± 3.1 years (PCOS no intervention group)	34.27 ± 2.40 nmol/L (PCOS intervention group)	sRAGE and AMH serum levels	8 weeks	50,000 IU 1,25 dihydroxyvitamin D3 weekly vs no intervention	131.29 ± 21.39 nmol/L (PCOS intervention group)	Significant increase in sRAGE and decrease in AMH in the PCOS group
Asadi <i>et al.</i> (62)	RCT	101 PCOS women	26.29 ± 3.80 years vs 26.09 ± 3.46 years	31.35 ± 2.72 nmol/L (vitamin D group) vs 30.53 ± 14.62 nmol/L (placebo group)	Endometrial thickness	12 weeks	300,000 IU cholecalciferol at study start vs placebo	Not available	Significantly higher endometrial thickness
Irani <i>et al.</i> (59)	RCT	68 PCOS women	30.5 ± 1.0 years (vitamin D group) vs 29.6 ± 1.7 years (placebo group)	40.7 ± 2.2 nmol/L (vitamin D group) vs 42.4 ± 4.2 nmol/L (placebo group)	Serum TGF-β1, sENG, lipid profile, testosterone, DHEA-S, insulin resistance	8 weeks	50,000 IU cholecalciferol weekly vs placebo	107.8 ± 6.0 nmol/L (vitamin D group) vs 43.4 ± 4.7 nmol/L (placebo group)	Significant decrease in time between menstrual periods, FG-score, triglycerides, TGF-β1 to sENG ratio
Garg <i>et al.</i> (57)	RCT	36 PCOS women	22.0 ± 4.61 years (vitamin D group) vs 22.8 ± 4.56 years (placebo group)	19.22 ± 15.1 nmol/L (vitamin D group) vs 16.97 ± 6.14 nmol/L (placebo group)	Whole-body insulin sensitivity, Matsuda index, HOMA-IR, insulinogenic index	6 months	120,000 IU cholecalciferol monthly plus metformin 1500 mg daily vs placebo plus metformin 1500 mg daily	78.62 ± 34.64 nmol/L (vitamin D group) vs 16.72 ± 5.77 nmol/L (placebo group)	No significant effect
Razavi <i>et al.</i> (61)	RCT	60 PCOS women	Not shown	35.9 ± 7.2 nmol/L (intervention group) vs 36.4 ± 13.7 nmol/L (placebo group)	Several endocrine biomarkers	8 weeks	200 IU of vitamin D, 90 µg of vitamin K2, 500 mg calcium twice daily vs placebo	49.2 ± 7.7 nmol/L (intervention group) vs 36.2 ± 15.0 nmol/L (placebo group)	Significant decrease in free testosterone and DHEA-S Significant increase in total antioxidant capacity and plasma malondialdehyde

Maktabi <i>et al.</i> (55)	RCT	70 PCOS women	22.0 ± 1.6 years (vitamin D group) vs 23.1 ± 3.3 years (placebo group)	31.9 ± 11.2 nmol/L (vitamin D group) vs 36.2 ± 12.7 nmol/L (placebo group)	Markers of insulin resistance and androgens	12 weeks	50,000 IU cholecalciferol every 2 weeks vs placebo	68.6 ± 24.5 nmol/L (vitamin D group) vs 35.9 ± 13.0 nmol/L (placebo group)	Significant decrease in FPG, insulin, HOMA-IR, HOMA-B, hs-CRP, plasma malondialdehyde Significant increase in QUICKI (non-significant after adjustment) Significant decrease in VEGF concentrations
Irani <i>et al.</i> (60)	RCT	68 PCOS women	30.5 ± 1.0 years (vitamin D group) vs 29.6 ± 1.7 years (placebo group)	40.7 ± 2.2 nmol/L (vitamin D group) vs 42.4 ± 4.5 nmol/L (placebo group)	VEGF	8 weeks	50,000 IU cholecalciferol weekly vs placebo	107.8 ± 6.0 nmol/L (vitamin D group) vs 43.4 ± 4.7 nmol/L (placebo group)	
Foroozanfard <i>et al.</i> (54)	RCT	90 PCOS women	Not shown	33.7 ± 7.7 nmol/L (4000 IU group) vs 34.9 ± 11.5 nmol/L (1000 IU group) vs 34.9 ± 8.7 nmol/L (placebo group)	Various hormonal parameters	12 weeks	4000 IU cholecalciferol vs 1000 IU cholecalciferol vs placebo	60.7 ± 9.2 nmol/L (4000 IU group) vs 51.7 ± 15.5 nmol/L (1000 IU group) vs 35.2 ± 9.0 nmol/L (placebo group)	Significant decrease in FPG, serum insulin, HOMA-IR, triglycerides, VLDL, total cholesterol, LDL-cholesterol, total cholesterol/HDL-cholesterol ratio
Jamilian <i>et al.</i> (53)	RCT	90 PCOS women	28 ± 5 years (4000 IU group) vs 26 ± 5 years (1000 IU group) vs 25 ± 5 years (placebo group)	31.4 ± 6.7 nmol/L (4000 IU group) vs 31.4 ± 8.5 nmol/L (1000 IU group) vs 32.2 ± 6.0 nmol/L (placebo group)	Various hormonal parameters	12 weeks	4000 IU cholecalciferol daily vs 1000 IU cholecalciferol daily vs placebo	61.4 ± 8.2 nmol/L (4000 IU group) vs 46.2 ± 12.2 nmol/L (1000 IU group) vs 32.7 ± 6.2 nmol/L (placebo group)	Significant decrease in FPG, serum insulin, HOMA-IR, TT, FAI, and hirsutism Significant increase in SHBG and total antioxidant capacity

Data are given as mean ± s.d. or median (IQR) unless otherwise stated.

25(OH)D, 25-hydroxyvitamin D; AMH, antimüllerian hormone; BMI, body mass index; DHEA-S, dehydroepiandrosterone-sulfate; FAI, free androgen index; FG-score, Ferriman-Gallwey-score; FPG, fasting plasma glucose; FSH, follicle-stimulating hormone; HDL-cholesterol, high density lipoprotein-cholesterol; HOMA-B, homeostasis model assessment-estimated beta cell function; HOMA-IR, homeostasis model assessment-insulin resistance; hs-CRP, high-sensitive C-reactive protein; IR, insulin resistance; IU, international units; LDL cholesterol, low-density lipoprotein-cholesterol; LH, luteinizing hormone; OR, odds ratio; PCOS, polycystic ovary syndrome; QUICKI, quantitative insulin sensitivity check index; RCT, randomized controlled trial; sENG, soluble endoglin; SHBG, sex hormone-binding globulin; sRAGE, soluble receptor for advanced glycation end products; TGF-β1, transforming growth factor-β1; TT, total testosterone; VEGF, vascular endothelial growth factor; VLDL, very low density lipoprotein.

environment (26), it has been speculated that vitamin D supplementation may also improve ovulatory dysfunction and thereby fertility in PCOS patients.

Observational studies

In the following paragraph, we will discuss observational studies evaluating the association of vitamin D and PCOS. So far, several observational studies investigated a possible association of vitamin D status with biochemical and clinical parameters in PCOS patients (summarized in Table 1). These studies have yielded, however, inconsistent results.

Since insulin resistance is a prominent feature in both PCOS pathophysiology and phenotype (45), several authors aimed to investigate possible associations of vitamin D status with parameters of glycemic control. A study conducted by Mishra and coworkers (46) found negative correlations of 25(OH)D with insulin concentrations and HOMA-IR. This finding was confirmed by another study (47), which furthermore reported a positive association of 25(OH)D with fasting glucose:insulin ratio, but no significant correlation with fasting glucose. In another study by Joham and coworkers (48), hyperinsulinemic–euglycemic clamps were performed in 42 PCOS patients and 34 control women. In the PCOS group, 25(OH)D concentrations were significantly associated with insulin resistance measured by hyperinsulinemic–euglycemic clamp independently of body fat percentage, whereas no significant association could be shown within the control group. However, other studies were unable to find a significant association of 25(OH)D with various parameters of insulin resistance or glycemic control in PCOS patients (49, 50, 51).

Observational studies investigating possible associations of vitamin D with endocrine parameters in PCOS women have yielded more unanimous results: Apart from one study reporting a positive correlation with sex hormone-binding globulin (SHBG) (47), other authors were unable to detect a significant correlation of vitamin D with e.g. total testosterone (TT) and free testosterone (FT) (46, 49, 50), free androgen index (FAI) (49), LH and FSH (50).

In addition to observational studies aiming to evaluate the relationship of vitamin D with endocrine and metabolic parameters, some authors investigated the association of vitamin D status with outcome parameters of fertility treatment. A retrospective analysis of the pregnancy in PCOS I (PPCOS I) randomized controlled trial (RCT), evaluating the effect of 50 mg of clomiphene citrate, metformin 1000 mg twice daily or a combination of both in PCOS women, revealed a 44% reduced likelihood

for live birth if 25(OH)D concentrations were <75 nmol/L (OR 0.58 (0.35–0.92)) (47). Progressive improvement in the odds for live birth was found at thresholds of ≥ 95 , ≥ 100 and ≥ 112.5 nmol/L, respectively. Vitamin D status was also an independent predictor of live birth and ovulation after ovulation induction. Similarly, in a prospective cohort study in 91 anovulatory PCOS women who underwent clomiphene citrate stimulation, vitamin D deficiency (defined as a 25[OH]D concentration <25 nmol/L) was a significant predictive parameter for both follicle development and pregnancy (52).

Intervention studies

Within recent years, several interventional trials aimed to investigate potential effects of vitamin D supplementation on metabolic and clinical parameters associated with fertility in PCOS patients. These studies showed a broad variation regarding study design, population, study medication as well as study outcome (summarized in Table 2).

Jamilian and coworkers (53) reported significant decreases in fasting plasma glucose, serum insulin and HOMA-IR in 90 PCOS women after supplementation of 4000 international units (IU) of cholecalciferol daily for 12 weeks in comparison to 1000 IU daily or placebo. A similar RCT conducted by Foroozanfar and coworkers (54) found a significant effect of 4000 IU cholecalciferol daily over 12 weeks on fasting plasma glucose, serum insulin, HOMA-IR, VLDL, total cholesterol and total cholesterol to HDL-cholesterol ratio compared to groups receiving either 1000 IU of cholecalciferol daily or placebo ($n=90$ PCOS patients, 30 patients per group). Another RCT (55), which investigated the effect of 50000 IU cholecalciferol every 2 weeks over 12 weeks vs placebo in 70 PCOS patients, found significant decreases in fasting plasma glucose, HOMA-IR, homeostasis model assessment-estimated beta cell function (HOMA-B), high-sensitive C-reactive protein and plasma malondialdehyde in the intervention group, whereas no significant change was observed in the placebo group. An increase in quantitative insulin sensitivity check index (QUICKI) in the intervention group did not remain significant after controlling for baseline values of biochemical parameters, age and baseline BMI.

Nevertheless, other interventional studies were unable to find significant effects of vitamin D supplementation on metabolic parameters: Raza-Khan and coworkers (56) aimed to investigate the effect of 12,000 IU cholecalciferol daily vs placebo on insulin sensitivity (QUICKI) over 12 weeks ($n=28$ PCOS patients). However, there was no significant effect on primary or secondary outcome

measures (including HOMA-IR, fasting glucose and fasting insulin). Similar results were found in a study by Garg and coworkers (57), where 36 PCOS women either received either 120,000IU of cholecalciferol monthly or placebo over 6 months. Of note, patients in both groups also received 1500mg of metformin daily. Several parameters of insulin resistance and insulin sensitivity did not significantly change after vitamin D supplementation, including area under the curve glucose and insulin, insulin:glucose ratio, HOMA-IR, Matsuda index and insulinogenic index. Of note, both of the above mentioned trials (56, 57) included a comparatively small patient collective, therefore, potentially limiting the proposed implications derived from these studies.

As elevated AMH levels in PCOS reflect abnormal ovarian folliculogenesis, Irani and coworkers (58) designed an intervention study to investigate the effects of vitamin D supplementation on AMH. In this trial in 22 PCOS patients and 45 healthy control women with vitamin D deficiency ($25[\text{OH}]\text{D} < 50\text{nmol/L}$), the participants received 50,000IU of $1,25(\text{OH})_2\text{D}$ over 8 weeks (16 PCOS women and 35 controls) or no treatment. Vitamin D supplementation significantly decreased AMH in PCOS women, but not in controls. In the same study, vitamin D supplementation also significantly increased the level of the soluble receptor for advanced glycation end products (sRAGE) in the PCOS group. Advanced glycation end products might be involved in the pathogenesis of PCOS, and their production is accelerated by DM, insulin resistance and aging, while their binding to sRAGE prevents adverse intracellular effects. Therefore, the authors hypothesized that a rise in sRAGE might improve follicular growth and insulin resistance (58). In another RCT by Irani and coworkers (59) in 68 vitamin D-deficient PCOS women who were randomized to either 50,000IU of cholecalciferol vs placebo over 8 weeks, a significant decrease in transforming growth factor- $\beta 1$ (TFG- $\beta 1$) bioavailability, correlating with a possible improvement in several adverse parameters associated with PCOS was reported. Another finding of that RCT was a significant reduction of interval between menstrual periods and Ferriman–Gallwey scores in the treatment vs the placebo group. A *post hoc* analysis derived from the same trial (60) found a significant decrease in vascular endothelial growth factor (VEGF) levels correlating with a decrease in triglyceride levels after vitamin D supplementation.

Some studies also reported a significant effect of vitamin D supplementation on androgen concentrations: The already mentioned RCT by Jamilian and coworkers (53) found a significant reduction in TT, FAI and hirsutism

after cholecalciferol supplementation. In a study by Razavi and coworkers (61), supplementation of 200IU cholecalciferol, 90 μg of vitamin K2 and 500mg of calcium twice daily (vs placebo) led to a significant decrease in FT and dehydroepiandrosterone-sulfate (DHEA-S) in 60 PCOS women.

In addition, Asadi and coworkers (62) aimed to investigate the effects of vitamin D supplementation on the success rate of intra uterine insemination in 110 infertile PCOS women (300,000IU cholecalciferol once vs placebo, 2-month interval between vitamin D administration and insemination). While there was no treatment effect on pregnancy outcomes, the endometrial thickness was significantly higher in the vitamin D when compared to the placebo group.

Meta-analyses

A recent meta-analysis (63) including 16 studies involving 315 participants on vitamin D effects in PCOS women found that vitamin D supplementation significantly decreased PTH and triglyceride levels but had no effect on HOMA-IR, QUICKI, LDL, DHEAS, FT and TT levels. Another meta-analysis including 9 studies involving 502 PCOS women found that vitamin D significantly improved follicular development in PCOS women (64). Further, a positive effect on menstrual cycle regulation as observed when metformin plus vitamin D was compared with metformin alone. Further, Azadi-Yazdi and coworkers (65) conducted a meta-analysis including 6 clinical trials involving 184 participants investigating vitamin D effects on androgen levels in PCOS women. The authors found a significant effect on TT levels, whereas no effect was found on FT or SHBG levels.

In conclusion, recent interventional trials investigating the effects of vitamin D supplementation in PCOS have yielded inconsistent results. This may be, at least in part, explained by the differences in study population sizes, study design (e.g. study durations of 8 weeks vs 12 weeks vs 6 months) or by different dosing regimens (e.g. daily administration vs weekly administration vs one-time administration at study start). Additionally, some studies recruited PCOS patients regardless of their vitamin D status while others included only vitamin D-deficient participants (in most cases defined as $25[\text{OH}]\text{D} < 50\text{nmol/L}$), further limiting the comparability and generalizability of currently available interventional trials. Thus, further RCTs in clearly defined large patient collectives are needed to potentially draw conclusions for clinical practice.

Table 3 Summary of clinical observational studies investigating the association of vitamin D and androgen levels in men.

Author/study	Subjects	Age	Hypogonadism	Androgens	Other endocrine parameters	Adjustment
Wehr <i>et al.</i> /LURIC study (3)	2299 men at high cardiovascular risk	62 ± 11 years	OR 2.47 (1.55–3.93) for men with 25(OH)D <25 nmol/L compared to >75 nmol/L	↑TT, ↑FAI	↓SHBG	Age, BMI, wine consumption, smoking, beta-blocker use, statin use and diabetes
Heijboer <i>et al.</i> (86)	183 men (101 men with chronic heart failure; 76 male nursing home residents; 43 overweight non-Western immigrants)	20–86 years (range)	na	↑TT		Unadjusted
Jorde <i>et al.</i> the Tromsø study (84)	893 men	60.6 ± 9.8 years	na	↑TT, no association with FT		Age, BMI, season, presence of cardiovascular disease and diabetes, and physical activity
Chin <i>et al.</i> (87)	382 Chinese and Malay men	≥20 years	na	No independent association with TT	↑SHBG	Age, ethnicity, BMI
Wulaningsih <i>et al.</i> NHANES III (94)	1412 men	≥20 years	na	No independent association with TT or FT		Age, race/ethnicity, % body fat, diabetes, cigarette smoking, alcohol intake, vigorous physical activity, and serum levels of 25(OH)D, calcium, and creatinine
Anic <i>et al.</i> NHANES III and NHANES 2001–2004 (90)	1315 men (NHANES III) and 318 men (NHANES 2001–2004)	≥20 years	na	↑TT	↑SHBG	Adjusting for age, race/ethnicity, body fat percentage, and smoking
Blomberg Jensen <i>et al.</i> the Copenhagen-Bone-Gonadal Study (91)	1427 infertile men	34.1 (31–38) years	na	Higher FT in men with 25(OH)D levels <25 nmol/L compared to men with 25(OH)D levels >75 nmol/L	Lower SHBG and T/estradiol ratios and higher estradiol in men with 25(OH)D levels <25 nmol/L compared to men with 25(OH)D levels >75 nmol/L	Age, BMI, smoking, season

Lerchbaum <i>et al.</i> (95)	225 men	35 (30–41) years	U-shaped association of vitamin D status and risk of hypogonadism. Significantly increased risk of hypogonadism in men within the highest 25(OH)D quintile (>102 nmol/L) compared to men in quintile 4 (reference, 82–102 nmol/L). (OR 9.21, 2.27–37.35, $P=0.002$)	No independent association with TT and FT	No independent association with SHBG	Adjusted for age, BMI, ethnic background, study site
Wang <i>et al.</i> (85)	2854 Chinese men	53.0 ± 13.5 years	Increasing quartiles of 25(OH)D were associated with significantly decreased odds ratios of hypogonadism. OR 1.50 (95% CI, 1.14, 1.97) for men in the lowest compared to men in highest 25(OH)D quartile	↑TT	↑Estradiol	Age, residence area, economic status, smoking, BMI, homeostasis model assessment-insulin resistance, DM and systolic pressure
Tak <i>et al.</i> (88)	652 Korean men	56.7 ± 7.9 years	Vitamin D deficiency (<50 nmol/L) was associated with an increased risk of TT (odds ratio (OR): 2.65; 95% confidence interval (CI): 1.21–5.78, $P=0.014$) and FT deficiency (OR: 1.44; 95% CI: 1.01–2.06 $P=0.048$)	↑TT, ↑FT		TT: body fat, WC, BMI, FPG, DM and dyslipidemia FT: adjusted age, total muscle mass, smooth muscle mass, TC, DM, dyslipidemia and alcohol use Hypogonadism: adjusting for age, season, body mass index, body composition, chronic disease, smoking, and alcohol use
Rafiq <i>et al.</i> (89)	Older Dutch individuals ($n=643$)	65–89 years (range)	No independent association of 25(OH)D levels with hypogonadism	↑TT and bioavailable testosterone		Adjusted for age, BMI, alcohol consumption, smoking status, season of blood collection, number of chronic diseases, serum creatinine and physical performance

(Continued)

Table 3 Continued.

Author/study	Subjects	Age	Hypogonadism	Androgens	Other endocrine parameters	Adjustment
Zhao <i>et al.</i> (92)	3016 older men	62.1 ± 10.2 years	no independent association of 25(OH)D levels with hypogonadism	↑FT, no independent association with TT	↓SHBG	Adjusting for age, race/ethnicity, and study site, BMI, smoking, education, intentional physical exercise, and self-reported health status, diabetes, systolic blood pressure, use of antihypertensive medications, eGFR, total cholesterol, HDL cholesterol, use of lipid lowering medication usage, and hsCRP
Lee <i>et al.</i> the European Male Aging Study, (83)	3369 community-dwelling men	Aged 40–79 years (range)	Independent association of 25(OH)D <50 nmol/L with compensated (relative risk ratio (RRR)=1.52, 1.03, 2.25) <i>P</i> =0.03) and secondary hypogonadism (RRR=1.16, 1.00–1.34, <i>P</i> =0.05) compared to men with sufficient vitamin D status (>75 nmol/L)	No independent association with TT or FT		Adjusted for age, centre, BMI, smoking, alcohol consumption, physical activity, physical function, heart conditions, hypertension, DM, and depression
Nimptsch <i>et al.</i> (82)	1362 male participants of the Health Professionals Follow-up Study	65.8 ± 7.4 years	Comparing participants in the highest vs lowest quintile of 25(OH) vitamin D had a significantly decreased relative risk of hypogonadism of 0.50 (95% CI 0.31–0.93; <i>P</i> -trend=0.01)	Independent association with TT and FT		Age (at blood collection), batch, time of blood collection, season, BMI at blood collection, smoking status, geographical region, physical activity
Hammoud <i>et al.</i> (93)	170 healthy men	29.0 ± 8.5 years	na	No independent association with TT or FT		Age, BMI, season, alcohol intake and smoking

Data are given as mean ± s.d. or median (IQR) unless otherwise stated. 25(OH)D, 25 hydroxyvitamin D; BMI, body mass index; DM, type 2 diabetes mellitus; eGFR, estimated glomerular filtration rate; FAI, free androgen index; FPG, fasting plasma glucose; FT, free testosterone; HDL, high density lipoprotein, hsCRP, high sensitive C-reactive protein; OR, odds ratio; SHBG, sex hormone binding globulin; TT, total testosterone; TC, total cholesterol; WC, waist circumference.

Vitamin D and testosterone in men

The association of vitamin D and testosterone in men has been investigated in various studies including studies on molecular mechanisms, observational studies analyzing the relationship of vitamin D status and circulating testosterone levels in men and clinical intervention studies evaluating the effect of vitamin D treatment on testosterone levels in men. The following paragraph will provide an overview on possible molecular mechanisms regarding the association of vitamin D and testosterone.

Background and mechanisms

In addition to its important regulatory effect on spermatogenesis, testosterone is an anabolic hormone with a wide range of beneficial effects on men's health, including important physiological effects on brain, muscle, bone and fat mass (66). There is accumulating evidence suggesting that androgen deficiency may contribute to the onset and progression of cardiovascular disease and play an important role in the development of the metabolic syndrome in men (67). Interestingly, men with combined vitamin D and androgen deficiencies are at high risk for all-cause and cardiovascular mortality, suggesting that a parallel deficiency of both hormones is a marker of poor overall health (68). Thus, a causal relationship between vitamin D and testosterone and in particular a potential increase of testosterone levels after vitamin D treatment is of high clinical interest.

Testosterone is produced in the Leydig cells following pituitary pulsatile LH secretion, but its production is also modulated by paracrine and autocrine signals supplied by growth factors and cytokines secreted within the testis (69, 70). The VDR is almost ubiquitously expressed in human cells, which underlines the clinical significance of the vitamin D endocrine system (1, 2, 71). VDR and vitamin D-metabolizing enzymes are concomitantly expressed in the entire reproductive male tract, including Leydig cells (72). This VDR expression suggests local autocrine as well as paracrine action of vitamin D and indicates that vitamin D is involved in regulation of testis function. Obviously, vitamin D metabolites are locally synthesized and degraded and vitamin D metabolism seems to be regulated by local as well as systemic factors (22). The negative effect of orchiectomy as well as testis dysfunction on circulating 25(OH)D levels, supports the hypothesis of vitamin D synthesis in the testis (73, 74, 75).

Of note, it has been shown in an experimental study that mouse Leydig cells basally secrete 25(OH)D, which is also stimulated by human chorionic gonadotropin (hCG) (76). This notion is supported by the fact that hCG treatment in men with late-onset hypogonadism increased circulating 25(OH)D levels (76). Despite this hypothesis of a local 25(OH)D production in the testis driven by Leydig cells, the exact regulation of testis vitamin D metabolism is largely unknown. Some evidence suggests that regulatory mechanisms resembling renal vitamin D metabolism exist. These mechanisms include PTH-related molecules and fibroblast growth factor (FGF-23) pathways (22).

Interestingly, it has been shown that androgens increase 1- α -hydroxylase, a key enzyme in vitamin D metabolism that converts 25(OH)D to 1,25(OH)₂D (77). In addition, it has also been demonstrated that the regulation of gene expression by vitamin D metabolites is modified according to androgen levels (78). On the other hand, vitamin D significantly increased testosterone production in a human primary testicular cell culture model (79). After 1,25(OH)₂D supplementation, 63 genes were significantly upregulated in human testicular cells, such as IGF-1, ALPL, DPP4 and other bone- and immune system-associated genes (79).

In male VDR-knockout mice, high LH and FSH levels indicate the presence of hypergonadotropic hypogonadism (71). Vitamin D may be critical for testicular function because vitamin D treatment upregulates certain testis-specific genes in mice (35) including ABCA1 (ATP-binding cassette transporter 1). ABCA1-knockout mice have significantly reduced intratesticular testosterone levels as well as reduced sperm counts compared with wild-type animals (80). Experimental studies also indicate that testosterone secretion might be modulated by vitamin D-induced changes in intracellular calcium homeostasis in Leydig cells in animals (22) mediated via calbindin-D28k (81). Calbindin-D 28k is a cytosolic calcium-binding protein involved not only in the regulation of intracellular calcium homeostasis but also in testis hormone production (81). Vitamin D effects on testosterone production might also be mediated via osteocalcin, which is produced by osteoblasts and involved in bone metabolism. It has been postulated that vitamin D-induced stimulation of osteocalcin expression might have an indirect relevant role in modulating testosterone production by the testis (22). Further, in humans, a direct stimulatory genomic effect of vitamin D on steroidogenesis enzymes has been postulated (22).

Table 4 Summary of clinical intervention studies investigating the effects of vitamin D treatment on androgen levels in men.

Author/Study	Study design	Subjects	Age	25(OH)D levels (baseline)	TT levels (baseline)
Pilz <i>et al.</i> (20)	RCT	54 obese men (vitamin D $n=31$, placebo $n=23$)	48.1 ± 11.1 years	<50 nmol/L; 31.1 ± 21.9 nmol/L	11.4 nmol/L
Jorde <i>et al.</i> the Tromsø study (84)	RCT (pooled data from 3 independent RCTs)	Study 1 (vitamin D and obesity): 129 men with BMI 28–47 kg/m ² , study 2 (insulin sensitivity): 53 men, study 3 (depression study): 100 men	Study 1: 48.9 ± 10.6 years; study 2: 51.2 ± 10.0 years; study 3: 53.0 ± 11.1 years	Study 1: 52.6 ± 17.8 nmol/L, study 2: 39.9 ± 14.0 nmol/L (25(OH)D <50 nmol/L), study 3: 45.8 ± 15.0 nmol/L (25(OH)D ≤ 55 nmol/L)	Study 1: 13.5 ± 4.2 nmol/L, study 2: 16.9 ± 6.4 nmol/L, study 3: 14.2 ± 4.9 nmol/L
Heijboer <i>et al.</i> (86)	RCT (pooled data from 3 independent RCTs)	92 male patients with heart failure (study 1), 49 male nursing home residents (study 2) and 42 male non-Western immigrants in the Netherlands (study 3)	Study 1: 63 (range 42–86) years, study 2: 82 (range 71–97) years, study 3: 53 (range 20–70) years	Study 1: 46.5 (38.5–62.5) nmol/L, study 2: 27.0 (23.0–31.5) nmol/L, study 3: (25OHD <50 nmol/L), 27.5 (18–33) nmol/L	Study 1: 15 (11–19) nmol/L, study 2: 11 (8–15.8) nmol/L, study 3: 13 (11–17) nmol/L
Ferlin <i>et al.</i> (99)	Uncontrolled intervention study	20 men with Klinefelter syndrome (substudy of a cross-sectional study of 127 Klinefelter men and 60 healthy controls); 12 men treated with testosterone + calcifediol (group 1), 8 men treated with calcifediol (group 2)	31.5 ± 8.5 years (all 127 men with Klinefelter syndrome)	Baseline 25(OH)D <50 nmol/L; 31.3 ± 8.9 nmol/L (group 1: testosterone+calcifediol); 23.2 ± 18.9 nmol/L (group 2: calcifediol)	10.5 ± 4.9 nmol/L (all 127 men with Klinefelter syndrome)
Foresta <i>et al.</i> (100)	Uncontrolled intervention study	66 patients with hypogonadism (classic hypogonadism (TT < 12 nmol/L, LH ≥ 8 IU/L) ($n=26$) and subclinical hypogonadism (TT ≥ 12 nmol/L, LH ≥ 8 IU/L) ($n=40$))	34.5 ± 6.8 years	Baseline 25(OH)D <50 nmol/L; cholecalciferol group: 33.8 ± 10.5 nmol/L; calcidiol group: 33.4 ± 9.5 nmol/L	Not given
Canguven <i>et al.</i> (101)	Uncontrolled intervention study	102 middle-aged men	53.2 ± 10.4 years	Baseline 25(OH)D <75 nmol/L; 37.9 ± 11.6 nmol/L	12.46 ± 3.30 nmol/L
Lerchbaum <i>et al.</i> Graz Vitamin D&TT-RCT (102)	RCT	100 healthy men with TT levels ≥ 10.4 nmol/L (vitamin D $n=50$, placebo $n=50$)	37 (27–50) years	<75 nmol/L; 52 (42–66) nmol/L	18.0 (15.8–21.5) nmol/L

Data are given as mean ± s.d. or median (IQR) unless otherwise stated.

25(OH)D, 25 hydroxyvitamin D; BMI, body mass index; IU, international unit; LH, luteinizing hormone; RCT, randomized controlled trial; TT, total testosterone.

Primary outcome	Study duration	Study medication	TT levels (study end)	25(OH)D levels (study end) vitamin D group
Weight loss	1 year	3332 IU/day vs placebo	Significant increase in TT, bioactive testosterone, and FT levels	86.4 ± 68.8 nmol/L
Study 1: weight loss, study 2: change in insulin sensitivity as evaluated with a hyperglycemic glucose clamp, study 3: change in depression scores	Study 1: 1 year, study 2: 6 months, study 3: 6 months	Study 1: 40 000 IU/week vitamin D (~5714 IU/day), 20 000 IU/week vitamin D (~2857 IU/day), or placebo for 1 year (all subjects: 500 mg calcium/day); study 2 & 3: 40 000 IU vitamin D/week (~5714 IU/day) vs placebo for 6 months	No significant effect	118.8 ± 52.2 nmol/L
Vitamin D effects on the renin-angiotensin-aldosterone system (study 1), effects of different vitamin D doses (study 2), and vitamin D effects on insulin sensitivity (study 3)	Study 1: 6 weeks, study 2: 16 weeks, study 3: 16 weeks	Study 1: 2000 IU/day, study 2: 600 IU per day, 4200 IU per week or 18 000 IU/month, study 3: 1200/d vs placebo (+500 mg calcium carbonate: all subjects)	No significant effect	Study 1: 73.5 nmol/L, study 2: 57 nmol/L, study 3: 49 nmol/L
Maintain 25(OH)D levels above 50 nmol/L	2 years	Calcifediol treatment with Didrogyl, Calcifediol starting dose of 4000 IU/week, adjusting the dosage to maintain 25(OH)D levels >50 nmol/L, by determining the levels every 6 months	No significant effect	Group 1: 94.3 ± 12.9 nmol/L, group 2: 102.5 ± 28.7 nmol/L
Effects of cholecalciferol vs calcidiol on 25(OH)D levels	3 months	5000 IU cholecalciferol per week (~714 IU/d; <i>n</i> =20) or 4000 IU calcidiol per week (~571 IU/d; <i>n</i> =36)	No significant effect	Cholecalciferol: 42.6 ± 11.6 nmol/L; calcidiol: 81.4 ± 25.0 nmol/L
Not specifically stated (vitamin D effects on biochemical and hormonal parameters as well as on erectile dysfunction)	1 year	Men received an initial vitamin D dose (Ergocalciferol; oral solution 600 000 IU/1.5 ml), and followed a vitamin D treatment regime thereafter	Significant increase in TT levels (12.46 ± 3.30 to 15.99 ± 1.84 nmol/L)	121.4 ± 29.1 nmol/L
TT measured using mass spectrometry	12 weeks	20,000 IU/week (~2,857 IU/day) vs placebo	No significant effect	107 (89–119) nmol/L

Observational studies

In the following paragraph, we will discuss observational studies on vitamin D status and circulating androgen levels in men (summarized in Table 3). The majority of studies found an independent association of vitamin D with circulating androgen levels or hypogonadism in men. In 2011, some of us demonstrated for the first time a significant association of 25(OH)D and TT levels in men (19). Similarly, Nimptsch and coworkers (82) reported an independent association of 25(OH)D with TT and FT levels in 1362 male participants of the Health Professionals Follow-up study. Lee and coworkers (83) observed no independent association of TT or FT with 25(OH)D in 3369 community-dwelling men aged 40–79 years from the European Male Aging study. There was, however, an independent association of 25(OH)D levels <50 nmol/L with compensated as well as with secondary hypogonadism (83). Similarly, Jorde and coworkers (84) found a significant positive association of vitamin D and TT levels (adjusted for age, BMI, season, presence of cardiovascular disease and DM and physical activity) in 893 men from the Tromsø Study. In that study, no significant association was found between 25(OH)D and FT, SHBG, FSH or LH (84). In 2854 Chinese men, 25(OH)D was positively associated with TT and estradiol after adjustments for age, residence area, economic status, smoking, BMI, HOMA-IR, DM and systolic pressure (85). Further, increasing quartiles of 25(OH)D were associated with significantly decreased odds ratios of hypogonadism (85). Heijboer and coworkers (86) observed a significant positive association of 25(OH)D and TT levels in 183 men (unadjusted). In addition, a significant positive association of TT and SHBG with 25(OH)D levels was reported in 382 Chinese and Malaysian men (87). The results lost, however, significance after adjustment for BMI (87). Tak and coworkers (88) found an independent association of 25(OH)D levels with TT (adjusted for body fat, WC, BMI, fasting plasma glucose, DM and dyslipidemia) and FT levels (adjusted for age, total muscle mass, smooth muscle mass, total cholesterol, DM, dyslipidemia and alcohol use) in 652 Korean men aged 56.7±7.9 years. Further, vitamin D deficiency (<50 nmol/L) was associated with an increased risk of TT and FT deficiency (adjusting for age, season, BMI, body composition, chronic disease, smoking and alcohol use) (88). Data from the Longitudinal Aging Study Amsterdam, an ongoing population-based cohort study of older Dutch individuals (*n*=643), documented an independent association of 25(OH)D levels with TT and bioavailable TT (adjusted for age, BMI, alcohol

consumption, smoking status, season of blood collection, number of chronic diseases, serum creatinine and physical performance) (89). Others reported a significant positive association of 25(OH)D and TT as well as SHBG in 1315 men (NHANES III) and 318 men (NHANES 2001–2004), respectively (adjusted for age, race/ethnicity, body fat percentage and smoking) (90). Moreover, data from 1427 infertile men indicated lower SHBG and TT/estradiol ratios but higher FT and estradiol in men with 25(OH)D levels <25 nmol/L compared to men with 25(OH)D levels >75 nmol/L (91). The authors observed no independent association of 25(OH)D levels with hypogonadism, estradiol, SHBG, LH or FSH, respectively (91). In 3016 older men, lower 25(OH)D levels were associated with lower SHBG and higher FT levels after adjusting for demographic and lifestyle variables, whereas no independent association with TT was observed (92).

Some authors failed, however, to demonstrate an independent association of vitamin D and serum androgens in men. Hammoud and coworkers (93) observed no independent association of 25(OH)D levels with TT or FT levels in 170 healthy men. Similarly, results from NHANES III suggested no significant association of 25(OH)D with TT, FT, SHBG or estradiol in 1412 middle-aged men (94). Of note, cross-sectional results from 225 middle-aged men suggest a U-shaped association of vitamin D status and risk of hypogonadism (95). In detail, we found a significantly increased risk of hypogonadism in men within the highest 25(OH)D quintile compared to men in quintile 4 (reference) as well as a trend towards increased risk of hypogonadism in men within the lowest 25(OH)D quintile. With respect to risk of male hypogonadism, these results suggest optimal serum 25(OH)D concentrations of 82–102 nmol/L. Those findings as well as previous studies showing no association of vitamin D and androgens in linear analyses might therefore be supported by previous inconsistent results with possible U-shaped or non-linear associations that have been suggested for vitamin D and cancer (5), cardiovascular disease (96) and mortality (97, 98). Furthermore, inconsistent results reported in observational studies might be related to different study populations with respect to sample size, age, comorbidities, ethnicity as well as to various statistical methods used for data analyses.

Intervention studies

In addition to the above mentioned observational studies, there are some intervention studies evaluating vitamin D effects on androgen levels in men (summarized in Table 4).

Uncontrolled intervention studies

A small study investigating men with Klinefelter syndrome found no significant effect of vitamin D supplementation (vitamin D treatment $n=8$, vitamin D+testosterone treatment $n=12$) on androgen levels in men with baseline $25(\text{OH})\text{D} < 50 \text{ nmol/L}$ (99). Similarly, Foresta and coworkers (100) investigated the effect of cholecalciferol (5000 IU per week) ($n=20$) or calcidiol (4000 IU per week) ($n=46$) in 66 patients with hypogonadism and $25(\text{OH})\text{D} < 50 \text{ nmol/L}$ and found no significant effect on TT levels. In contrast, Canguven and coworkers (101) observed a significant increase in TT levels (12.46 ± 3.30 to $15.99 \pm 1.84 \text{ nmol/L}$) in 102 middle-aged men with $25(\text{OH})\text{D} < 75 \text{ nmol/L}$ who received an initial vitamin D dose (Ergocalciferol; oral solution 600,000 IU/1.5 mL) and followed a vitamin D treatment regime thereafter. Interestingly, the authors observed an increase of erectile dysfunction scores as well as a decrease in estradiol levels after treatment (101).

RCTs

Evidence from RCTs on vitamin D and TT is sparse. Pilz and coworkers (20) investigated the effects of 1 year of vitamin D supplementation (3332 IU daily) on androgens in 54 men (vitamin D $n=31$, placebo $n=23$) undergoing a weight reduction program. The authors observed a significant increase in TT levels, bioactive testosterone levels, and FT levels compared to baseline levels, whereas no significant change was found in the placebo group. The study included men with vitamin D deficiency ($25[\text{OH}]\text{D} < 50 \text{ nmol/L}$) and relatively low baseline TT levels (11.4 nmol/L). Of note, no statistical analysis on treatment effect (between group differences) was performed. In contrast, Heijboer and coworkers (86) failed to find an effect of vitamin D supplementation on serum TT concentrations in 3 independent intervention studies including male patients with heart failure (study 1), male nursing home residents (study 2) and male non-Western immigrants in the Netherlands (study 3). Those studies were designed to investigate vitamin D effects on the renin-angiotensin-aldosterone system (study 1), effects of different vitamin D doses (study 2) and vitamin D effects on insulin sensitivity (study 3). In study 1, 92 subjects received vitamin D (2000 IU daily) or placebo for 6 weeks. In study 2, 49 vitamin D-deficient subjects were randomized to vitamin D (600 IU daily) or placebo for 16 weeks. In study 3, 43 vitamin D-deficient subjects received 1200 IU vitamin D daily or placebo for 16 weeks. Similarly, Jorde and coworkers (84) failed to show a significant vitamin D effect on androgen concentrations

(TT and FT) in pooled data from 3 vitamin D RCTs performed in Tromsø with weight reduction, insulin sensitivity and depression scores as endpoints. In that study, serum $25(\text{OH})\text{D}$ and androgens were measured in 282 men at baseline and after 6–12 months of vitamin D supplementation (20,000–40,000 IU weekly vs placebo).

Recently, we presented results of the first RCT (Graz Vitamin D&TT-RCT) designed for the evaluation of vitamin D effects on serum androgens in men (102). In detail, we randomly assigned 100 men with TT levels $\geq 10.4 \text{ nmol/L}$ and $25(\text{OH})\text{D}$ levels $< 75 \text{ nmol/L}$ to receive 20,000 IU/week of vitamin D3 ($n=50$) or placebo ($n=50$) for 12 weeks. We found no significant effect on androgen levels in these healthy men with relatively high TT levels at baseline. These results are in line with the Jorde and coworkers (84) as well as with the Heijboer and coworkers study (86) but contradict previous promising findings from the Pilz and coworkers (20) study. Those differences might be related to different study designs and subjects. Although both studies used similar doses (2857 IU/day Lerchbaum and coworkers (102) vs 3332 IU/day Pilz and coworkers (20)), respectively, the Graz Vitamin D&TT-RCT (102) investigated vitamin D effects after 12 weeks vs vitamin D effects after 1 year in the Pilz and coworkers study (20). Further, the Graz Vitamin D&TT-RCT (102) investigated men with $25(\text{OH})\text{D}$ levels $< 75 \text{ nmol/L}$, whereas Pilz and coworkers (20) included subjects with $25(\text{OH})\text{D}$ levels $< 50 \text{ nmol/L}$. Other differences are related to the study participants (healthy men vs significant weight loss in obese subjects), baseline TT levels (19.1 nmol/L vs 11.4 nmol/L) and the method used for TT measurements (mass spectrometry vs immunoassay) for the Graz Vitamin D&TT-RCT report and the Pilz and coworkers study, respectively.

In summary, to date, there is only 1 RCT showing increased androgen levels after vitamin D supplementation (20); however, vitamin D effects on androgen levels have not been the primary outcome of this study. It should, therefore, be noted that only 1 RCT was specifically designed for the investigation of vitamin D effects on TT levels (102). As this RCT investigated men with relatively high baseline TT levels, a vitamin D effect on TT levels in hypogonadal men or men with low normal TT levels cannot be excluded. This issue is addressed in the second arm of the Graz Vitamin D&TT-RCT (ClinicalTrials.gov Identifier NCT01748370), results are expected in 2018. Further, the Graz Vitamin D&TT-RCT investigated a cohort of men with relatively high baseline $25(\text{OH})\text{D}$ levels. Thus, vitamin D effects in men with severe vitamin D deficiency cannot be excluded. Given the fact that in healthy middle-aged men a U-shaped association of

vitamin D levels with hypogonadism has been observed (95), one might speculate that a RCT aiming at target 25(OH)D levels between 75 and 100nmol/L would provide different results.

In summary, evidence from RCTs on vitamin D and TT is sparse and revealed conflicting results. We therefore recommend further investigation of potential vitamin D effects on androgen levels in various cohorts including hypogonadal men with severe vitamin D deficiency.

Conclusion

As outlined above, vitamin D might play an important role in androgen metabolism as well as in metabolic and reproductive processes in PCOS women. Indeed, in PCOS women some RCTs revealed promising results regarding vitamin D effects on metabolic as well as on fertility aspects. Nevertheless, considering previous inconsistent results as well as the large differences in study design and PCOS populations (i.e. PCOS phenotypes), large-scale RCTs in well-defined PCOS populations with clearly defined end points are needed before a general recommendation regarding 25(OH)D measurement and vitamin D supplementation in PCOS can be made. Similarly, existing evidence from available RCTs evaluating the effect of vitamin D supplementation on androgen levels in men is insufficient to recommend measurement of 25(OH)D levels or vitamin D supplementation in hypogonadal men. Still, we cannot exclude vitamin D effects on androgen levels in men with low TT levels or in men with severe vitamin D deficiency. This question remains to be answered in future RCTs.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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