Dissertation

Effects of vitamin D supplementation on endocrine and metabolic parameters in women with and without polycystic ovary syndrome

submitted by

Dr.med.univ. Christian Georg TRUMMER

for the Academic Degree of

Doctor of Medical Science

(Dr. scient. med.)

at the

Medical University of Graz

Division of Endocrinology and Diabetology,
Department of Internal Medicine
&
Department of Obstetrics and Gynecology

under the Supervision of

Priv.-Doz.in Dr.in med.univ. et scient.med. Elisabeth LERCHBAUM

Univ.-Prof. in Dr. in med.univ. Barbara OBERMAYER-PIETSCH

Priv.-Doz.in Dr.in med.univ. Verena THEILER-SCHWETZ, PhD

Assoz.Prof. Priv.-Doz. Dr.med.univ. Philipp KLARITSCH

2018

Statutory Declaration

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this thesis. Due acknowledgment has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the "Standard of Good Scientific Practice and Ombuds Committee at the Medical University of Graz".

Disclosures

Part of this thesis has been published in "Trummer C[1], Schwetz V[1], Kollmann M[2], Wölfler M[2], Münzker J[3], Pieber TR[1], Pilz S[1], Heijboer AC[4,5], Obermayer-Pietsch B[1], Lerchbaum E[1]. Effects of vitamin D supplementation on metabolic and endocrine parameters in PCOS: a randomized-controlled trial. Eur J Nutr. 2018 Jun 26; doi: 10.1007/s00394-018-1760-8".

Author information

[1]Division of Endocrinology and Diabetology, Department of Internal Medicine, Medical University of Graz, Graz, Austria

[2]Department of Obstetrics and Gynecology, Medical University of Graz, Graz, Austria

[3]Department of Medicine, Integrated Research and Treatment Centre for Adiposity Diseases, Leipzig University, Leipzig, Germany

[4]Endocrine Laboratory, Department of Clinical Chemistry, VU University Medical Center, Amsterdam, The Netherlands

[5]Laboratory of Endocrinology, Academic Medical Center, Amsterdam, The Netherlands

I confirm that all co-authors have explicitly agreed to the use of their data in the thesis and that I have obtained permission to reproduce figures and tables published in Azziz et al., Fertil Steril. 2009;91(2):456-488 with the permission of copyright holder Elsevier Inc.; Holick, N Engl J Med. 2007;357(3):266-281 with the permission of copyright holder Massachusetts Medical Society; Thomson et al., Clin Endocrinol (Oxf). 2012;77(3):343-350 with the permission of copyright holder

John Wiley and Sons; and Trummer et al., Eur J Nutr. 2018; doi:10.1007/s00394-018-1760-8 with the permission of publisher Springer Nature.

Graz, July 13, 2018

Acknowledgments

Doctoral student Christian Georg Trummer received funding from the Austrian Science Fund (FWF), project no.: KLI 274, and the Medical University of Graz through the Doctoral School “Sustainable Health Research”. The underlying project of this dissertation was supported by funding from the Austrian Science Fund (FWF), project no.: KLI 274 (recipient: Elisabeth Lerchbaum). I would like to thank the members of my dissertation committee for their continuous support in making this dissertation possible. Furthermore, I would like to thank Roswitha Gumpold for the recruitment of patients, Cornelia Missbrenner and the Endocrinology Lab platform for their support in biochemical analyses as well as Fresenius Kabi for providing the study medication.

Table of Contents

Abbreviations and Definitions

$1,25(OH)_2D$ ..... 1,25-dihydroxyvitamin D
25(OH)D ..... 25-hydroxyvitamin D
AE-PCOS Society ..... Androgen Excess and PCOS Society
AMH ..... anti-Müllerian hormone
AUCgluc ..... plasma glucose area under the curve
BMI ..... body-mass index
CAH ..... congenital adrenal hyperplasia
Cholecalciferol ..... Vitamin D3
CI ..... confidence interval
CRP ..... C-reactive protein
CV ..... coefficient of variation
DBP ..... vitamin D binding protein
DHEAS ..... dehydroepiandrostendione-sulfate
Ergocalciferol ..... Vitamin D2
FAI ..... free androgen index
FSH ..... follicle-stimulating hormone
FT ..... free testosterone
GDM ..... gestational diabetes mellitus
GnRH ..... gonadotropin releasing hormone
GWAS ..... genome-wide association study
HbA1c ..... glycated hemoglobin
HC ..... hormonal contraceptive
HDL-cholesterol ..... high-density lipoprotein-cholesterol
HOMA-IR ..... homeostatic model assessment – insulin resistance
ID-LC-MS/MS ..... Isotope-Dilution Liquid Chromatography Tandem Mass Spectrometry
IGT ..... impaired glucose tolerance
IVF ..... in vitro fertilization
LDL-cholesterol ..... low-density lipoprotein-cholesterol
NHANES ..... National Health and Nutrition Survey
NIH ..... National Institutes of Health

LH.....luteinizing hormone
OGTT.....oral glucose tolerance test
PCOS.....polycystic ovary syndrome
PTH.....parathyroid hormone
QUICKI.....quantitative insulin sensitivity check index
RCT.....randomized-controlled trial
SD.....standard deviation
SHBG.....sex hormone-binding globulin
T2DM.....type 2 diabetes mellitus
TC.....total cholesterol
TT.....total testosterone
VDR.....vitamin D receptor
VLDL.....very low-density lipoprotein
WHR.....waist-to-hip ratio

List of Figures

List of Tables

Zusammenfassung

Einleitung: Rezente Untersuchungen des polyzystischen Ovar-Syndroms (PCOS) suggerieren einen Zusammenhang zwischen dem Vitamin D-Status und den typischen Merkmalen des Syndroms wie Insulinresistenz, gestörter ovarieller Funktion oder Hyperandrogenämie. Das Ziel dieser Dissertation war daher, die Effekte einer Vitamin D-Supplementation auf endokrine und metabolische Parameter bei Frauen mit PCOS zu untersuchen. Diese Effekte wurden zudem auch in einer Studienpopulation bestehend aus Frauen ohne PCOS untersucht.

Material und Methoden: Insgesamt 180 Frauen mit PCOS und 150 Frauen ohne PCOS mit einem 25-Hydroxyvitamin D [25(OH)D] Serumspiegel <75 nmol/L wurden rekrutiert und erhielten nach 2:1-Randomisierung 20 000 wöchentliche Einheiten Cholecalciferol oder Placebo über 24 Wochen. Primäres Studienziel waren die Unterschiede zwischen Interventions- und Placebogruppe hinsichtlich der Plasmaglukose area under the curve (AUCgluc) zu Studienende.

Ergebnisse: Insgesamt beendeten 123 Teilnehmerinnen aus der PCOS-Gruppe und 127 Teilnehmerinnen aus der Gruppe ohne PCOS die Studie. Frauen mit und ohne PCOS unterschieden sich zu Studienbeginn signifikant hinsichtlich bestimmter Parameter, inklusive anthropometrischer Maße, Parameter des Glukosestoffwechsels sowie Androgenspiegel. In beiden Gruppen hatte eine Vitamin D-Supplementation keinen signifikanten Effekt auf das primäre Studienziel (AUCgluc), jedoch führte die Intervention in der PCOS-Gruppe zu einer signifikanten Reduktion der Plasmaglukose nach 60 Minuten im oralen Glukosetoleranztest (OGTT), während es in der Gruppe ohne PCOS zu einem signifikanten Anstieg des homeostatic model assessment – insulin resistance (HOMA-IR) und zu einem signifikanten Abfall des quantitative insulin sensitivity check index (QUICKI) kam.

Schlussfolgerungen: Eine Vitamin D-Supplementierung zeigte bei Frauen mit und ohne PCOS keinen signifikanten Effekt auf AUCgluc, während es zu einer Beeinflussung gewisser sekundärer Zielgrößen kam.

Abstract

Introduction: Growing evidence suggests a possible association of vitamin D status with clinical features of polycystic ovary syndrome (PCOS), including insulin resistance, ovulatory dysfunction, and hyperandrogenemia. The aim of this dissertation was to evaluate the effects of vitamin D supplementation on endocrine and metabolic parameters in PCOS. Furthermore, these effects were also assessed in a study group of premenopausal women without PCOS.

Material and Methods: In total, 180 PCOS patients and 150 women without PCOS with a 25-hydroxyvitamin D [25(OH)D] serum concentration <75 nmol/L were recruited. Participants were 2:1 randomized to receive 20,000 IU of cholecalciferol or placebo over 24 weeks. Primary study outcome was the difference in plasma glucose area under the curve (AUCgluc) between the vitamin D and the placebo group at study end with adjustment for baseline values.

Results: In the PCOS group, 123 participants completed the study, while 127 participants completed the study in the group without PCOS. At baseline, we found significant differences in several parameters between the groups with and without PCOS, including anthropometric measurements, parameters of glucose homeostasis, and serum androgen levels. Vitamin D supplementation had no significant effect on our primary outcome measure (AUCgluc) in both groups. However, vitamin D supplementation lead to a significant decrease in plasma glucose after 60 minutes during oral glucose tolerance test (OGTT) in the PCOS group, while it lead to a significant increase in homeostatic model assessment – insulin resistance (HOMA-IR) and a significant decrease in quantitative insulin sensitivity check index (QUICKI) in the group without PCOS.

Conclusions: Vitamin D supplementation had no significant effect on AUCgluc in women with and without PCOS, while it had a significant impact on some of our secondary outcome parameters.

1 Introduction

1.1 Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of reproductive age with a prevalence of up to 20% of all women (1,2). The syndrome is a very heterogeneous condition and has significant implications on reproductive, metabolic, and psychological features and therefore possesses great clinical and public health importance (3).

1.1.1 Diagnosis

The first description of PCOS as a syndrome was made by Stein and Leventhal in 1935. The authors reported seven cases of women with amenorrhea, hirsutism, and bilateral polycystic ovaries. As a result, the syndrome was first known as "Stein-Leventhal-Syndrome" (4). Since then, several revisions of the definition of PCOS were made. In the last few decades, the three most popular definitions were published: the National Institutes of Health (NIH) criteria in 1990, the Rotterdam criteria in 2003, and the most recent Androgen Excess and PCOS Society (AE-PCOS Society) criteria in 2006 (5–7).

1.1.1.1 NIH criteria

The NIH criteria originated from an expert conference in April 1990. The attendees of this conference were queried which criteria they believed to be relevant for the diagnosis of PCOS (5). According to the results, the following definition for the diagnosis of PCOS was made:

1) Hyperandrogenemia and/or clinical hyperandrogenism
2) Chronic oligo-/anovulation (5)

Disorders with similar symptoms like Cushing's syndrome, congenital adrenal hyperplasia (CAH) or hyperprolactinemia need to be excluded before a diagnosis of PCOS can be made, making it a diagnosis of exclusion. Unlike other diagnostic

criteria, the presence of polycystic ovaries on ultrasound was not considered for the NIH criteria (5,8).

1.1.1.2 Rotterdam criteria

The Rotterdam criteria derived from another expert meeting held by the European Society for Human Reproduction and the American Society for Reproductive Medicine in 2003. One of the major concerns of the consensus meeting was the rising evidence that not every clinical expression of PCOS was covered by the preexisting NIH criteria (6,9). Therefore, two out of the following three criteria must be met for diagnosis of PCOS according to the Rotterdam criteria:

1) Oligo-/anovulation
2) Clinical and/or biochemical signs of hyperandrogenism
3) Polycystic ovaries (diagnosed by ultrasound) (6)

Comparable to the NIH criteria, disorders that may cause a similar clinical presentation must be excluded before a definite diagnosis of PCOS can be made. The consensus group specifically recommended screening for CAH, Cushing's syndrome, hyperprolactinemia, thyroid dysfunction, hypogonadotropic hypogonadism, and androgen-secreting tumors (6).

Oligo-/anovulation was defined as the presence of oligomenorrhea or amenorrhea, respectively. Hirsutism was considered as the primary indicator for clinical hyperandrogenism, the sole presence of acne or androgenetic alopecia was found to be less indicative (6,10–12). For diagnosis of hyperandrogenemia, the measurement of total testosterone (TT) and free testosterone (FT) was recommended, as they were felt to be the most sensitive parameters. Sonographic polycystic ovary morphology was defined as the presence of 12 or more follicles in each ovary measuring 2-9 mm in diameter and/or increased ovarian volume (>10 mL) (6,13).

1.1.1.3 AE-PCOS Society criteria

In 2006, the AE-PCOS Society appointed a task force to evaluate whether the existing definitions of PCOS were overly narrow or unjustifiably broad. The task force recognized four key features in PCOS: ovulatory and menstrual dysfunction, hyperandrogenemia, clinical features of hyperandrogenism, and polycystic ovaries. Ovulatory dysfunction was considered as a prominent, but not universal symptom of PCOS. It was recognized that some patients demonstrate regular ovulation ("ovulatory PCOS") (7).

In the data reviewed by the task force, elevated androgen levels were observed in 60-80% of PCOS women, with FT being the most frequently elevated parameter. The task force also noted that the measurement of androgen levels is only to be used as an adjuvant for the diagnosis of hyperandrogenic disorders since not every PCOS patient shows elevated androgen levels and androgen assays tend to be highly variable (7,14). Approximately 60% of women with PCOS in the available data were found to be hirsute; the modified Ferriman-Gallwey score was suggested as the measurement of choice (7,15). A significant fraction of PCOS patients were considered to suffer from acne and/or androgenetic alopecia. However, the task force found the regarding study data not sufficient to include either acne or androgenetic alopecia as a diagnostic criterion. Even though the rate of polycystic ovaries in ultrasound was reported to be 75%, the task force recognized a relatively high rate of false-positive results. Thus, the authors noted that strict criteria should be used (7).

Therefore, according to the AE-PCOS Society task force, the diagnosis of PCOS can be made if the following two criteria are met:

1) Hyperandrogenism: Hirsutism and/or hyperandrogenemia
2) Ovarian dysfunction: Oligo-/anovulation and/or polycystic ovaries (7)

Like the NIH and Rotterdam criteria, the AE-PCOS Society criteria also require exclusion of other sources of androgen excess or related disorders (7).

1.1.1.4 Clinical use

Depending on the presence or absence of the main features of PCOS, several phenotypes can be identified. Figure 1 gives an overview on the possible phenotypes and their coverage by the different diagnostic criteria (16).

Figure 1 Phenotypes and diagnosis criteria of PCOS. Reproduced from (16) with permission of publisher Elsevier Inc.

	Potential Phenotypes															
Features	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**
Hyperandrogenemia	+	+	+	+	−	−	+	−	+	−	+	−	−	−	+	−
Hirsutism	+	+	−	−	+	+	+	+	−	−	+	−	−	+	−	−
Oligo-anovulation	+	+	+	+	+	+	−	−	−	+	−	−	+	−	−	−
Polycystic ovaries	+	−	+	−	+	−	+	+	+	+	−	+	−	−	−	−
NIH 1990 criteria	√	√	√	√	√	√										
Rotterdam 2003 criteria	√	√	√	√	√	√	√	√	√	√						
AE-PCOS 2006 criteria	√	√	√	√	√	√	√	√	√							

Azziz. AE-PCOS Society report on PCOS phenotype. Fertil Steril 2009.

When the NIH criteria were established in 1990, they represented the first attempt for a universally accepted definition of PCOS. However, it became evident that polycystic ovaries were a consistent finding in women showing biochemical and clinical aspects of the syndrome (17–19). Thus, the sonographic presence of polycystic ovaries was added as a diagnostic feature in the Rotterdam criteria, which led to the acknowledgement of previously undiagnosed phenotypes (6). Nevertheless, the Rotterdam criteria were since discussed controversially, as certain phenotypes can be diagnosed without showing any kind of androgen excess or menstrual irregularity. As a consequence, the AE-PCOS Society criteria require patients to show biochemical and/or clinical hyperandrogenism. However, they do not acknowledge mild variants of PCOS in which little evidence exists about metabolic status or long-time health risks (7,17).

The current PCOS clinical practice guideline by the Endocrine Society (20) recommends use of the Rotterdam criteria to establish the diagnosis of PCOS. The authors noted that these criteria allow the diagnosis to be made clinically as well as biochemically. Additionally, PCOS phenotypes with present

hyperandrogenism may show a higher association with metabolic abnormalities whereas phenotypes with irregular menses and polycystic ovaries may be associated with a higher rate of infertility. The task force deployed by the Endocrine Society also referred to a recent NIH-sponsored Evidence-Based Methodology workshop on PCOS (21) that came to the same conclusions.

1.1.2 Pathophysiology and etiology

PCOS is a complex condition with a broad spectrum of features. Therefore, no single etiologic factor for its development can be identified. Instead, several processes are involved in the pathogenesis of PCOS (22).

1.1.2.1 Gonadotropins and androgens

PCOS patients show significant abnormalities in gonadotropin secretion and plasma concentration. Generally, PCOS is characterized by an increased luteinizing hormone (LH) pulse amplitude and an exaggerated LH response to exogenous gonadotropin releasing hormone (GnRH). Additionally, follicle-stimulating hormone (FSH) levels in PCOS patients are usually low (23). In a study conducted by Taylor et al. (24), 75% of the included women had elevated LH levels and 94% showed an increased LH:FSH ratio. In PCOS patients, a persistently rapid GnRH pulsation of about one pulse per hour favors synthesis and secretion of LH over FSH, therefore increasing the measured plasma levels of LH and increasing the LH:FSH ratio (23,25).

In response to the increased secretion of LH, the ovarian theca cell is stimulated to synthesize a higher quantity of androgens. Cytochrome P-450c17, which possesses 17α-hydroxylase and 17,20-lyase activities that are required to form androstendione, mediates the androgen biosynthesis. It is then converted by 17β-hydroxysteroid dehydrogenase for the synthesis of testosterone or aromatized by the aromatase enzyme (cytochrome P-450arom) for the synthesis of estrone (22). Contrary to LH, FSH is responsible for aromatase activity in ovarian granulosa cells, therefore determining the estrogen synthesis from androgenic precursors.

Thus, the higher the LH concentration is in comparison to the FSH concentration, the more the ovaries prefer to synthesize androgens (22).

1.1.2.2 Insulin resistance and hyperinsulinemia

PCOS patients present with a high prevalence of insulin resistance beyond predictions by their body-mass index (BMI) (26). In a study by DeUgarte et al. (27), 64% of the study population showed insulin resistance according to the homeostatic model assessment-insulin resistance (HOMA-IR). As a consequence, hyperinsulinemia is a common finding in PCOS patients (22). Insulin acts synergistically with LH in promoting androgen biosynthesis in the ovarian theca cells. Furthermore, it inhibits the hepatic production sex hormone-binding globulin (SHBG), thus increasing the proportion of freely available, biologically active testosterone. As a result, PCOS patients often show elevated FT levels whereas the TT concentration lies within the upper normal range or is only slightly elevated (22).

Several studies confirmed the androgen-increasing effect of insulin in PCOS women (26). Insulin infusion during euglycemic clamp studies lead to elevated levels of androgens without influencing gonadotropin levels, suggesting a direct effect on androgen synthesis. Furthermore, a suppression of insulin levels with diazoxide resulted in decreased testosterone concentrations without any effect on LH release (26).

1.1.2.3 Genetics

PCOS shows an increased prevalence among family members; its prevalence is estimated to be 20-40% among female first-degree family members of a PCOS patient (2,28). Additionally, twin studies showed an increased heritability, also suggesting a genetic component to the syndrome (29). A wide array of studies aimed to identify specific genes associated with the development of PCOS. Candidate gene studies and genome-wide association studies (GWASs) reported a broad spectrum of polymorphisms in genetic regions encoding SHBG, androgen receptors, and gonadotropic receptors as well as polymorphisms in regions responsible for insulin sensitivity and obesity (2,22,30). Studies reporting an up-

regulation of proto-oncogenic factors in the endometrium and a shortening of telomeres in leukocytes might indicate an involvement of genes responsible for cell growth (31,32).

In the first GWAS conducted in PCOS among Chinese women, three gene loci were significantly associated with PCOS (33). One of the identified loci, located on chromosome 2, contains the information for the LH/human chorionic gonadotropin receptor (33). Another GWAS reported that variants found in the THADA and DENND1A gene in Chinese women also affected the risk of developing PCOS in European women, therefore suggesting that PCOS might be an ancient disorder (34).

1.1.2.4 Environmental factors

Environmental factors, especially dietary and lifestyle influences, have a significant impact on the PCOS phenotype. Obesity promotes several aspects of PCOS, including insulin resistance, menstrual irregularities, and hyperandrogenism (2). As a consequence, regional differences due to lifestyle influences exist: PCOS women from the USA show an increased prevalence of metabolic disturbances compared to women from other countries (2,35). Weight loss may lead to an alleviation of several symptoms in PCOS, as studies reported a correlation of weight loss with lower circulating insulin and androgen levels and an improvement of hirsutism, dyslipidemia and menstrual and ovulatory dysfunction (36–40).

Additionally, environmental disrupting chemicals might play a role in the pathogenesis of PCOS. Bisphenol A, an industrial estrogenic plasticizer, induced an increased ovarian androgen production and was associated with insulin resistance in animal studies (41,42). Due to the decreased hepatic clearance in PCOS patients as a consequence of androgen excess, Bisphenol A might accumulate to high levels, therefore raising concerns of potentially aggravating the PCOS phenotype in affected women (41,42).

1.1.3 Clinical features

1.1.3.1 Hirsutism

Hirsutism is defined as excess terminal hair that appears in a typical male pattern in women. It must be distinguished from hypertrichosis, which describes an excessive hair growth in a generalized manner without preference of a male pattern and is often caused by hereditary factors or certain medications (43). Sexual hair growth is entirely dependent on the presence of androgens (44). As a response to increased androgen levels after the onset of puberty, vellus follicles in certain body areas develop into terminal hair, which appears larger, curlier and darker than vellus hair. In other body areas (e.g., forehead, cheeks), the increase in androgen levels does not lead to terminal hair growth but instead increases the size of sebaceous glands (44).

Hirsutism is a result of circulating androgen levels and the sensitivity of the hair follicle to androgens. This sensitivity partially depends on the local metabolism of androgens, in particular the conversion of testosterone to dihydrotestosterone by 5α-reductase and subsequent binding of these molecules to the androgen receptor. Therefore, some women show hirsutism in spite of normal serum androgen levels (43).

Hirsutism is a clinical diagnosis. It is usually assessed by the Ferriman-Gallwey score, which depicts the nine body areas most sensitive to androgens. For each area, depending on the intensity of terminal hair in the region, up to four possible points are added to the total score (i.e., a maximum of 36 points can be accumulated). A total score of eight points and more is indicative of the prevalence of hirsutism (43,45). Nevertheless, this scoring system has some limitations, including its subjective nature, the neglect of focally increased hair growth that does not raise the total score to an abnormal extent, the lack of consideration of other androgen-sensitive areas (e.g. sideburns or buttocks), and the lack of normative data on other populations (43).

PCOS is the most common etiology of moderate to severe hirsutism (often in conjunction with elevated testosterone levels). Nevertheless, several other

disorders may also lead to terminal hair growth in a male pattern (e.g. Cushing's syndrome, thyroid dysfunction, or acromegaly) (43).

1.1.3.2 Acne

Acne, as another potential sign of hyperandrogenism, is less common in PCOS patients than hirsutism. It shows an overall prevalence of about 12% in all women, with an increased rate of 23-35% in PCOS patients. Vice versa, PCOS shows a high prevalence among women with acne (46,47). Interestingly, several acne-related conditions, namely hydradenitis suparitiva, dissecting cellulitis of the scalp, or pilonidal sinuses, are not increased in PCOS (46,47).

The correlation of increased androgen levels with acne is controversial. However, androgens from both the adrenals and the ovaries as well as target tissue produced androgens play a role in the pathogenesis of acne, since they increase sebum production causing an abnormal follicular epithelial turnover, resulting in comedone formation (46,48,49). A possible explanation for the difference in prevalence of hirsutism and acne may be the different expression of 5α-reductase in the hair follicle and the sebaceous glands (47).

1.1.3.3 Androgenetic alopecia

Isolated alopecia appears to be a relatively poor marker of androgen excess, even though concise study data are scarce (6). Androgenetic alopecia describes a progressive non-scarring pattern loss of scalp terminal hair. It usually diffusely involves the crown, sparing the occiput as well as the frontal hairline (46,47). Studies suggest an implication of adrenal and ovarian androgens in the process (46,50).

1.1.3.4 Impaired glucose tolerance

Several studies investigated the prevalence of impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM) in PCOS patients. Among three different studies (51–53), the prevalence of IGT was 23-35% and the prevalence of T2DM was 4-10% (26). Additionally, the prevalence rate of IGT in PCOS patients was 3-fold higher than the population prevalence rate reported in women of similar age in

the National Health and Nutrition Survey (NHANES) II and about twice the prevalence rate in weight- and age-comparable women (26,51–53). The prevalence rate of T2DM was 7.5 to 10-fold higher than the prevalence rate of age-comparable women reported in the NHANES II study. However, these studies potentially even underestimated the prevalence of diabetes mellitus in PCOS since they excluded women with a preexisting diagnose of diabetes mellitus type 1 or type 2 (26,51–53).

As expected, the prevalence of dysglycemia (i.e., fasting glucose ≥100 mg/dL or blood glucose ≥140 mg/dL two hours after ingestion of 75 g glucose) increased with BMI and showed the highest frequency in women with a BMI ≥30 kg/m^2. Nevertheless, even lean women with PCOS showed an increased prevalence of IGT and T2DM (26,51,53). Interestingly, these rates were evenly increased in adolescents with PCOS (54). A meta-analysis in PCOS patients was able to confirm the increased prevalence of IGT and T2DM in both BMI- and non-BMI-matched studies (26,55). Additionally, PCOS is recognized as risk factor for the development of T2DM by the American Diabetes Association (56).

Follow-up studies to assess the conversion rate from normal glucose tolerance to IGT and from IGT to T2DM are scarce. Existing studies estimate the conversion rate from normal glucose tolerance to IGT to range from 2.5 to 3.6% annually over a time of three to eight years (55,57–60).

Typically, women with PCOS show postprandial dysglycemia as a sign of peripheral insulin resistance but rarely fasting dysglycemia as a sign of increased endogenous glucose production (26). Therefore, a 75 g oral glucose tolerance test (OGTT) appears to be the most fitting tool for the diagnosis of glycemic disturbances in PCOS patients (51,61). Consequently, the AE-PCOS Society recommended a 75 g OGTT to be performed in obese PCOS patients with a BMI ≥30 kg/m^2 or in lean PCOS women with an age >40 years who have a family history of T2DM or gestational diabetes mellitus (GDM) (62). The Endocrine Society Guidelines (20) even recommend the performance of a 75 g OGTT in all women with PCOS.

1.1.3.5 Dyslipidemia

Dyslipidemia is very common in PCOS and is estimated to affect approximately 70% of PCOS patients in the United States (62,63). It may present in different patterns, ranging from low levels of high-density lipoprotein (HDL)-cholesterol, increased low-density lipoprotein (LDL)-cholesterol, total cholesterol (TC), and triglycerides to altered LDL-cholesterol quality (62,63).

PCOS patients commonly show the so-called atherogenic lipoprotein phenotype, which consists of hypertriglyceridemia, increased small dense LDL-cholesterol concentration, and decreased HDL-cholesterol concentration. This pattern is comparable to the findings described in T2DM and is considered to be mainly caused by insulin resistance prohibiting insulin to act suppressive on lipolysis and therefore mobilizing free fatty acids from adipose stores (62,63). In consequence, an increased hepatic delivery of free fatty acids suppresses insulin inhibition of very low-density lipoprotein (VLDL) 1 synthesis in the liver, which leads to an altered catabolism of VLDL. Since insulin resistance increases with adipose tissue mass, this pattern is very common in PCOS (62,63).

The prevalence of dyslipidemia shows regional differences. It is generally more common in the United States, where it is estimated to affect about 70% of all women with PCOS and less prevalent in countries with a lower average BMI. However, even in Mediterranean countries, about 50% of PCOS patients show a lipoprotein pattern with low HDL-cholesterol and a small dense LDL-cholesterol phenotype. Therefore, guidelines recommend measurement of triglycerides and HDL-cholesterol at least in obese PCOS patients (6,62).

Increased levels of LDL-cholesterol were also found in women with PCOS, even though the prevalence of increased LDL-cholesterol in PCOS is generally lower than that found for atherogenic dyslipidemia. Its prevalence is estimated to range from 24 to 40% and depends on body weight and could be at least partially associated with hyperandrogenism (62).

1.1.3.6 Obesity

Obesity is a very prevalent feature in PCOS, although representative population data are missing. Additionally, the rate of obese PCOS patients seems to vary depending on geographic differences: studies from the United States reported an increasing prevalence of obesity up to 74% during the last decades, while the prevalence of obesity in Italian PCOS patients was reported to be 14% (64,65).

Increased body weight worsens some of the key features of PCOS, including hyperandrogenism, infertility, menstrual disturbances, dyslipidemia, and insulin resistance. It also increases the risk to develop IGT and T2DM. However, studies failed to reproduce consistent results. PCOS patients – even those who are normal-weight - usually show a high prevalence of abdominal body fat distribution, which is associated with a more pronounced insulin resistance and hyperandrogenism than a peripheral body fat distribution (64,65).

1.1.3.7 Cardiovascular risk

Studies described a predisposition for macrovascular disease and thrombosis in PCOS patients. Women with PCOS show a higher prevalence of coronary artery calcification detected by electron-beam computed tomography. Additionally, several biochemical markers for cardiovascular risk were adversely altered in PCOS: data suggests increased levels of C-reactive protein (CRP), homocysteine, plasminogen activator inhibitor-1 and its activity, vascular endothelial growth factor, asymmetric dimethylarginine, advanced glycation end products, and lipoprotein(a), while showing a great deal of heterogeneity. As a clinical marker of cardiovascular risk, the lipid accumulation product (based on a combination of waist circumference and fasting triglycerides) is typically elevated in PCOS patients (22,66,67).

1.1.3.8 Infertility

Infertility was one of the original symptoms of PCOS described by Stein and Leventhal and is a common complaint at presentation. According to population-based studies, anovulatory infertility occurs in 25-40% of PCOS patients (68,69). Thus, PCOS is considered to be the most common cause of ovulatory dysfunction,

since it accounts for 70-90 % of all ovulatory disorders (20,70). In PCOS patients, studies suggest a monthly spontaneous ovulation rate of about 32% (20).

1.1.4 Treatment

1.1.4.1 Lifestyle intervention

In the general population, cardiovascular fitness – measured as maximal oxygen consumption during exercise – is an independent predictor of cardiovascular mortality, even after adjusting for age, smoking, cholesterol, diabetes, or blood pressure (71). In PCOS, only a few studies aiming to evaluate the efficacy of lifestyle intervention are available. However, these studies suggest that exercise improves body composition, hyperandrogenism, and insulin resistance (72).

Weight loss, whether achieved by lifestyle intervention or through certain medications or bariatric surgery, showed the possibility of normalizing serum androgens in obese PCOS patients. Menstrual irregularities were shown to be improved in some women even after a weight loss of only 5-10% of total body weight (20,73). There is also some evidence for improved pregnancy rates and reduced requirement of fertility increasing treatments after weight reduction, even though there are no randomized-controlled trials (RCTs) to confirm these findings. According to the currently available data, the effects of weight loss on hirsutism seem to be negligible (20).

Despite the limited evidence of lifestyle interventions in PCOS patients, the current clinical practice guidelines published by the Endocrine Society (20) recommended lifestyle intervention to improve metabolic disease in PCOS. It should be initiated with calorie-reduced diets for adolescents and obese or overweight PCOS patients (20). Of note, there is no evidence for superiority of a certain diet. The society concluded that weight loss is probably effective for metabolic and reproductive outcome in this certain setting. However, it is most likely insufficient as treatment for normal-weight PCOS women (20).

1.1.4.2 Hormonal contraceptives

In PCOS patients, the progestin contained in hormonal contraceptives (HCs) suppresses LH levels and consequently ovarian androgen production. Estrogen additionally increases the serum level of SHBG, therefore reducing bioavailable androgens. Furthermore, some progestins possess antiandrogenic properties by antagonizing effects on the androgen receptor and/or by inhibition of 5α-reductase activity (20,74). There is no clear evidence whether oral or parenteral HCs provide better outcome. However, risk-benefit ratios may vary among preparations and with different progestins. Extended-cycle HCs might provide greater hormonal suppression and also prevent rebound ovarian function during the contraceptive-free interval (20).

The effect of HCs on glucose metabolism is not entirely clear, since previous studies had small study populations and were short-term. Predominantly cross-sectional studies in healthy women reported reduced insulin sensitivity and increased glucose response to a glucose load while HCs were used. However, these results varied depending on the contained estrogen and the used progestin (20). Data on the long-term effect of HCs on glucose tolerance in women with PCOS are currently not available. A Cochrane meta-analysis came to the conclusion that HCs do not significantly affect glucose tolerance, although it was based on limited and low-quality evidence (20,75). Nevertheless, long-term studies in healthy women using HCs did not report an increased incidence of T2DM in the general population or in women with a history of GDM (76–78). Furthermore, the use of HCs was not associated with increased complications in women with type 1 diabetes mellitus (77).

The effect of HCs on lipid metabolism is also dependent on the used formulation. If a predominantly estrogenic formulation is used, an increase in HDL-cholesterol and a decrease in LDL-cholesterol levels can be observed (20). The possibility of HCs increasing HDL-levels in PCOS patients seems to be the most promising effect and generally outweighs the negative effect on LDL-cholesterol and triglycerides, since HDL-cholesterol may be the critical link between PCOS and metabolic syndrome (20).

The impact of HCs on body weight is similar in PCOS patients and healthy women. Studies reported unchanged or occasionally improved BMI and waist-to-hip ratio (WHR), depending on existing obesity (20,79,80).

In the current clinical practice guidelines by the Endocrine Society (20), HCs are recommended as first-line therapy for menstrual irregularities and hyperandrogenic symptoms (e.g. hirsutism, acne) in PCOS. The Task Force further recommended screening for possible contraindications and did not favor a certain formulation for the use in PCOS patients (20).

1.1.4.3 Insulin sensitizers

The most commonly used insulin sensitizer used in PCOS is metformin (20). Current guidelines (20) do not suggest the use of metformin to treat hirsutism, while previous studies evaluating the effect on acne were not sufficiently powered (20,81). In a meta-analysis and a systematic review, PCOS patients under metformin treatment lost significantly more weight when compared to placebo. Nevertheless, metformin did not improve weight loss in patients using diet and exercise programs (20,82). Thus, if lifestyle modifications are used to treat obesity, the addition of metformin does not lead to further improvements. Consequently, lifestyle intervention should be the first line intervention in obese women with PCOS, with metformin remaining a treatment option for patients who fail to improve with diet and exercise (20).

The most important change reported under treatment with metformin is improvement of menstrual cyclicity (20). A systematic review and meta-analysis suggested an improvement of ovulation rates in women taking metformin (81). However, studies comparing metformin to HCs revealed that metformin is not as effective in menstrual regulation as HCs (20). In patients with IGT, lifestyle intervention was more successful in preventing a conversion to T2DM than metformin (83). Furthermore, lifestyle intervention appears to be the only available treatment to restore normal glucose tolerance in patients with IGT (83). However, similar trials in PCOS patients with IGT are too small and limited in duration to determine whether metformin prevented a conversion to T2DM or caused a

regression to normal glucose tolerance (20). Nevertheless, metformin is recommended in PCOS women for the prevention of T2DM when lifestyle intervention was not successful (20).

The use of other insulin sensitizing drugs, such as thiazolinediones or inositols, is currently not recommended for the treatment of PCOS. This is a result of current safety concerns regarding thiazolinediones and concerns regarding the formulation and limited evidence of efficacy of D-chiro-inositol (20).

1.1.4.4 Infertility treatment

The best-studied drugs for infertility treatment in PCOS are metformin and clomiphene, with several multi-center studies evaluating their efficacy and safety. In nearly all of these studies, the use of clomiphene resulted in higher pregnancy rates compared to metformin while having comparable rates to injectable gonadotropins (20,84).

A meta-analysis conducted by Tang et al. (81) came to the conclusion that the use of metformin for infertility treatment in PCOS appears to be limited. The authors furthermore reported no evidence for improved live birth rates under metformin treatment, regardless if used in combination with clomiphene or alone. Commonly, metformin has been recommended for infertility treatment, since it is thought to be less associated with multiple pregnancy rates when compared to clomiphene (20). However, studies adequately powered to identify differences in multiple pregnancy rates are still missing. Nevertheless, reported multiple pregnancies under metformin treatment are rare (≤5 % of all pregnancies) and more common when using clomiphene (about 5%) (20).

Metformin might prove helpful as an adjuvant agent in infertility treatment in PCOS (20). However, its use is probably more effective in obese patients than lean PCOS women (85). In a systematic review, clomiphene-resistant women had higher live-birth rates when treated in combination with metformin than with clomiphene alone. The treatment with metformin was also associated with higher live birth rates than laparoscopic ovarian drilling (20,86). Currently, the use of

metformin during pregnancy is unwarranted, however, it might be useful for the treatment of GDM. Metformin also showed no effect on abortion rate, the prevalence of pre-eclampsia or preterm delivery. As expected, it was associated with a higher rate of gastrointestinal disturbances during pregnancy, but no serious maternal or fetal adverse effects (20).

Thus, clomiphene is currently recommended as the first-line therapy of anovulatory infertility in PCOS, while metformin is suggested as an adjuvant agent (20).

1.2 Vitamin D

The discovery of vitamin D resulted from the efforts to find a cure for rickets. Since the discovery of fat-soluble vitamin A by McCollum and Davis at the beginning of the 20th century (87), the ability of cod oil to cure rachitic conditions was considered another property of vitamin A (88,89). However, McCollum was able to shut down the activity of vitamin A in cod oil, but the ability to cure rickets remained. He therefore concluded that this represented the discovery of a new vitamin, and called it vitamin D (89). It was discovered with many other vitamins at this time and is classified as a vitamin even until now. However, findings from the second half of the 20th century showed that vitamin D is in fact a prohormone. It cannot be found in plant materials (e.g., fruit, vegetables) and is only present in low abundance in meat and other animal-derived sources (with the exception of some rare cases, such as fish liver oil) (89).

1.2.1 Synthesis and metabolism

Humans get vitamin D from exposure to sunlight, from their diet, and from dietary supplements (90). Solar ultraviolet B radiation with a wavelength of 290 to 315 nm penetrates the skin and converts a derivative of cholesterol, 7-dehydrocholesterol, to previtamin D3, which is further converted to cholecalciferol (vitamin D3) (90). Excessive exposure to sunlight does not lead to vitamin D intoxication, since any excess previtamin D3 or cholecalciferol is destroyed by sunlight (90). Cholecalciferol represents the natural form of vitamin D produced by the skin, whereas ergocalciferol (vitamin D2) is synthesized by ultraviolet irradiation of ergosterol. This process occurs to some degree in plankton and is used to produce ergocalciferol from the mold ergot (89).

Vitamin D produced in the skin and gained from diet is metabolized to 25-hydroxyvitamin D [25(OH)D] by the liver (90). As it increases in proportion to intake, the 25(OH)D level in the serum is used to measure a patient's vitamin D status (91). However, 25(OH)D itself possesses no metabolic function and needs to be modified. Thus, 25(OH)D can be converted to its active form, 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], in the kidneys by the enzyme 25-hydroxyvitamin

D-1α-hydroxylase (CYP21B1) (89,90). Under certain circumstances (e.g., pregnancy, chronic kidney failure, sarcoidosis), $1,25(OH)_2D$ can also be synthesized by extrarenal cells. However, hormones produced this way often serve as an autocrine/paracrine factor with cell-specific functions (91).

The activity of the renal 1α-hydroxylase and therefore the renal production of $1,25(OH)_2D$ is a highly regulated process. Dietary calcium affects the homeostasis directly by increasing serum calcium and indirectly by its influence on parathyroid hormone (PTH) (91,92). Dietary phosphate restriction increases the renal 1α-hydroxylase activity independent from serum calcium and PTH levels (91). Fibroblast growth factor 23, a hormone secreted from the bone, exerts phosphaturic effects on the kidney, while impairing the formation of $1,25(OH)_2D$ (93). A summary of the mechanisms underlying vitamin D synthesis and metabolism is depicted in Figure 2.

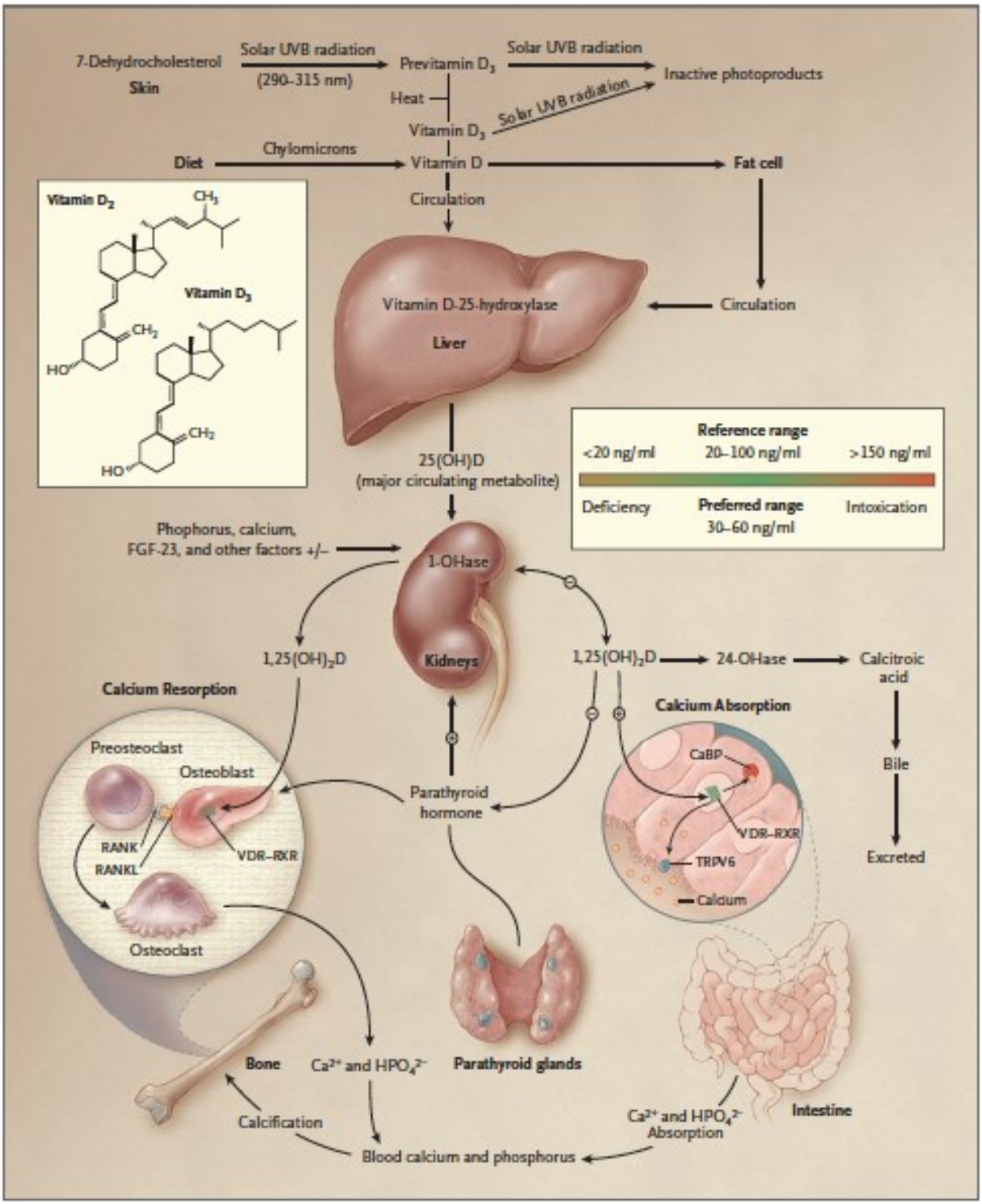
Vitamin D metabolites are lipophilic molecules with low aqueous solubility and thus need to be transported bound to plasma proteins. The most important of these proteins is the so-called vitamin D binding protein (DBP) (91). It binds vitamin D metabolites with a high affinity and its plasma levels are about 20 times higher than the levels of vitamin D metabolites combined. More than 99% of all vitamin D compounds are bound to proteins; while most of the metabolites are bound to DBP, albumin and lipoproteins also play an important role (91). Vitamin D bound to DBP has limited access to target cells and is therefore less susceptible to hepatic metabolism and consequently excretion, resulting in a longer half-life in circulation. There is evidence that only unbound metabolites are able to enter target cells to be further metabolized or to exert biological activity (91,94).

Most of the biological effects of $1,25(OH)_2D$ are mediated by the vitamin D receptor (VDR), a member of the superfamily of nuclear receptors for steroid hormones. Therefore, it acts as a ligand-activated transcription factor with a high affinity for $1,25(OH)_2D$ (91). Many polymorphisms of the VDR gene located on chromosome 12 are described in humans, with substantial differences between races and ethnic groups (95,96). These variants were associated with alterations in bone mass density, propensity to hyperparathyroidism, resistance to vitamin D

supplementation, and susceptibility to autoimmune diseases, infections, and cancer (91).

Currently, a universally accepted normal range for 25(OH)D serum concentrations does not exist. Thus, it remains unsure whether, e.g., 50 nmol/L or 75 nmol/L should be considered as sufficient 25(OH)D levels. The Institute of Medicine report on dietary reference intakes for calcium and vitamin D (97) considers a 25(OH)D serum concentration of 50 nmol/L sufficient to cover the requirements of 97.5% of the population and a serum concentration of 40 nmol/L sufficient to cover the requirements of 50% of the population. The Endocrine Society defines vitamin D deficiency as 25(OH)D serum levels below 50 nmol/L, while serum concentrations between 52.5 and 72.5 nmol/L were defined as vitamin D insufficiency (98). For the treatment of vitamin D deficiency, the use of cholecalciferol is generally recommended (99), since it showed improved efficacy in raising serum concentrations of 25(OH)D when compared to ergocalciferol (100). In general, the supplementation of 1,000 IU of cholecalciferol daily leads to an increase in 25(OH)D serum levels by approximately 25-50 nmol/L. This increase, however, is highly dependent on baseline 25(OH)D concentrations, body weight, and calcium intake, leading to a significant inter-individual variation (99,101–103).

Figure 2 Synthesis and metabolism of vitamin D and its effect on calcium, phosphorus, and bone metabolism. Reproduced with permission from (90), Copyright Massachusetts Medical Society



1.2.2 Biological actions of vitamin D

Vitamin D plays an important role in the interaction of kidney, bone, parathyroid gland, and intestine, maintaining extracellular calcium levels within narrow limits, with vital consequences for cellular physiology and skeletal integrity (91).

Without the effects of vitamin D, only 10-15% of dietary calcium and 60% of dietary phosphorus can be absorbed in the intestine. Binding of $1,25(OH)_2D$ to the VDR increases calcium absorption to 30-40% and the efficiency of phosphorus absorption to about 80% (90). Study data suggests a relation of vitamin D levels to bone mass density, with a maximum density at a 25(OH)D serum level of 100 nmol/L (divide by 2.496 to convert nmol/L to ng/mL) and above (104). When 25(OH)D levels were below 75 nmol/L, the intestinal calcium absorption was significantly decreased and associated with increased serum levels of PTH (105,106). PTH causes an activation of osteoblasts in the bone, which stimulate the maturation of preosteoclasts into osteoclasts. Mature osteoclasts are able to dissolve the mineralized collagen matrix in the bone, leading to osteopenia and eventually osteoporosis associated with an increased risk of fractures (90). Progressive vitamin D deficiency leads to a maximum stimulation of the parathyroid gland with the result of secondary hyperparathyroidism (107). PTH increases the transformation of 25(OH)D to $1,25(OH)_2D$ and therefore further exacerbates the measured vitamin D deficiency. Increased levels of PTH also lead to phosphaturia, causing low-normal or decreased serum phosphate concentrations. This leads – as a consequence of an adequate calcium-phosphorus product – to a diminished mineralization of the collagen matrix and therefore to the classic signs of rickets in children and osteomalacia in adults (90).

As already mentioned, vitamin D is closely linked to osteoporosis and fractures. Osteoporosis affects approximately 33% of women within an age range of 60 to 70 years and 66% of women older than 80 years (90,108,109). An estimated 47% of women and 22% of men over an age of 50 years will experience an osteoporotic fracture in their remaining lifetime (90). Several studies reported a reduction of hip fractures and nonvertebral fractures under supplementation of vitamin D and calcium. In studies using daily doses of 700 to 800 IU of cholecalciferol, the

relative risk of hip fractures was reduced by 26% and the relative risk of nonvertebral fractures by 23% when compared with calcium or placebo (90,104). An optimal prevention of both hip and nonvertebral fractures only occurred in patients whose initial vitamin D serum level was below 42.5 nmol/L and who received 700 to 800 IU of vitamin D per day to raise their serum level to approximately 100 nmol/L (90,104). However, it must be noted that some study data suggests a higher risk of falls in elderly populations following high-dose vitamin D supplementation (110).

After the discovery of the VDR, one of the most important findings was the detection of the receptor in other cells than osteoblasts, enterocytes, and distal renal tube cells (89). Overall, $1,25(OH)_2D$ directly or indirectly controls more than 200 genes, among which are genes responsible for angiogenesis and regulation of cell proliferation, differentiation, and apoptosis (90). This led to the investigation of vitamin D effects not primarily linked to bone metabolism (89,90). $1,25(OH)_2D$ is a potent immunomodulator and therefore plays a role in several immunologic processes. Studies discovered an up-regulation of VDR gene and the D-1α-hydroxylase gene in macrophages and monocytes exposed to a lipopolysaccharide or to Mycobacterium tuberculosis. Furthermore, an increased production of $1,25(OH)_2D$ leads to the synthesis of cathelicidin, a peptide able to destroy Mycobacterium tuberculosis and several other infectious agents. Vitamin D deficiency seems to affect T cell-mediated immunity in particular, whereas excess vitamin D levels were discovered to suppress certain immunologic aspects (89,90).

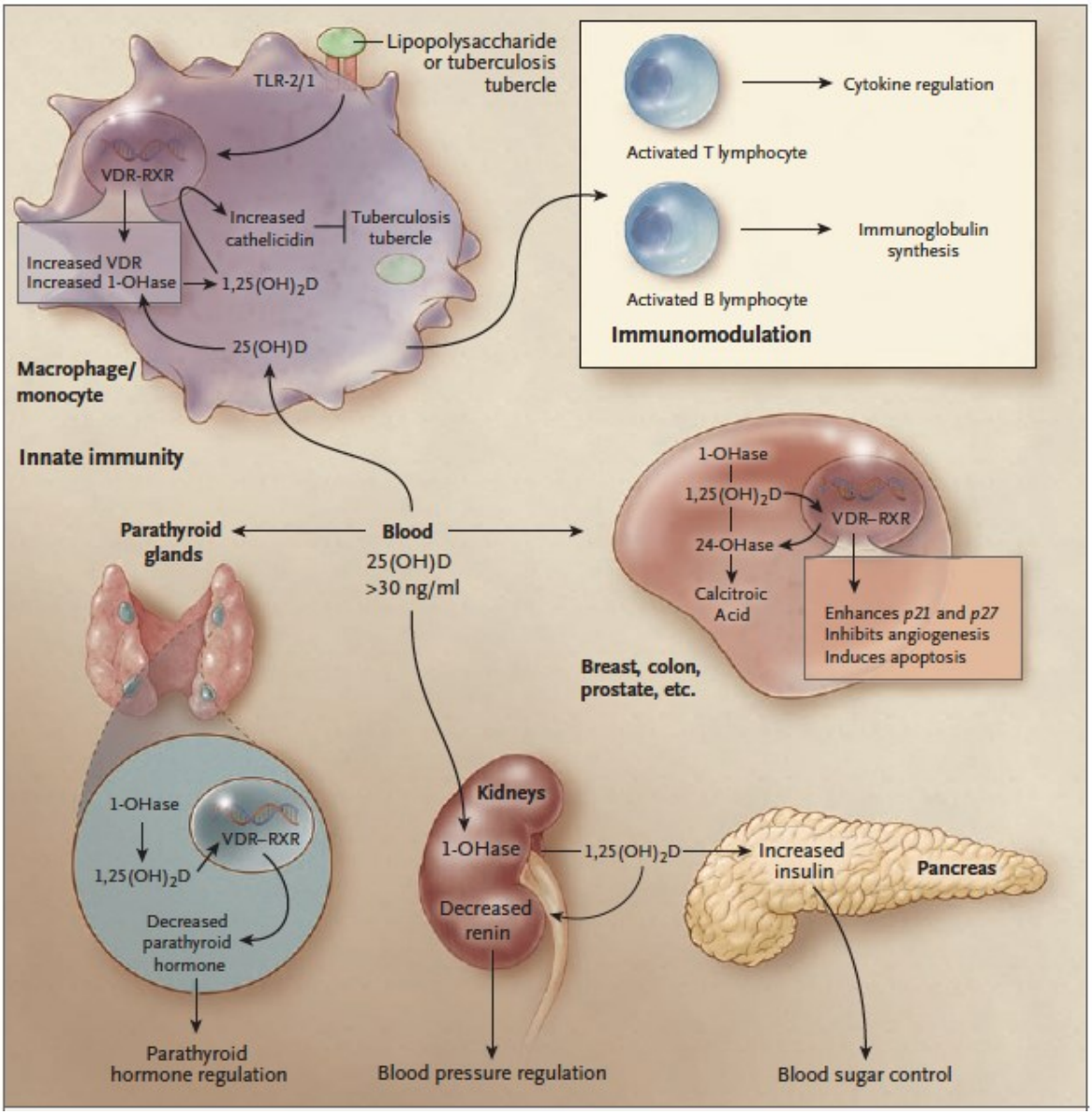
Already in the 20^{th} century, observations of a correlation between higher sunlight exposure and reduced cancer mortality were made. Therefore, as UV exposure is required to produce vitamin D in the skin, it was postulated that vitamin D could protect against cancer (111). It was furthermore observed that people living at a higher latitude possess a higher risk of developing Hodgkin's lymphoma a well as pancreatic, colon, prostate, breast, ovarian, and other cancers and have a higher mortality resulting from these malignancies when compared to people living at lower latitudes (90,112). The activation of the VDR appears to initiate various molecular effects that protect against cancer and alter its progression. In

epidemiological studies, low 25(OH)D levels were associated with various manifestations of cancer. Patients already diagnosed with cancer showed a significantly reduced survival if they also were vitamin D deficient (111). Nevertheless, data from RCTs regarding cancer incidence and mortality are scarce and have mostly failed to prove any impact. However, two recent meta-analyses of RCTs reported a modest, statistically significant reduction of overall cancer mortality in association with vitamin D supplementation (90,111,113).

Several studies aimed to evaluate the impact of vitamin D on cardiovascular health. Activation of the VDR leads to suppression of renin, therefore influencing the renin-angiotensin-aldosterone system (114). Other effects of vitamin D considered to act antihypertensive include renoprotective actions, anti-inflammatory properties, direct effects on the vasculature, and regulation of calcium homeostasis including a suppressive effect on PTH (114). Observational studies reported an association of vitamin D deficiency with an adverse cardiovascular risk profile. However, data derived from RCTs on vitamin D status and cardiovascular risk factors were less in favor of showing significant effects (90,114,115).

Figure 3 gives an overview over non-skeletal effects of vitamin D.

Figure 3 Non-skeletal effects of vitamin D. Reproduced with permission from (90), Copyright Massachusetts Medical Society



1.2.3 Effects of vitamin D on glucose metabolism

The VDR and vitamin D metabolizing enzymes are expressed in pancreatic beta-cells, indicating a possible role of vitamin D in beta-cell function (116,117). Additionally, $1,25(OH)_2D$ improves the insulin sensitivity of target cells and may protect beta-cells from autoimmune damage (118,119). Several experimental studies implicate that impaired glucose mediated insulin secretion could be

improved in vitamin D deficient subjects by vitamin D supplementation. However, data regarding basal insulin secretion are not concise and do not necessarily implicate an impact of VDR activation (116). Additionally, in studies with VDR knockout mice, a development of glucose intolerance could not be consistently demonstrated (117).

The effect of vitamin D on calcium metabolism may also have a significant influence on glucose metabolism, since calcium is necessary for insulin synthesis and secretion as well as for physiological glucose metabolism (116,117,120). Therefore, probable antidiabetic effects of vitamin D may be mediated by changing calcium levels, which consequently might stimulate insulin secretion. Thus, direct effects on calcium levels as well as calcium independent effects of the VDR may play a role in glucose metabolism (116). Another possible effect of vitamin D on glucose metabolism derives from its influence on PTH levels, since elevated PTH levels were associated with increased insulin resistance (121). As VDR activation leads to a multitude of effects on cytokine secretion and immune function, anti-autoimmunological features of vitamin D were investigated in regard to type 1 diabetes, whereas the anti-inflammatory effects of vitamin D may prove to be relevant for T2DM (116).

Observational studies on the role of vitamin D in glucose metabolism yielded mixed results. Higher sun exposure was associated with a decreased incidence of T2DM, in line with possible seasonal variations in parameters of glycemic control (116,122,123). It was also shown that the incidence of T2DM is significantly lower in women with high daily vitamin D intake compared to women with low daily vitamin D intake (124). Additionally, higher levels of 25(OH)D were associated with a lower risk of incident T2DM, even after multivariate adjustments (125). However, vitamin D deficiency is at least partially a consequence of obesity and low physical activity. Therefore, regardless of careful adjustments, residual confounding related to these factors cannot be entirely ruled out (116).

RCTs in the general population showed no effect of vitamin D on glucose metabolism. These studies reported no significant impact of vitamin D supplementation on glucose, insulin, and parameter of insulin resistance and

sensitivity when compared to placebo (126,127). Furthermore, RCTs in subjects with obesity or prediabetes also predominantly failed to prove any beneficial effects of vitamin D supplementation on glucose metabolism, as slight improvements in insulin resistance reported by a few studies could not be confirmed by the majority of the conducted trials (116,126,127).

1.2.4 Effects of vitamin D on fertility

In both males and females, the VDR can be found across various tissues of the reproductive system. In females, VDR mRNA is expressed in ovarian cells, suggesting a possible role in the synthesis of sex steroids (128,129). The VDR can also be found in endometrial cells as well as the human pituitary gland (128,130). Moreover, the VDR and CYP27B1 (which encodes 1α-hydroxylase) are expressed by the human placenta (131,132). In cultured human ovary cells, $1,25(OH)_2D$ lead to a 13% increased production of progesterone (129). It also increased estradiol production by 9% and estrone production by 21% (129). Furthermore, $1,25(OH)_2D$ regulates human chorionic gonadotropin expression and its secretion in syncytiotrophoblasts as well as increasing placental production of sex steroids (133).

In male rodents, the VDR is expressed in Sertoli cells, spermatogonia and smooth muscle cells of the epididymis, suggesting a possible involvement of vitamin D in spermatogenesis and sperm maturation (133–135). In humans, the VDR was found in testicular tissue and sperm (136,137). Testosterone appears to down-regulate VDR expression in testis cells (133). In human osteoblasts, androgens increase the activity of 1α-hydroxylase, therefore stimulating the conversion of precursors to $1,25(OH)_2D$ (133).

Several studies in humans suggest an association of vitamin D and fertility. Levels of 25(OH)D show seasonal differences with high levels in summer and autumn and low levels in winter and spring. In northern countries, an increased conception rate in summer could be observed, whereas the rate decreased in the winter months. Furthermore, in these countries, endometrial receptiveness and ovulation rates appear to be reduced during the prolonged winter season (138,139). Several

explanations were suggested for these circumstances, including brain transmitters such as serotonin and dopamine or alterations in the hypothalamic-pituitary axis (133,138). Nevertheless, this might also partly be an effect of the seasonal variation in vitamin D levels (133).

Studies in women undergoing in vitro fertilization (IVF) produced conflicting results. Certain studies suggested higher pregnancy rates in women with higher serum levels of 25(OH)D while undergoing IVF (140). However, in a study investigating over 100 women who underwent IVF, women with a sufficient vitamin D level in the follicular fluid (>75 nmol/L) showed a lower embryo quality and were less likely to achieve pregnancy when compared to women with insufficient or deficient vitamin D levels (133,141).

Since the pathogenesis of endometriosis is understood as an alteration in immunologic processes and inflammatory responses, an influence of vitamin D in these mechanisms has been discussed (133). Additionally, as mentioned previously, endometrial cells express the VDR (128). In studies investigating possible correlations, a higher expression of the VDR and 1α-hydroxylase in the endometrium could be found in women with endometriosis when compared to a healthy control group. However, there was no significant difference in 25(OH)D serum levels between the groups (128). Other study data suggests a possible correlation between high 25(OH)D levels and an increased risk of developing endometriosis (142), while another study found increased $1,25(OH)_2D$ levels in women with endometriosis, while 25(OH)D levels were comparable between these patients and a control group (143).

In men, vitamin D may be associated with semen quality, hypogonadism, spermatogenesis and testiculopathies (133). Calcium appears to play an important role within the male reproductive system, as it is essential for spermatogenesis, sperm motility, hyperactivation, and acrosome reaction (144). Since vitamin D is known to be an important regulator of the calcium metabolism (133), its impact on spermatogenesis and semen quality was assessed by several studies. Thus, evidence regarding a positive correlation between 25(OH)D levels and sperm motility and progressive motility was found (145). Men with 25(OH)D levels <25

nmol/L also showed a lower rate of motile, progressive motile, and morphologically unimpaired spermatozoa when compared to men with sufficient 25(OH)D status (serum levels >75 nmol/L) (145). In vitro, $1,25(OH)_2D$ increases intracellular calcium concentrations in human spermatozoa through VDR-mediated calcium release, leading to increased sperm motility and inducing the acrosome reaction (133,145). Furthermore, a study investigating the expression of CYP2R1 (which encodes 25-hydroxylase) in patients with testiculopathy and healthy controls and found a significantly decreased gene and protein expression of CYP2R1 and significantly lower 25(OH)D serum levels in patients with testiculopathy (146). These patients also showed osteoporosis and osteopenia with increased bone-turnover markers and decreased bone mineral density, despite having normal testosterone levels (133,146).

Both low androgens and low vitamin D levels are associated with an increased mortality in men (133,147,148). Since both are related to obesity, obesity might prove to be a confounder regarding the association of androgen and vitamin D status with mortality (133). However, in studies investigating the relation of these parameters with mortality, the results remained significant even after adjusting for BMI (147,148). Studies furthermore showed that androgens are able to increase 1α-hydroxylase as well as an influence of androgen levels on the regulation of gene expression by vitamin D metabolites (149,150). Moreover, studies reported an independent correlation of 25(OH)D levels with androgen levels underlying a seasonal variation (151,152). Additionally, vitamin D supplementation might lead to an increase in testosterone levels by yet unexplained mechanisms (133).

During pregnancy, vitamin D deficiency is a well-known issue, since pregnant women have significantly lower serum levels of 25(OH)D than non-pregnant women (153). In the United States, approximately two out of three pregnant women have suboptimal vitamin D status, with even higher prevalence among certain ethnic groups (154). Studies indicate a possible independent association of maternal vitamin D deficiency with an increased risk of developing GDM (155). In women with GDM, serum levels of 25(OH)D are significantly lower than in women without prevalent GDM (156). Moreover, vitamin D deficiency in pregnant women could also be related to an elevated risk of preeclampsia, bacterial vaginosis,

small-for-gestational age births, offspring rickets, reduced bone mineral density, and asthma (133). A study assessing the safety of vitamin D supplementation during pregnancy reported no adverse effects even with daily supplementation doses of 4,000 IU (157). In addition, vitamin D supplementation lead to a reduction of preterm deliveries by 50%, a reduction of maternal infections by 25%, and a 30% reduced rate in comorbidities (133,157).

1.3 Polycystic ovary syndrome and vitamin D

Vitamin D deficiency is a common problem in PCOS patients. Its prevalence in PCOS patients is estimated to be 67-85%, compared to 20-48% in the general adult population (158,159). However, studies that compared vitamin D levels in PCOS patients with healthy control women yielded varying results. In a study by Li et al. (160), PCOS patients showed lower vitamin D levels when compared with women without PCOS (27.5 nmol/L vs. 42.5 nmol/L), although this difference was not significant. However, the ovulatory group had a lower BMI and was significantly older than the PCOS group. Wehr et al. (161) also reported lower levels of vitamin D in PCOS women when compared with controls (64.3 nmol/L vs. 80.0 nmol/L). In contrast, Mahmoudi et al. (162) found higher levels of vitamin D in PCOS patients than in a control group matched for BMI and age.

Most studies agree upon an inverse relation between body weight (BMI, body fat or waist measurements) and serum levels of 25(OH)D in PCOS patients (159). These studies reported 27-56% lower levels of vitamin D in obese PCOS patients when compared to non-obese PCOS women (159). Data also suggests that low levels of 25(OH)D are determined by the degree of adiposity (BMI and total body fat mass) and not affected by whether patients developed insulin resistance (163). As a fat-soluble hormone, the high prevalence of vitamin D deficiency in PCOS may be a consequence of obesity, since a higher proportion of vitamin D is sequestered in adipose tissue, therefore lowering bioavailability. Obese patients may also spend less time outdoors exposed to sunlight and have different dietary preferences, which might be alternative explanations for their reduced serum vitamin D levels (159).

Evidence exists for a possible correlation of vitamin D deficiency and the development of insulin resistance and metabolic syndrome in PCOS. The effects of the VDR on SHBG, LH, testosterone, insulin resistance, and insulin levels haven been linked to the pathogenesis of PCOS (159,164). The synthesis of PTH is stimulated by vitamin D deficiency, and elevated serum levels of PTH are independently associated with PCOS, elevated testosterone levels, and anovulatory infertility (159,164). This state might also be aggravated by low dietary

calcium intake. A study investigating the impact of dietary calcium on PCOS found an independent association of high testosterone levels with low dietary calcium intake (165). Vitamin D furthermore regulates estrogen biosynthesis by directly regulating the expression of the aromatase gene and by maintaining extracellular calcium homeostasis. Thus, in human ovary tissue, the effect of $1,25(OH)_2D$ on estrogen and progesterone production and the lack of effect on testosterone may be explained by the changes in aromatase activity. In follicle cells of PCOS patients, aromatase gene expression was found to be decreased when compared to controls (159,166).

As highlighted previously, vitamin D status may play a role in the development of insulin resistance. As insulin resistance is a common problem in PCOS, several observational studies aimed to investigate a possible correlation with vitamin D. Hahn et al. (167) reported an association of low levels of 25(OH)D with insulin resistance and obesity in PCOS women. A possible confounding role of obesity on this association could be disproved by other studies, reporting increased insulin resistance in PCOS women with severe vitamin D deficiency independent from BMI or WHR (160). In a multivariate regression analysis conducted by Wehr et al. (168), levels of 25(OH)D were an independent predictor for HOMA-IR together with BMI.

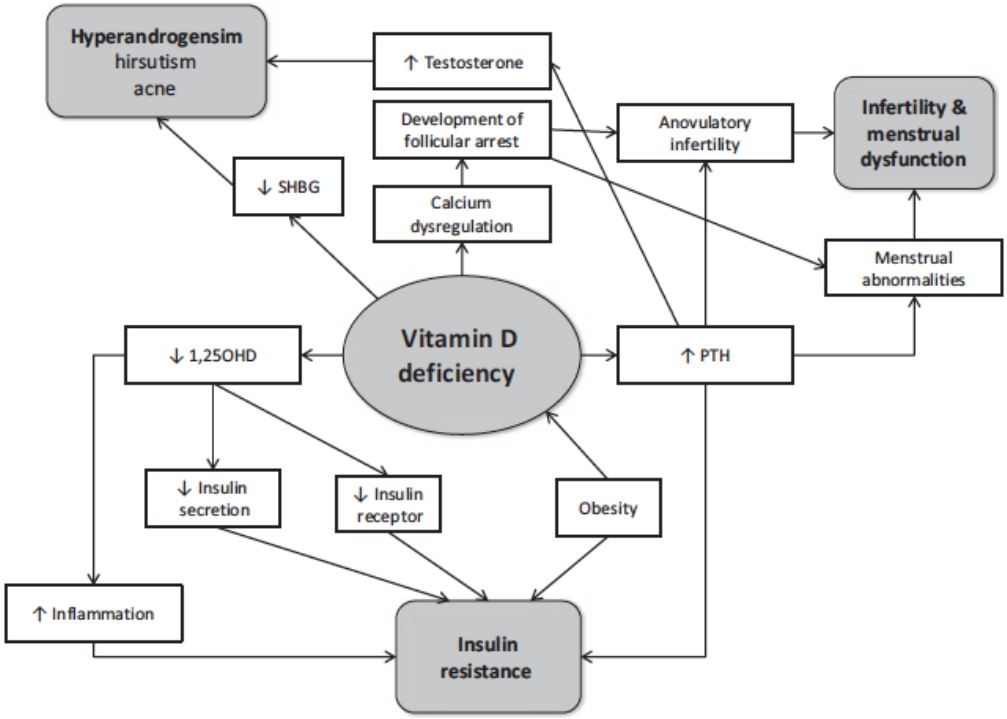
Accumulating evidence also supports the hypothesis of a possible role of vitamin D in ovulatory function and hyperandrogenemia in PCOS. Vitamin D deficiency is associated with calcium dysregulation, which leads to follicular arrest in PCOS and therefore to menstrual and fertility dysfunction. Some studies supplementing vitamin D deficient PCOS women with vitamin D and calcium reported a normalization of menstrual irregularities in the majority of patients, therefore supporting this hypothesis (159,169).

So far, several interventional trials were conducted in order to confirm possible effects of vitamin D supplementation on metabolic and endocrine parameters as suggested by cross-sectional studies. However, these studies mostly yielded mixed results. Jamilian et al. (170) reported a significant decrease in TT, FAI, hirsutism and CRP and a significant increase in SHBG and total antioxidant

capacity after supplementation of 4,000 IU of cholecalciferol daily when compared to 1,000 IU daily or placebo (n=90 PCOS women). Foroozanfard et al. (171) conducted a similar study in the same number of patients with a matching intervention scheme, and found a significant decrease in fasting plasma glucose, serum insulin, and HOMA-IR, as well as significant decreases in mean change of triglycerides, VLDL-cholesterol, LDL-cholesterol, and TC/HDL-cholesterol ratio. However, other studies like those conducted by Raja-Khan et al. (172) or Garg et al. (173) failed to demonstrate significant effects of vitamin D supplementation on metabolic and endocrine parameters in PCOS.

Possible effects of vitamin D in PCOS are summarized in Figure 4.

Figure 4 Possible effects of vitamin D on the pathogenesis of PCOS.
Reproduced from (159) with permission of publisher John Wiley and Sons



1.4 Aims

The aim of this dissertation was to evaluate the effects of vitamin D supplementation on metabolic and endocrine parameters in women with and without PCOS. Main outcome measure was the plasma glucose area under the curve (AUCgluc) during a 75 g OGTT, while secondary outcome measures were several other metabolic and endocrine parameters, including TT and changes in menstrual frequency (in PCOS patients) (174).

2 Material and Methods

2.1 Study design

This study was a single-center, double-blind, placebo-controlled randomized-controlled trial conducted at the Medical University of Graz, Austria. It was designed to investigate the effects of vitamin D supplementation over 24 weeks on metabolic and endocrine parameters in both women with and without PCOS. An additional follow-up visit after 12 weeks was scheduled for participants from both groups to investigate possible short-time effects of vitamin D supplementation (174). The study protocol was approved by the ethics committee of the Medical University of Graz. The design, conduction, and publication of this study adhere to the Consolidated Standards of Reporting Trials (CONSORT) 2010 statement (175). The trial was registered at http://www.clinicaltrialsregister.eu (EudraCT number, 2011-000994-30) and at clinicaltrials.gov (ClinicalTrials.gov Identifier NCT01721915). Parts of the described methods were published previously (174).

2.2 Subjects

In total, 180 patients with PCOS and 150 healthy control women without PCOS were recruited for this study. PCOS patients were recruited from women who were routinely referred to the outpatient clinic of the Division of Endocrinology and Diabetology, Department of Internal Medicine, Medical University of Graz, Austria (174). Healthy control women without PCOS were either also recruited from patients from the same outpatient clinic who met the respective inclusion criteria or were recruited from women responding to promotional material (postings at the Medical University of Graz, internet advertisements) after approval by the local ethics committee.

The diagnosis of PCOS was made according to the Rotterdam criteria, which require two out of the following three symptoms to be present in each patient: oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism, polycystic ovaries (diagnosed by ultrasound) (6,174). Different disorders with similar symptoms (e.g. CAH, Cushing's syndrome, hyperprolactinemia) were excluded before the diagnosis was made. Participants in the group without PCOS were required to show none of the above-mentioned criteria for the diagnosis of

PCOS (6,174). Participants from both group needed to be older than 18 years and premenopausal (evaluated by menstrual history and gonadotropin levels) and show serum concentrations of 25(OH)D <75 nmol/L (174). Exclusion criteria for both groups were:

- Hypercalcemia, defined as total serum calcium concentration >2.65 mmol/L
- Withdrawal of given informed consent
- Use of HCs (oral contraceptives, transdermal contraceptive patches, parenteral contraceptives, hormonal intrauterine devices) within three months before study participation
- Use of insulin sensitizers (metformin, incretins, thiazolidinedione, sulfonylurea) within six months before study participation
- Use of lipid-lowering drugs or other drugs known to affect insulin sensitivity (e.g., corticosteroids, beta blockers, calcium channel blockers, thiazide diuretics) or serum androgen levels (estrogens, anti-androgens, androgens) within three months before study participation
- Regular vitamin D intake within three months before study participation
- Prevalent T2DM (174)

All study participants gave written informed consent prior to any study related procedures.

2.3 Intervention

Study participants from both the vitamin D and the placebo group were allocated in a 2:1 manner to receive either vitamin D or placebo according to a computer-generated randomization list. This list was created by a web-based software (http://www.randomizer.at) with good clinical practice compliance confirmed by the Austrian Agency for Health and Food safety (AGES). The 2:1 ratio (vitamin D:placebo) was chosen to increase the sample size in the vitamin D group for further evaluation of possible vitamin D effects (174).

According to the computer-generated randomization list, both the study medication and the placebo were filled into numbered bottles. Participants with and without

PCOS who were allocated to the intervention group received 20,000 IU of cholecalciferol (vitamin D3) weekly as 50 oily drops per week (Oleovit D3-drops produced by Fresenius Kabi Austria GmbH, Linz, Austria) over 24 weeks (174). Participants who were allocated to the placebo group received 50 oily drops without cholecalciferol over 24 weeks that could not be distinguished from the study medication in smell, look, or taste. To improve participant compliance and to verify regular intake of the study medication, participants were asked to return the empty bottles at the final study visit after 24 weeks. All investigators who enrolled participants, collected data, or assigned intervention were masked to participant allocation (174).

2.4 Primary outcome measure

The primary outcome measure for participants with and without PCOS was the between-group difference (vitamin D vs. placebo) in AUCgluc during OGTT after 24 weeks (174).

2.5 Secondary outcome measures

Secondary outcome measures for both women with and without PCOS were between-group differences (vitamin D vs. placebo) in HOMA-IR, TC, glycated hemoglobin (HbA1c), TT, FT, insulin sensitivity assessed by QUICKI, and triglycerides after 24 weeks. In participants with PCOS, another secondary outcome measure was the between-group difference (vitamin D vs. placebo) in menstrual frequency. To investigate possible short-time effects of vitamin D supplementation, we additionally investigated effects of vitamin D supplementation on primary and secondary outcome parameters after a study visit after 12 weeks (174).

2.6 Procedures

At each study visit (baseline visit, follow-up visits after 12 and 24 weeks, respectively), patient interviews, physical examinations, and blood samplings were conducted between 8.00 and 9.00 a.m. after an overnight fast of more than 12 hours. From each study participant, anthropometric measurements (i.e., height, weight, blood pressure, waist and hip circumference) were obtained at each of the

study visits (174). BMI was calculated as body weight in kg divided by the height in meters squared. Blood pressure was measured after a sitting resting period of five minutes or more. Waist circumference was measured midway between the lower costal margin and the iliac crest in a standing position. Hip circumference was measured at the maximum circumference over the buttocks in a standing position. To assess the presence of clinical hyperandrogenism in participants in the PCOS group, hirsutism was classified according to the modified Ferriman-Gallwey-score (15,174).

Initially, both 25(OH)D and TT were measured by immunoassays to establish the diagnosis of PCOS (for participants in the PCOS group) or to rule out biochemical hyperandrogenism (for participants in the group without PCOS) as well as to assess the inclusion criterion of a 25(OH)D concentration <75 nmol/L (174). For this purpose, 25(OH) was measured by an enzyme immunoassay (IDS, Boldon, UK) with an intra- and interassay coefficient of variation (CV) of 5.6 and 6.4%, respectively. TT was initially measured by a luminescence immunoassay (Siemens, Erlangen, Germany) with intra- and interassay CVs of <10% (174). Additionally, both 25(OH)D and TT were measured by a well-standardized Isotope-Dilution Liquid Chromatography Tandem Mass Spectrometry (ID-LC-MS/MS) at the VU University Medical Center, Amsterdam, the Netherlands, in 2017 (174,176–178). For ID-LC-MS/MS measurements of 25(OH)D, the internal standards $^{13}C_5$-25(OH)D_3 and $^{2}H_6$-25(OH)D were added to the samples and 25(OH)D was released from its binding proteins with acetonitrile. Following liquid-liquid extraction by hexane, the samples were analyzed by LC-MS/MS (Acquity UPLC coupled to a Quattro Premier XE MS/MS, Waters Corp., Milford, MA, USA). The limit of quantification (LOQ) was 4.0 nmol/L. For concentrations between 17 and 160 nmol/L, intra- and interassay CVs were <4.5% and <5.5%, respectively (174). For ID-LC-MS/MS measurements of TT, the internal standard $^{13}C_3$-testosterone was added to the samples and testosterone was released from its binding proteins with acetonitrile. Following liquid-liquid extraction by hexane, the samples were analyzed by LC-MS/MS (Acquity 2D-UPLC coupled to a Xevo TQ-S tandem mass spectrometer, Waters Corp., Milford, MA, USA). The LOQ was 0.10 nmol/L. Interassay variation at 0.1 nmol/L was 10.6% and <6% between 0.9 and 14 nmol/L (174). In this dissertation, all 25(OH)D and TT values show parameters

derived from the ID-LC-MS/MS measurements. These values were also used for all statistical analyses.

Insulin was measured by luminescence immunoassay (Siemens, Erlangen, Germany) with intra- and interassay CVs of 4% and 2.6%, respectively. PTH was measured by ElectroChemiLuminescence immunoassay (ECLIA; Roche Diagnostics, Mannheim, Germany) with intra- and interassay CVs of 1.5-2.7% and 3.0-6.5%, respectively. For the calculation of FT and FAI, SHBG was measured by luminescence immunoassay (Cobas, Roche, Basel, Switzerland) with intra- and interassay CVs of 1.3% and 2.1%, respectively. Androstendione and DHEAS were measured by ELISA (DiaMetra, BioVendor, Brno, Czech Republic and LDN Labor Diagnostika Nord GmbH, Nordhorn, Germany) with intra- and interassay CVs of <10%. Estradiol was measured by chemiluminescence immunoassay (Siemens, Erlangen, Germany) with intra- and interassay CVs of 6.3-15% and 6.4-16%, respectively. FSH and LH were measured by enzyme immunoassay (DiaMetra S.r.l., Segrate (MI), Italy) with intra- and interassay CVs of <10%. $1,25(OH)_2D$ was measured by chemiluminescence immunoassay (IDS, Boldon, UK) with intra- and interassay CVs of 6.4-12.1% and 6.6-9.6%, respectively. Other parameters (i.e., plasma glucose, HbA1c, TC, HDL-cholesterol, LDL-cholesterol, CRP, plasma calcium) were measured by routine laboratory diagnostics. To avoid possible glycolysis, plasma glucose was measured form tubes containing sodium fluoride (174).

At each of the three study visits, a 75 g OGTT was performed in each patient. Blood samples for the measurement of plasma glucose and insulin were drawn at baseline and after 30, 60, and 120 minutes. AUCgluc was calculated following the trapezoidal method. As an estimate of insulin resistance, HOMA-IR was calculated as fasting plasma insulin [µU/mL] x fasting plasma glucose [mg/dL] / 405 (174). To estimate insulin sensitivity, QUICKI was calculated as 1/log fasting plasma insulin [µU/mL] + log fasting plasma glucose [mg/dL] (179). FT was calculated according to Vermeulen et al. (180).

During the screening visit, eligible participants were randomized according to the computer-generated randomization list and received the study medication as well

as appointments for the follow-up visits after 12 and 24 weeks. All study participants furthermore received printed menstrual calendars and were instructed to document the length and frequency of menstrual cycles during study participation. To evaluate possible menstrual changes, the participants were told to return the menstrual calendar at the final follow-up visit after 24 weeks (174).

2.7 Statistical analyses

Sample size calculation for this study was derived from the results of a pilot study conducted at our department from May 2009 to April 2010 (181). In this trial, 57 women with PCOS received 20,000 IU of cholecalciferol over 24 weeks, leading to a significant reduction of AUCgluc from 115±17 at baseline to 103±18 after vitamin D supplementation over 24 weeks. Thus, we calculated a sample size of 92 participants to detect a treatment difference at a two-sided 0.05 significance level with a probability of 90%, if the true difference between treatments is 12 with a standard deviation (SD) of 17. Due to the unexpectedly high drop-out rate in the PCOS group, the number of enrolled study participants in this group was increased from initially 150 to 180 to ensure an adequate power to detect differences regarding our primary outcome measure (174).

Distribution of data was analyzed by descriptive statistics and the Kolmogorov-Smirnov test. Continuous data with normal distribution are presented as means with SD. Continuous data with a skewed distribution are shown as medians with interquartile range. Categorical data are shown as percentages. Baseline parameters were compared between the vitamin D and the placebo group in both women with and without PCOS. These parameters were also compared between participants with and without PCOS. For comparisons of baseline parameters between the vitamin D and placebo groups and the PCOS and the non-PCOS group, unpaired Student's t test, Mann-Whitney U test, X^2 test, or Fisher's exact test were used, depending on data distribution and variable type (174). To evaluate possible changes in menstrual frequency, menstrual frequencies according to the returned menstrual calendars were categorized as follows: normal menstrual frequency (menstrual duration between 21-35 days), oligomenorrhea (menstrual cycle duration more than 35 days), hypermenorrhea (menstrual cycle

duration less than 21 days), or amenorrhea (absence of menstrual bleeding for more than six months) (174). Analysis of outcome variables was performed adhering to the intention-to-treat principle with no data imputation for missing values and inclusion of all participants with available baseline and follow-up values of the respective outcome parameter. We performed ANCOVA with adjustments for baseline values to test for differences in continuous outcome variables between the treatment and the placebo group in both women with and without PCOS at the respective follow-up visit (after 12 and 24 weeks, respectively). Skewed variables were log(e) transformed before they were used in ANCOVA. We used X^2 test to evaluate possible differences between the treatment and placebo groups regarding menstrual frequency in PCOS patients. For this purpose, an improvement in menstrual frequency was defined as a transition from amenorrhea to oligomenorrhea or hypermenorrhea or as a transition from amenorrhea, oligomenorrhea, or hypermenorrhea to normal menstrual frequency (174). A p-value <0.05 was considered statistically significant. All statistical analyses were performed with SPSS version 23 software (SPSS Inc., Chicago, IL, USA).

3 Results

3.1 Baseline comparisons between participants with and without PCOS

Table 1 shows baseline characteristics of the entire groups with (n=180) and without PCOS (n=150), regardless of randomization to either vitamin D or placebo. Participants in the PCOS group were significantly younger, had a significantly higher BMI, waist and hip circumference, systolic blood pressure, plasma glucose after 30, 60, and 120 minutes during OGTT, AUCgluc, plasma calcium, TT, FT, FAI, androstendione, DHEAS, and LH when compared to the group without PCOS. Additionally, participants with PCOS showed significantly lower HDL-cholesterol, 25(OH)D, PTH, estradiol, and FSH levels when compared to participants without PCOS (Table 1) (174).

Table 1 Baseline characteristics of participants with and without PCOS. Parts of this table (baseline characteristics of PCOS patients) were reproduced from (174) with permission of publisher Springer Nature

Characteristics	All (n=330)	PCOS (n=180)	No PCOS (n=150)	p-value
Age (years)	30.4±8.5	26.0±5.0	35.8±8.7	<0.001
Body-mass index (kg/m²)	26.5±6.8	27.6±7.5	25.2±5.5	0.001
Waist circumference (cm)	86.0 (76.3-99.8)	89.0 (78.3-104.0)	83.5 (74.0-93.8)	0.002
Hip circumference (cm)	100.0 (93.3-110.8)	102.0 (94.1-116.8)	98.5 (93-107)	0.005
WHR (cm/cm)	0.86±0.09	0.87±0.10	0.85±0.09	0.102
Systolic BP (mmHg)	120±13	122±13	118±12	0.007
Diastolic BP (mmHg)	81±10	81±10	80±10	0.312
Fasting glucose (mg/dL)	84±8	84±8	84±8	0.865
OGTT glucose 30 min (mg/dL)	125±27	130±26	118±27	<0.001
OGTT glucose 60 min (mg/dL)	107±36	117±37	96±32	<0.001
OGTT glucose 120 min (mg/dL)	91±23	97±25	84±20	<0.001
AUCgluc	209.19±46.45	222.09±44.5	193.71±44.07	<0.001
Fasting insulin (mU/L)	9.1 (5.8-13.9)	10.1 (5.8-16.1)	8.4 (5.6-13.3)	0.081
HbA1c (mmol/mol)	33 (31-35)	34 (31-35)	33 (31-35)	0.151
HOMA-IR	1.84 (1.17-3.04)	2.07 (1.18-3.47)	1.69 (1.14-2.79)	0.089
QUICKI	0.348 (0.324-0.374)	0.342 (0.318-0.373)	0.353 (0.328-0.375)	0.089
Triglycerides (mg/dL)	67 (51-92)	68 (50-94)	65 (53-90)	0.732
Total cholesterol (mg/dL)	177 (156-201)	175 (154-197)	184 (159-204)	0.732
HDL-cholesterol (mg/dL)	67±18	64±19	69±17	0.007
LDL-cholesterol (mg/dL)	97±32	96±33	99±31	0.350
CRP (mg/L)	1.0 (0.0-2.6)	1.1 (0.0-3.6)	0.9 (0.0-1.7)	0.052
25(OH)D (nmol/L)	52.7±19.0	50.4±19.0	55.4±18.9	0.019
PTH (pg/mL)	43.4 (35.7-54.6)	41.6 (34.1-52.5)	45.6 (38.6-58.5)	0.003
Plasma calcium (mmol/L)	2.34±0.08	2.36±0.08	2.32±0.08	<0.001
Total testosterone (nmol/L)	1.20 (0.90-1.60)	1.50 (1.10-1.95)	0.95 (0.68-1.30)	<0.001
Free testosterone (nmol/L)	0.015 (0.009-0.023)	0.021 (0.015-0.032)	0.010 (0.007-0.014)	<0.001
FAI	2.17 (1.22-3.55)	3.14 (2.18-5.26)	1.34 (0.87-2.05)	<0.001
Androstendione (ng/mL)	2.96 (2.11-4.02)	3.36 (2.51-4.44)	2.50 (1.80-3.38)	<0.001
DHEAS (µg/mL)	1.53 (1.06-2.40)	1.90 (1.34-2.78)	1.21 (0.83-1.66)	<0.001
Estradiol (pg/mL)	69.0 (49.5-123.0)	60.6 (44.6-96.0)	92.6 (59.2-137.3)	<0.001
FSH (mU/mL)	6.07 (4.45-8.23)	5.80 (4.24-7.36)	6.86 (4.73-9.64)	<0.001
LH (mU/mL)	7.39 (4.60-11.72)	8.86 (5.18-13.18)	6.28 (4.27-9.66)	<0.001

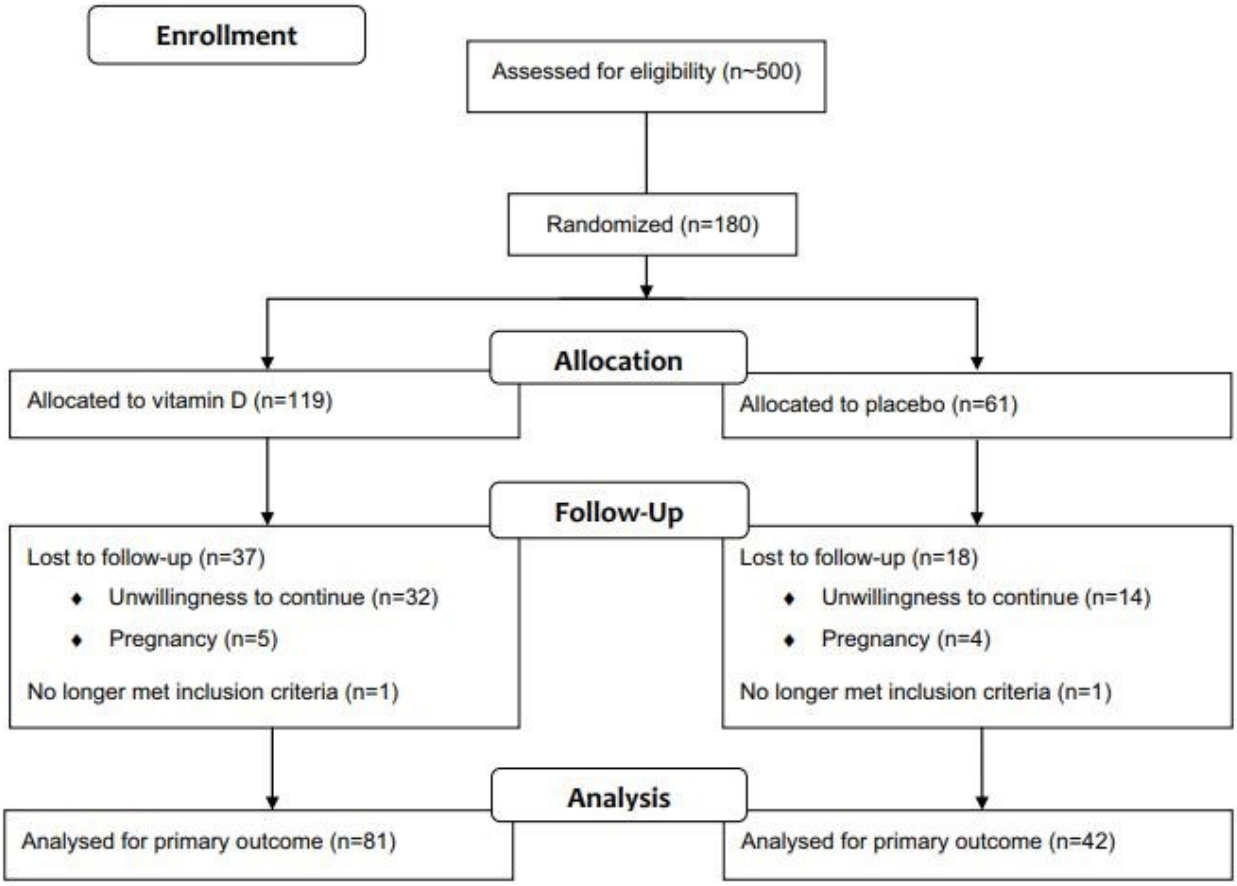
Data are shown as means with standard deviation or as medians and interquartile range, as appropriate. Comparisons of baseline characteristics between the vitamin D and the placebo group were performed using unpaired Student's t test or Mann-Whitney U test, as appropriate. 25(OH)D = 25-hydroxyvitamin D; AUCgluc = plasma glucose area under the curve; BP = blood pressure; CRP = C-reactive protein; DHEAS = dehydroepiandrostendione-sulfate; FAI = free androgen index; FSH = follicle-stimulating hormone; HbA1c = glycated hemoglobin; HDL-cholesterol = high density lipoprotein-cholesterol; HOMA-IR = homeostatic model assessment-insulin resistance; LDL-

cholesterol = low density lipoprotein-cholesterol; LH = luteinizing hormone; OGTT glucose 30 min = plasma glucose at 30 minutes during 75g oral glucose tolerance test; OGTT glucose 60 min = plasma glucose at 60 minutes during 75g oral glucose tolerance test; OGTT glucose 120 min = plasma glucose at 120 minutes during 75g oral glucose tolerance test; PTH = parathyroid hormone; QUICKI = quantitative insulin sensitivity check index; WHR = waist-to-hip ratio

3.2 PCOS patients

The results of the PCOS group were published previously (174). Out of approximately 500 patients who were referred to our outpatient clinic for evaluation of PCOS, 180 eligible patients were enrolled for study participation. Participants in the PCOS group were enrolled from December 2011 to July 2017 (time from first patient randomized to last follow-up visit). Figure 5 shows a participant flow-chart for the PCOS group (174).

Figure 5 Participant flow-chart for the PCOS group. Reproduced from (174) with permission of publisher Springer Nature



Baseline characteristics of all randomized participants of the PCOS group are shown in Table 2. Participants who were randomized to the vitamin D group were significantly younger and had higher plasma glucose concentrations after 60 minutes during OGTT than participants who were randomized to the placebo group. All other baseline parameters showed no significant differences between the vitamin D and the placebo group in PCOS patients (Table 2) (174).

Table 2 Baseline characteristics of all randomized study participants of the PCOS group. Reproduced from (174) with permission of publisher Springer Nature

Characteristics	All (n=180)	Vitamin D (n=119)	Placebo (n=61)	p-value
Age (years)	26.0±5.0	25.4±4.6	27.2±5.5	0.022
Body-mass index (kg/m²)	27.6±7.5	27.3±7.4	28.3±7.8	0.453
Waist circumference (cm)	89.0 (78.3-104.0)	87.0 (77.0-104.0)	93.0 (82.0-104.5)	0.210
Hip circumference (cm)	102.0 (94.1-116.8)	101.0 (94.0-115.0)	105.0 (95.5-118.5)	0.378
WHR (cm/cm)	0.87±0.10	0.86±0.08	0.88±0.12	0.245
Systolic BP (mmHg)	122±13	122±13	122±13	0.803
Diastolic BP (mmHg)	81±10	81±10	82±10	0.214
Fasting glucose (mg/dL)	84±8	84±8	84±7	0.859
OGTT glucose 30 min (mg/dL)	130±26	131±27	126±23	0.247
OGTT glucose 60 min (mg/dL)	117±37	121±39	109±32	0.044
OGTT glucose 120 min (mg/dL)	97±25	99±24	93±25	0.150
AUCgluc	222.09±44.5	226.71±46.12	213.07±40.03	0.051
Fasting insulin (mU/L)	10.1 (5.8-16.1)	10.3 (5.7-16.8)	9.9 (6.3-13.6)	0.845
HbA1c (mmol/mol)	34 (31-35)	33 (31-35)	34 (32-35)	0.683
HOMA-IR	2.07 (1.18-3.47)	2.10 (1.12-3.59)	2.04 (1.31-2.80)	0.825
QUICKI	0.342 (0.318-0.373)	0.341 (0.316-0.376)	0.343 (0.327-0.367)	0.825
Triglycerides (mg/dL)	68 (50-94)	66 (50-92)	72 (50-109)	0.388
Total cholesterol (mg/dL)	175 (154-197)	173 (157-191)	176 (149-203)	0.565
HDL-cholesterol (mg/dL)	64±19	63±19	65±20	0.720
LDL-cholesterol (mg/dL)	96±33	94±28	100±41	0.283
CRP (mg/L)	1.1 (0.0-3.6)	1.4 (0-0-3.9)	0.8 (0.0-3.3)	0.350
25(OH)D (nmol/L)	50.4±19.0	50.7±19.5	49.9±18.3	0.798
PTH (pg/mL)	41.6 (34.1-52.5)	41.9 (34.4-53.8)	40.2 (33.0-51.4)	0.595
Plasma calcium (mmol/L)	2.36±0.08	2.36±0.08	2.36±0.07	0.944
Total testosterone (nmol/L)	1.50 (1.10-1.95)	1.50 (1.10-2.10)	1.40 (1.10-1.80)	0.315
Free testosterone (nmol/L)	0.021 (0.015-0.032)	0.021 (0.016-0.032)	0.018 (0.013-0.032)	0.221
FAI	3.14 (2.18-5.26)	3.33 (2.26-5.29)	2.53 (2.04-5.15)	0.223
Androstendione (ng/mL)	3.36 (2.51-4.44)	3.41 (2.43-4.46)	3.32 (2.58-4.41)	0.850
DHEAS (µg/mL)	1.90 (1.34-2.78)	1.94 (1.34-2.70)	1.90 (1.42-2.79)	0.897
Estradiol (pg/mL)	60.6 (44.6-96.0)	59.1 (42.3-91.2)	64.0 (49.5-118.5)	0.164
FSH (mU/mL)	5.97±2.41	5.94±2.33	6.04±2.59	0.783
LH (mU/mL)	9.56±5.60	9.79±5.87	9.11±5.05	0.437
Menstrual irregularity (%)	89.4	89.9	88.5	0.801
Oligomenorrhea (%)	71.7	73.1	68.9	0.549
Hypermenorrhea (%)	2.2	1.7	3.3	0.605
Amenorrhea (%)	15.6	15.1	16.4	0.824

Data are shown as means with standard deviation, medians and interquartile range, or as percentages, as appropriate. Comparisons of baseline characteristics between the vitamin D and the placebo group were performed using unpaired Student's t test, Mann-Whitney U test, X^2 test, or Fisher's exact test, as appropriate. 25(OH)D = 25-hydroxyvitamin D; AUCgluc = plasma glucose

area under the curve; BP = blood pressure; CRP = C-reactive protein; DHEAS = dehydroepiandrostendione-sulfate; FAI = free androgen index; FSH = follicle-stimulating hormone; HbA1c = glycated hemoglobin; HDL-cholesterol = high density lipoprotein-cholesterol; HOMA-IR = homeostatic model assessment-insulin resistance; LDL-cholesterol = low density lipoprotein-cholesterol; LH = luteinizing hormone; OGTT glucose 30 min = plasma glucose at 30 minutes during 75g oral glucose tolerance test; OGTT glucose 60 min = plasma glucose at 60 minutes during 75g oral glucose tolerance test; OGTT glucose 120 min = plasma glucose at 120 minutes during 75g oral glucose tolerance test; PTH = parathyroid hormone; QUICKI = quantitative insulin sensitivity check index; WHR = waist-to-hip ratio

In the PCOS group, 123 study participants [age 25.9±4.7 years; BMI 27.5±7.3 kg/m²; baseline 25(OH)D 48.8±16.9 nmol/L] completed the entire study including the final follow-up visit after 24 weeks. One-hundred and forty participants [age 26.1±4.8 years; BMI 27.5±7.4 kg/m²; baseline 25(OH)D 48.1±17.7 nmol/L] completed the baseline visit and the first follow-up visit after 12 weeks. The mean (± SD) overall treatment period was 176±23 days in the vitamin D group and 176±21 days in the placebo group (174).

Regarding our primary outcome measure, there was no significant effect of vitamin D supplementation on AUCgluc at the final follow-up visit after 24 weeks. The mean treatment effect [with 95% confidence interval (CI)] was -9.19 (-21.40 to 3.02; p=0.139). For secondary outcome measures, we found a significant decrease in plasma glucose after 60 minutes during OGTT (Table 3). No significant effect of vitamin D supplementation could be observed on any of the other pre-specified continuous secondary outcome parameters (Table 3). Regarding menstrual frequency, the rates of improved menstrual regularity did not significantly differ between the vitamin D and the placebo group: at study end after 24 weeks, 49.4% of the participants in the vitamin D group and 42.1% of the participants in the placebo group showed improved menstrual regularity when compared to the screening visit (p=0.552) (174).

Table 3 Continuous secondary outcome variables at baseline and the final follow-up visit after 24 weeks in participants of the PCOS group with available values at both study visits. Reproduced from (174) with permission of publisher Springer Nature

	Baseline	Follow-Up (24 weeks)	Treatment Effect (95% CI)	p-value
Fasting glucose [mg/dL]				
Vitamin D (n=81)	84±8	82±8	-1.2 (-3.6 to 1.3)	0.353
Placebo (n=42)	84±8	83±7		
OGTT glucose 30 min [mg/dL]				
Vitamin D (n=80)	133±24	130±23	-1.6 (-10.0 to 6.8)	0.711
Placebo (n=42)	128±25	129±26		
OGTT glucose 60 min [mg/dL]				
Vitamin D (n=80)	123±39	105±31	-10.2 (-20.2 to -0.3)	0.045
Placebo (n=42)	107±31	107±34		
OGTT glucose 120 min [mg/dL]				
Vitamin D (n=81)	98±24	88±24	0.5 (-7.6 to 8.6)	0.903
Placebo (n=42)	93±24	85±24		
*HbA1c [mmol/mol]**				
Vitamin D (n=74)	33 (31-35)	33 (32-35)	-0.4 (-0.9 to 0.2)	0.192
Placebo (n=38)	34 (32-35)	33 (32-35)		
*HOMA-IR**				
Vitamin D (n=81)	1.95 (1.09-3.51)	2.29 (1.43-3.47)	-0.26 (-0.80 to 0.27)	0.935
Placebo (n=42)	2.15 (1.28-3.00)	2.31 (1.28-3.81)		
*QUICKI**				
Vitamin D (n=81)	0.345 (0.317-0.378)	0.337 (0.318-0.362)	-0.004 (-0.028 to 0.019)	0.823
Placebo (n=42)	0.340 (0.324-0.369)	0.337 (0.314-0.368)		
*Triglycerides [mg/dL]**				
Vitamin D (n=79)	62 (49-85)	71 (52-93)	3 (-7 to 12)	0.455
Placebo (n=42)	78 (50-118)	74 (48-106)		
*Total cholesterol [mg/dL]**				
Vitamin D (n=79)	173 (158-188)	172 (158-189)	4 (-3 to 11)	0.180
Placebo (n=42)	179 (148-203)	172 (143-204)		
*Total testosterone [nmol/L]**				
Vitamin D (n=78)	1.60 (1.10-2.20)	1.55 (1.28-2.00)	0.09 (-0.11 to 0.28)	0.616
Placebo (n=41)	1.40 (1.15-1.80)	1.40 (1.20-1.90)		
*Free testosterone [nmol/L]**				
Vitamin D (n=77)	0.020 (0.016-0.032)	0.021 (0.015-0.029)	0.002 (-0.002 to 0.005)	0.445
Placebo (n=41)	0.019 (0.015-0.035)	0.021 (0.013-0.028)		

Data are shown as means with standard deviation or medians and interquartile range, as appropriate. Treatment effects with 95% confidence interval and p-values were calculated by ANCOVA for group differences at follow-up with adjustment for baseline values.

*Skewed variables for which logarithmic transformed values were used in ANCOVA, but untransformed values are shown in the table.

HbA1c = glycated hemoglobin; HOMA-IR = homeostatic model assessment-insulin resistance; OGTT glucose 30 min = plasma glucose at 30 minutes during 75g oral glucose tolerance test;

OGTT glucose 60 min = plasma glucose at 60 minutes during 75g oral glucose tolerance test;
OGTT glucose 120 min = plasma glucose at 120 minutes during 75g oral glucose tolerance test;
QUICKI = quantitative insulin sensitivity check index

To confirm the effectiveness and safety of our intervention, we furthermore investigated the effects of vitamin D supplementation on several parameters of bone and mineral metabolism. Vitamin D supplementation significantly increased serum concentrations of 25(OH)D and $1,25(OH)_2D$ and significantly decreased serum levels of PTH (Table 4). There was no significant effect of vitamin D supplementation of plasma calcium concentrations (Table 4) (174).

Table 4 Selected parameters of bone and mineral metabolism at baseline and the final follow-up visit after 24 weeks in participants of the PCOS group with available values at both study visits. Reproduced from (174) with permission of publisher Springer Nature

	Baseline	Follow-Up (24 weeks)	Treatment Effect (95% CI)	p-value
25(OH)D [nmol/L]				
Vitamin D (n=79)	48.8±16.8	90.2±20.1	33.4 (24.5 to 42.2)	<0.001
Placebo (n=41)	48.8±17.5	56.8±29.5		
*PTH [pg/mL]**				
Vitamin D (n=81)	41.9 (34.4-53.8)	40.6 (32.4-51.1)	-6.6 (-11.3 to -1.9)	0.004
Placebo (n=42)	40.2 (33.0-51.4)	45.7 (37.6-55.5)		
1,25(OH)$_2$D [pmol/L]				
Vitamin D (n=75)	114±48	141±52	27 (8 to 46)	0.006
Placebo (n=41)	110±43	113±48		
Plasma calcium [mmol/L]				
Vitamin D (n=79)	2.35±0.08	2.32±0.07	0.02 (-0.003 to 0.05)	0.081
Placebo (n=41)	2.36±0.07	2.32±0.07		

Data are shown as means with standard deviation or medians and interquartile range, as appropriate. Treatment effects with 95% confidence interval and p-values were calculated by ANCOVA for group differences at follow-up with adjustment for baseline values.

*Skewed variables for which logarithmic transformed values were used in ANCOVA, but untransformed values are shown in the table.

1,25(OH)$_2$D = 1,25-dihydroxy vitamin D; 25(OH)D = 25-hydroxyvitamin D; PTH = parathyroid hormone

At the follow-up visit after 12 months, we were able to confirm the already shown treatment effect of vitamin D supplementation on plasma glucose after 60 minutes during OGTT. Additionally, at this follow-up visit, vitamin D supplementation also lead to a significant decrease in AUCgluc (Table 5) (174).

Table 5 Primary outcome measure and continuous secondary outcome variables at baseline and the final follow-up visit after 12 weeks in participants of the PCOS group with available values at both study visits. Reproduced from (174) with permission of publisher Springer Nature

	Baseline	Follow-Up (12 weeks)	Treatment Effect (95% CI)	p-value
AUCgluc				
Vitamin D (n=92)	228.05±46.45	210.59±46.35	-12.89 (-24.70 to -1.08)	0.033
Placebo (n=45)	210.04±40.39	209.77±47.54		
Fasting glucose [mg/dL]				
Vitamin D (n=94)	84±8	83±9	-0.5 (-2.9 to 1.9)	0.681
Placebo (n=46)	84±8	84±7		
OGTT glucose 30 min [mg/dL]				
Vitamin D (n=91)	133±25	131±29	-3.0 (-11.0 to 4.9)	0.452
Placebo (n=45)	127±24	130±23		
OGTT glucose 60 min [mg/dL]				
Vitamin D (n=91)	122±40	108±35	-10.8 (-20.0 to -1.6)	0.022
Placebo (n=45)	107±31	108±39		
OGTT glucose 120 min [mg/dL]				
Vitamin D (n=92)	99±24	87±21	-2.8 (-9.9 to 4.3)	0.430
Placebo (n=45)	91±24	86±25		
*HbA1c [mmol/mol]**				
Vitamin D (n=42)	33 (31-35)	34 (32-35)	0.4 (-0.2 to 1.0)	0.166
Placebo (n=85)	34 (32-35)	33 (32-35)		
*HOMA-IR**				
Vitamin D (n=94)	1.92 (1.09-3.49)	2.32 (1.34-3.62)	0.52 (-0.96 to 1.99)	0.134
Placebo (n=46)	2.15 (1.28-3.00)	2.02 (1.06-3.23)		
*QUICKI**				
Vitamin D (n=94)	0.346 (0.318-0.378)	0.336 (0.316-0.366)	-0.009 (-0.022 to 0.003)	0.129
Placebo (n=46)	0.340 (0.324-0.369)	0.343 (0.321-0.380)		
*Triglycerides [mg/dL]**				
Vitamin D (n=91)	63 (49-90)	69 (53-94)	5 (-4 to 15)	0.455
Placebo (n=45)	74 (51-114)	76 (58-95)		
*Total cholesterol [mg/dL]**				
Vitamin D (n=91)	172 (156-190)	179 (151-194)	0.3 (-7 to 7)	0.780
Placebo (n=45)	175 (142-203)	172 (148-197)		
*Total testosterone [nmol/L]**				
Vitamin D (n=92)	1.55 (1.13-2.18)	1.50 (1.10-2.00)	-0.14 (-0.34 to 0.06)	0.164
Placebo (n=44)	1.40 (1.20-1.80)	1.60 (1.13-1.88)		
*Free testosterone [nmol/L]**				
Vitamin D (n=91)	0.021 (0.016-0.032)	0.021 (0.014-0.029)	-0.001 (-0.004 to 0.002)	0.271
Placebo (n=44)	0.019 (0.015-0.035)	0.022 (0.015-0.031)		

Data are shown as means with standard deviation or medians and interquartile range, as appropriate. Treatment effects with 95% confidence interval and p-values were calculated by ANCOVA for group differences at follow-up with adjustment for baseline values.

*Skewed variables for which logarithmic transformed values were used in ANCOVA, but untransformed values are shown in the table.

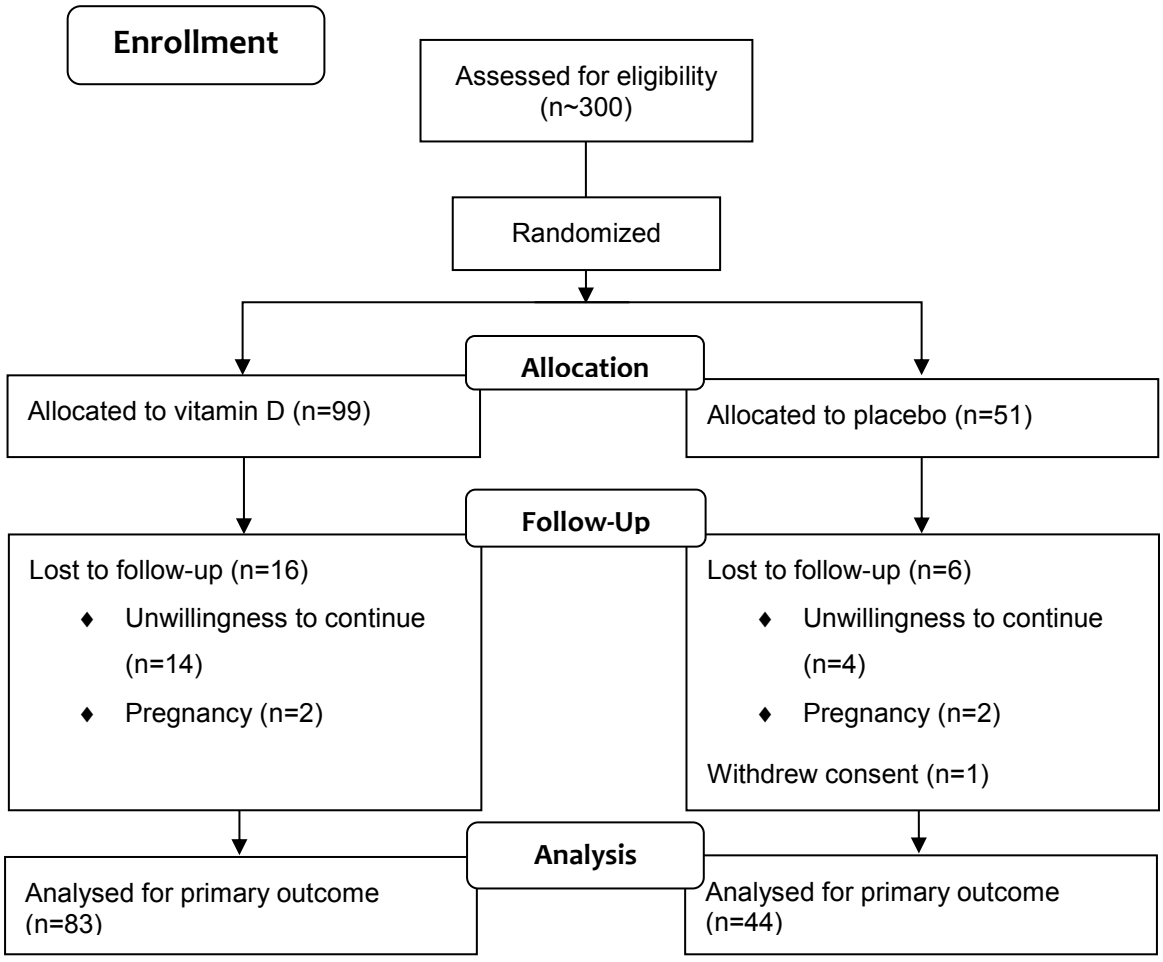
AUCgluc = plasma glucose area under the curve; HbA1c = glycated hemoglobin; HOMA-IR = homeostatic model assessment-insulin resistance; OGTT glucose 30 min = plasma glucose at 30 minutes during 75g oral glucose tolerance test; OGTT glucose 60 min = plasma glucose at 60 minutes during 75g oral glucose tolerance test; OGTT glucose 120 min = plasma glucose at 120 minutes during 75g oral glucose tolerance test; QUICKI = quantitative insulin sensitivity check index

We observed no unintended treatment effects or serious adverse events (e.g., hospitalizations) in PCOS patients during the study. No PCOS patient treated with vitamin D developed hypercalcemia (defined as total plasma calcium >2.65 mmol/L) at the follow-up visits (174).

3.3 Participants without PCOS

Approximately 300 women who were referred to our outpatient department or who responded to promotional material were screened for study eligibility. Of these women, 150 were enrolled for study participation. The first participant was enrolled in March 2013; the last follow-up visit took place in October 2017. Figure 6 shows a participant flow-chart for participants without PCOS.

Figure 6 Participant flow-chart for participants without PCOS



Baseline characteristics of all randomized participants without PCOS are shown in Table 6. At baseline, participants in the vitamin D group had significantly lower plasma glucose after 30 minutes during OGTT as well as a significantly lower estradiol serum concentration than participants in the placebo group. All other parameters showed no significant differences between the two groups (Table 6).

Table 6 Baseline characteristics of all randomized participants without PCOS

Characteristics	All (n=150)	Vitamin D (n=99)	Placebo (n=51)	p-value
Age (years)	35.8±8.7	35.7±8.9	36.1±8.4	0.826
Body-mass index (kg/m²)	25.2±5.5	25.5±5.3	24.7±5.8	0.398
Waist circumference (cm)	83.5 (74.0-93.8)	84.0 (77.0-93.5)	80.0 (72.0-96.0)	0.516
Hip circumference (cm)	98.5 (93-107)	99 (94-108)	97.0 (91.0-105.0)	0.269
WHR (cm/cm)	0.85±0.09	0.85±0.07	0.86±0.12	0.419
Systolic BP (mmHg)	118±12	118±12	118±12	1.000
Diastolic BP (mmHg)	80±10	80±9	79±12	0.550
Fasting glucose (mg/dL)	84±8	84±8	85±8	0.255
OGTT glucose 30 min (mg/dL)	118±27	115±28	125±24	0.032
OGTT glucose 60 min (mg/dL)	96±32	94±32	99±31	0.302
OGTT glucose 120 min (mg/dL)	84±20	83±20	86±19	0.362
AUCgluc	193.71±44.07	189.62±45.16	201.64±41.14	0.114
Fasting insulin (mU/L)	8.4 (5.6-13.3)	8.7 (6.2-13.3)	7.7 (5.3-13.4)	0.660
HbA1c (mmol/mol)	33 (31-35)	32 (31-35)	33 (31-35)	0.572
HOMA-IR	1.69 (1.14-2.79)	1.75 (1.16-2.80)	1.58 (1.10-2.74)	0.758
QUICKI	0.353 (0.328-0.375)	0.351 (0.327-0.374)	0.356 (0.329-0.377)	0.758
Triglycerides (mg/dL)	65 (53-90)	69 (53-92)	65 (51-75)	0.169
Total cholesterol (mg/dL)	184 (159-204)	185 (159-209)	184 (164-198)	0.574
HDL-cholesterol (mg/dL)	69±17	69±18	70±16	0.750
LDL-cholesterol (mg/dL)	99±31	100±32	97±29	0.602
CRP (mg/L)	0.9 (0.0-1.7)	1.0 (0.0-1.7)	0.7 (0.0-2.1)	0.345
25(OH)D (nmol/L)	55.4±18.9	55.4±18.9	55.3±18.9	0.966
PTH (pg/mL)	45.6 (38.6-58.5)	45.1 (37.5-58.4)	47.4 (39.1-59.0)	0.745
Plasma calcium (mmol/L)	2.32±0.08	2.31±0.08	2.32±0.07	0.830
Total testosterone (nmol/L)	0.95 (0.68-1.30)	0.90 (0.60-1.30)	1.10 (0.80-1.30)	0.100
Free testosterone (nmol/L)	0.010 (0.007-0.014)	0.010 (0.007-0.014)	0.010 (0.007-0.014)	0.974
FAI	1.34 (0.87-2.05)	1.37 (0.91-2.02)	1.21 (0.82-2.12)	0.634
Androstendione (ng/mL)	2.50 (1.80-3.38)	2.40 (1.70-3.58)	2.61 (1.90-3.19)	0.565
DHEAS (µg/mL)	1.21 (0.83-1.66)	1.20 (0.81-1.72)	1.23 (0.88-1.63)	0.301
Estradiol (pg/mL)	92.6 (59.2-137.3)	83.4 (50.5-123.0)	114.0 (74.4-156.0)	0.014
FSH (mU/mL)	6.86 (4.73-9.64)	6.94 (4.90-9.52)	6.65 (4.51-10.40)	0.728
LH (mU/mL)	6.28 (4.27-9.66)	6.28 (3.87-9.29)	6.48 (4.99-10.20)	0.659

Data are shown as means with standard deviation or as medians and interquartile range, as appropriate. Comparisons of baseline characteristics between the vitamin D and the placebo group were performed using unpaired Student's t test or Mann-Whitney U test, as appropriate. 25(OH)D = 25-hydroxyvitamin D; AUCgluc = plasma glucose area under the curve; BP = blood pressure; CRP = C-reactive protein; DHEAS = dehydroepiandrostendione-sulfate; FAI = free androgen index; FSH = follicle-stimulating hormone; HbA1c = glycated hemoglobin; HDL-cholesterol = high density lipoprotein-cholesterol; HOMA-IR = homeostatic model assessment-insulin resistance; LDL-cholesterol = low density lipoprotein-cholesterol; LH = luteinizing hormone; OGTT glucose 30 min = plasma glucose at 30 minutes during 75g oral glucose tolerance test; OGTT glucose 60 min = plasma glucose at 60 minutes during 75g oral glucose tolerance test; OGTT glucose 120 min =

plasma glucose at 120 minutes during 75g oral glucose tolerance test; PTH = parathyroid hormone; QUICKI = quantitative insulin sensitivity check index; WHR = waist-to-hip ratio

In the group without PCOS, 127 participants [age 36.2±8.7 years; BMI 25.3±5.6 kg/m²; baseline 25(OH)D 55.8±19.7 nmol/L] completed the entire study up to the final follow-up visit after 24 weeks. One-hundred and thirty-three participants [age 36.1±8.6 years; BMI 25.3±5.6 kg/m²; baseline 25(OH)D 55.6±19.3 nmol/L] completed the baseline visit and the first follow-up visit after 12 weeks. In participants without PCOS, the mean (± SD) overall treatment period was 174±44 days in the vitamin D group and 173±23 days in the placebo group.

Regarding the primary outcome measure, we were unable to find a significant effect of vitamin D supplementation on AUCgluc at the final follow-up visit after 24 weeks. The mean treatment effect (95% CI) was 11.70 (-0.91 to 24.31; p=0.069). Effects of vitamin D supplementation on secondary outcome measures are summarized in Table 7. Vitamin D supplementation significantly increased HOMA-IR and significantly decreased QUICKI when compared to placebo, while there was no significant effect on any of the other secondary outcome measures (Table 7).

Table 7 Secondary outcome variables at baseline and the final follow-up visit after 24 weeks in participants without PCOS with available values at both study visits

	Baseline	Follow-Up (24 weeks)	Treatment Effect (95% CI)	p-value
Fasting glucose [mg/dL]				
Vitamin D (n=83)	84±9	85±8	1.5 (-0.9 to 3.8)	0.214
Placebo (n=44)	86±8	84±7		
OGTT glucose 30 min [mg/dL]				
Vitamin D (n=83)	116±28	123±33	6.9 (-2.5 to 16.3)	0.147
Placebo (n=44)	127±25	123±27		
OGTT glucose 60 min [mg/dL]				
Vitamin D (n=82)	94±33	101±37	4.2 (-5.6 to 14.0)	0.396
Placebo (n=43)	100±33	101±33		
OGTT glucose 120 min [mg/dL]				
Vitamin D (n=83)	83±21	84±19	3.0 (-2.8 to 8.8)	0.307
Placebo (n=44)	86±20	83±18		
*HbA1c [mmol/mol]**				
Vitamin D (n=82)	33 (31-35)	33 (31-35)	0.2 (-0.5 to 0.9)	0.641
Placebo (n=43)	33 (31-36)	33 (32-35)		
*HOMA-IR**				
Vitamin D (n=83)	1.77 (1.16-2.80)	1.76 (1.26-2.63)	0.31 (-0.19 to 0.74)$^{+}$	0.019
Placebo (n=43)	1.54 (1.09-2.72)	1.42 (0.76-2.29)		
*QUICKI**				
Vitamin D (n=83)	0.350 (0.327-0.374)	0.351 (0.330-0.371)	-0.019 (-0.033 to -0.004)	0.013
Placebo (n=43)	0.358 (0.329-0.378)	0.362 (0.337-0.402)		
*Triglycerides [mg/dL]**				
Vitamin D (n=81)	69 (53-91)	70 (54-97)	9 (-3 to 21)	0.242
Placebo (n=42)	63 (51-73)			
*Total cholesterol [mg/dL]**				
Vitamin D (n=81)	185 (160-209)	180 (163-196)	-3 (-10 to 4)	0.242
Placebo (n=42)	179 (158-193)	180 (156-206)		
*Total testosterone [nmol/L]**				
Vitamin D (n=82)	0.90 (0.60-1.30)	0.90 (0.60-1.30)	-0.01 (-0.15 to 0.13)	0.463
Placebo (n=44)	1.00 (0.80-1.30)	1.10 (0.80-1.28)		
*Free testosterone [nmol/L]**				
Vitamin D (n=81)	0.010 (0.006-0.014)	0.010 (0.006-0.015)	0.001 (-0.001 to 0.003)	0.451
Placebo (n=43)	0.009 (0.007-0.014)	0.009 (0.007-0.012)		

Data are shown as means with standard deviation or medians and interquartile range, as appropriate. Treatment effects with 95% confidence interval and p-values were calculated by ANCOVA for group differences at follow-up with adjustment for baseline values.

*Skewed variables for which logarithmic transformed values were used in ANCOVA, but untransformed values are shown in the table. Thus, $^{+}$shows the untransformed treatment effect and confidence interval for HOMA-IR, which remained non-significant for untransformed values.

HbA1c = glycated hemoglobin; HOMA-IR = homeostatic model assessment-insulin resistance;

OGTT glucose 30 min = plasma glucose at 30 minutes during 75g oral glucose tolerance test;
OGTT glucose 60 min = plasma glucose at 60 minutes during 75g oral glucose tolerance test;
OGTT glucose 120 min = plasma glucose at 120 minutes during 75g oral glucose tolerance test;
QUICKI = quantitative insulin sensitivity check index

Regarding parameters of bone and mineral metabolism, vitamin D supplementation significantly increased 25(OH)D concentrations, while there was no significant effect on PTH, 1,25$(OH)_2$D, and plasma calcium (Table 8).

Table 8 Selected parameters of bone and mineral metabolism at baseline and the final follow-up visit after 24 weeks in participants without PCOS with available values at both study visits

	Baseline	Follow-Up (24 weeks)	Treatment Effect (95% CI)	p-value
25(OH)D [nmol/L]				
Vitamin D (n=82)	55.8±19.9	95.3±26.2	28.5 (19.3 to 37.7)	<0.001
Placebo (n=44)	56.2±19.3	67.0±24.8		
*PTH [pg/mL]**				
Vitamin D (n=83)	43.9 (38.7-58.9)	46.5 (37.4-55.6)	-4.4 (-9.3 to 0.6)	0.099
Placebo (n=44)	48.8 (39.1-58.8)	51.4 (43.0-63.8)		
1,25(OH)$_2$D [pmol/L]				
Vitamin D (n=80)	101±33	124±47	12 (-5 to 29)	0.173
Placebo (n=41)	107±43	113±41		
Plasma calcium [mmol/L]				
Vitamin D (n=82)	2.31±0.08	2.31±0.08	-0.01 (-0.03 to 0.02)	0.705
Placebo (n=42)	2.31±0.07	2.31±0.07		

Data are shown as means with standard deviation or medians and interquartile range, as appropriate. Treatment effects with 95% confidence interval and p-values were calculated by ANCOVA for group differences at follow-up with adjustment for baseline values.

*Skewed variables for which logarithmic transformed values were used in ANCOVA, but untransformed values are shown in the table.

1,25(OH)$_2$D = 1,25-dihydroxy vitamin D; 25(OH)D = 25-hydroxyvitamin D; PTH = parathyroid hormone

Table 9 shows the effects of vitamin D supplementation on primary and secondary outcome measures in participants without PCOS at the first follow-up visit 12 weeks after randomization. Vitamin D supplementation did not have a significant effect on any of the shown parameters at this time when compared to placebo (Table 9)

Table 9 Primary and secondary outcome variables at baseline and the final follow-up visit after 12 weeks in participants without PCOS with available values at both study visits

	Baseline	Follow-Up (12 weeks)	Treatment Effect (95% CI)	p-value
AUCgluc				
Vitamin D (n=85)	187.47±42.72	192.32±42.98	2.23 (-9.92 to 14.4)	0.717
Placebo (n=46)	201.90±42.50	199.49±43.43		
Fasting glucose [mg/dL]				
Vitamin D (n=87)	84±9	84±9	1.2 (-1.1 to 3.4)	0.304
Placebo (n=46)	86±8	84±8		
OGTT glucose 30 min [mg/dL]				
Vitamin D (n=84)	114±25	124±31	5.3 (-2.4 to 14.1)	0.232
Placebo (n=45)	126±25	122±27		
OGTT glucose 60 min [mg/dL]				
Vitamin D (n=83)	91±29	95±31	1.7 (-7.8 to 11.1)	0.729
Placebo (n=45)	100±32	99±33		
OGTT glucose 120 min [mg/dL]				
Vitamin D (n=85)	83±21	81±21	-3.4 (-9.3 to 2.5)	0.252
Placebo (n=46)	85±20	86±18		
*HbA1c [mmol/mol]**				
Vitamin D (n=87)	33 (31-35)	33 (32-35)	-0.2 (-0.9 to 0.5)	0.515
Placebo (n=45)	33 (31-36)	33 (32-36)		
*HOMA-IR**				
Vitamin D (n=87)	1.77 (1.16-2.83)	1.85 (1.18-2.76)	0.11 (-0.28 to 0.50)	0.460
Placebo (n=45)	1.53 (1.08-2.70)	1.60 (1.00-2.86)		
*QUICKI**				
Vitamin D (n=87)	0.350 (0.327-0.374)	0.348 (0.328-0.373)	-0.006 (-0.020 to 0.008)	0.410
Placebo (n=45)	0.358 (0.329-0.379)	0.356 (0.326-0.383)		
*Triglycerides [mg/dL]**				
Vitamin D (n=85)	69 (53-91)	72 (56-95)	4 (-5 to 13)	0.357
Placebo (n=45)	63 (51-74)	61 (49-84)		
*Total cholesterol [mg/dL]**				
Vitamin D (n=85)	185 (160-209)	185 (157-209)	1 (-5 to 8)	0.962
Placebo (n=45)	180 (156-194)	173 (165-187)		
*Total testosterone [nmol/L]**				
Vitamin D (n=87)	0.90 (0.60-1.30)	0.80 (0.60-1.30)	-0.04 (-0.17 to 0.09)	0.657
Placebo (n=45)	1.00 (0.80-1.30)	1.00 (0.70-1.25)		
*Free testosterone [nmol/L]**				
Vitamin D (n=86)	0.010 (0.007-0.014)	0.010 (0.006-0.014)	0.0001 (-0.002 to 0.002)	0.809
Placebo (n=45)	0.009 (0.007-0.013)	0.009 (0.006-0.015)		

Data are shown as means with standard deviation or medians and interquartile range, as appropriate. Treatment effects with 95% confidence interval and p-values were calculated by ANCOVA for group differences at follow-up with adjustment for baseline values.

*Skewed variables for which logarithmic transformed values were used in ANCOVA, but untransformed values are shown in the table.
AUCgluc = plasma glucose area under the curve; HbA1c = glycated hemoglobin; HOMA-IR = homeostatic model assessment-insulin resistance; OGTT glucose 30 min = plasma glucose at 30 minutes during 75g oral glucose tolerance test; OGTT glucose 60 min = plasma glucose at 60 minutes during 75g oral glucose tolerance test; OGTT glucose 120 min = plasma glucose at 120 minutes during 75g oral glucose tolerance test; QUICKI = quantitative insulin sensitivity check index

Similar to the PCOS group, we did not observe any unintended treatment effects or serious adverse events in the participant group without PCOS. No participant treated with vitamin D developed hypercalcemia (defined as total plasma calcium >2.65 mmol/L) at any of the follow-up visits.

4 Discussion

The aim of this dissertation was to investigate the effects of vitamin D supplementation on endocrine and metabolic parameters in women with and without PCOS.

At baseline, participants with and without PCOS showed several significant differences, including certain anthropometric parameters, measures of glycemic control, and androgen levels. Vitamin D supplementation had no significant effect on our primary study measure (AUCgluc) in both women with and without PCOS. However, in the PCOS group, vitamin D supplementation lead to a significant decrease in glucose after 60 minutes during OGTT. In PCOS patients, there was no significant effect of vitamin D supplementation on menstrual frequency when compared to placebo (174). In participants without PCOS, vitamin D supplementation significantly increased HOMA-IR and significantly decreased QUICKI when compared to placebo, while we found no significant effects on any of the other secondary outcome measures.

The pathophysiological backgrounds of a possible association of vitamin D and metabolic or endocrine parameters are still not entirely understood. $1,25(OH)_2D$ may play several roles in glucose homeostasis: it has been shown to increase the insulin sensitivity of target cells (e.g., liver, adipose tissue, or skeletal muscle) and to be able to increase and enhance the function of beta-cells. Furthermore, $1,25(OH)_2D$ may protect beta-cells from autoimmune damage by indirectly influencing different cells of the immune system, including macrophages, dendritic cells, and T cells (118,119). PTH serum concentrations, which are typically high or even elevated in subjects with particularly low serum 25(OH)D levels, were associated with impaired pancreatic insulin secretion (182), while vitamin D is also a key player in calcium homeostasis, which is important for intracellular insulin-mediated procedures in insulin-responsive tissues (126). On a molecular level, the transcription of the insulin gene is activated by $1,25(OH)_2D$ while the vitamin D-responsive element is present in the promoter region of the human insulin gene, therefore vitamin D might improve insulin responsiveness for glucose transport by the stimulation of insulin receptor expression (183–185). In PCOS women, the

relationship between vitamin D status and insulin resistance does not seem to be confounded by obesity, as study data suggests an association of insulin resistance with severe vitamin D deficiency regardless of BMI or WHR (160).

Possible vitamin D effects on androgens or ovulatory function in women with or without PCOS might be explained by the ubiquitous expression of the VDR within the female reproduction system. Studies found VDR mRNA expression in placenta, ovarian, deciduae, and endometrium cells (129,133,186). Furthermore, in cultured human placenta and ovary cells, $1,25(OH)_2D$ directly leads to the production of estrogen and progesterone, thereby potentially leading to an increased granulosa cell luteinization as well as an improved endometrial environment (185,187). The possible correlation of vitamin D with fertility is also underscored by its association with anti-Müllerian hormone (AMH). AMH is a glycoprotein that plays a fundamental role in the regression of Müllerian ducts in male embryos, while Müllerian ducts develop into female inner reproductive organs in its absence. It is produced by granulosa cells of small follicles in the ovary in females and is commonly used as a tool to assess ovarian follicular reserve (185,188). Across seasons, AMH shows changes mimicking those in serum 25(OH)D concentrations, while vitamin D supplementation might prevent seasonal changes in AMH concentrations (189). In PCOS, ovulatory function is closely linked to the metabolic features of syndrome, including insulin resistance (168,187). Thus, improvement of insulin resistance and other adverse metabolic features in PCOS might also improve ovarian physiology.

Our results in PCOS patients, i.e. that there were no significant effects on relevant metabolic or endocrine parameters, are in line with the findings of several previous RCTs: Raja-Kahn et al. (172) randomized 28 women with PCOS to receive either 12,000 IU of cholecalciferol or placebo daily over 12 weeks; primary outcome measure was difference in QUICKI between the groups, secondary outcome measures included glucose and insulin levels during OGTT as well as blood pressure. Patients were recruited regardless of their 25(OH)D serum concentration, although only six subjects had baseline 25(OH)D serum concentrations >75 nmol/L. The authors reported no significant changes in insulin sensitivity after vitamin D supplementation but a trend towards decreased 2-hour

insulin as well as a protective effect on blood pressure. Another RCT by Garg et al. (173) aimed to investigate the effect of 120,000 IU of cholecalciferol monthly vs. placebo over six months in 36 PCOS patients. Notably, participants from both the intervention and the placebo group concomitantly received 1,500 mg of metformin per day. Vitamin D deficiency or insufficiency was no criterion for study inclusion, however, the mean baseline 25(OH)D serum concentration was 18.3±11.1 nmol/L. After vitamin D supplementation, there was no significant difference in any of the pre-defined parameters of insulin sensitivity or insulin resistance (AUCgluc, serum insulin area under the curve, insulin:glucose ratio, HOMA-IR, Matsuda index, insulinogenic index, and disposition index).

Other RCTs, however, reported significant effects of vitamin D supplementation in PCOS patients: In a study in PCOS women by Jamilian et al. (170), supplementation of 4,000 IU of cholecalciferol daily over 12 weeks lead to a significant decrease in TT, FAI, hirsutism, and CRP as well as a significant increase in SHBG and total antioxidant capacity when compared to supplementation of 1,000 IU of cholecalciferol daily or placebo (n=90). Participants from each group additionally received 1,000 mg of metformin daily. To be eligible for the study, participants needed a HOMA-IR >2.5, while there was no restriction for baseline 25(OH)D serum concentrations. A similar study by Foroozanfard et al. (171) reported a significant decrease in fasting plasma glucose, serum insulin, and HOMA-IR, as well significant decreases in mean change of triglycerides, VLDL-cholesterol, LDL-cholesterol, and TC/HDL-cholesterol ratio. Comparable to the previously mentioned study (170), the significant results were found when supplementation of 4,000 IU of cholecalciferol daily (n=30) was compared to 1,000 IU of cholecalciferol daily (n=30) or placebo (n=30). Participants from each group received 1,500 mg of metformin; 25(OH)D serum concentrations were not relevant for study inclusion (171). Maktabi et al. (190) investigated the effects of 50,000 IU of vitamin D (type of supplement not defined) every two weeks vs. placebo in 70 vitamin D deficient PCOS women [25(OH)D serum concentrations <50 nmol/L]. Markers of insulin resistance and androgens were considered as primary outcome parameters, while lipid profiles and biomarkers of inflammation and oxidative stress were defined as secondary outcome measures. Compared to placebo, vitamin D supplementation lead to a significant decrease in fasting plasma

glucose, HOMA-IR, CRP, plasma malondialdehyde, and homeostasis model of assessment – estimated beta-cell function, while it significantly increased QUICKI (non-significant after adjustment). Razavi et al. (191) reported a significant decrease in FT and DHEAS as well as a significant increase in plasma total antioxidant capacity after supplementation of 200 IU of vitamin D (type of supplement not defined), 90 µg of vitamin K2, and 500 mg of calcium twice daily for eight weeks vs. placebo over eight weeks [n=60 PCOS patients, 30 participants per group, 25(OH)D serum concentrations not relevant for study eligibility]. All participants concomitantly received 1,500 mg of metformin daily during study participation. Recently, Jafari-Sfidvajani et al. (192) investigated the effect of 50,000 IU of cholecalciferol weekly vs. placebo in 60 PCOS patients with a 25(OH)D serum concentration <50 nmol/L. Participants from each group additionally received weight-loss intervention during study participation. In this study, vitamin D supplementation lead to a significant improvement in menstrual frequency, while there were no significant effects regarding androgens.

A plethora of studies investigated the effects of vitamin D supplementation in premenopausal women without PCOS. However, only a few studies addressed the effects of vitamin D supplementation on metabolic parameters in this particular population, as most of the studies were conducted in older participants or in study groups including both male and female subjects. Furthermore, most of the previous RCTs yielded mixed results: In a study in women with former GDM, Yeow et al. (193) found a significant decrease in plasma glucose after 120 minutes during OGTT as well as a significant increase in fasting insulin, but no significant effects on e.g. AUGgluc, fasting glucose, or QUICKI. The study population consisted of 26 participants who received 4,000 IU of cholecalciferol or placebo over six months and had a baseline 25(OH)D serum concentration of 15-50 nmol/L. In another study in 77 healthy overweight and obese women with a mean age of 38±8 years with no restrictions regarding baseline 25(OH)D serum levels (194), there was no significant effect of 1,000 IU of cholecalciferol daily vs. placebo over 12 weeks on glycemic parameters (including HbA1c, fasting plasma glucose, plasma glucose after 120 minutes during OGTT, and HOMA-IR). Ramly et al. (195) aimed to investigate the effect of 50,000 IU of cholecalciferol vs. placebo over two months on cardiometabolic risk factors in 192 vitamin D deficient

[25(OH)D <50 nmol/L] premenopausal women. The authors reported no significant effect of vitamin D supplementation on HOMA-IR, serum lipid profiles, or blood pressure. Our findings of significant vitamin D effects on QUICKI and HOMA-IR in women without PCOS are in line with some previous findings in different study populations: In 100 healthy men with a mean age of 39±13 years and baseline 25(OH)D concentrations <75 nmol/L, supplementation of 20,000 IU of cholecalciferol per week over three months significantly decreased QUICKI when compared to placebo (196). In a study in 42 patients with T2DM (197), a single intramuscular dose of 300,000 IU lead to a significant increase HOMA-IR after three months when compared to the placebo group. To the best of our knowledge, previous RCTs investigating the effect of vitamin D supplementation on androgens in healthy premenopausal women are unavailable, as these studies were preferably conducted in PCOS populations.

Differences between previous RCTs and our study results may be explained by significant differences in study design between the trials. While we used a threshold of serum 25(OH)D <75 nmol/L to recruit study participants, other studies recruited subjects with no restrictions regarding serum 25(OH)D concentrations or used lower thresholds, e.g., <50 nmol/L. The majority of previous RCTs were conducted in significantly smaller study populations, sometimes limiting their conclusions. Furthermore, previous studies varied in regard of their study duration (eight weeks vs. 12 weeks vs. 24 weeks), dose of study medication, or interval of vitamin D supplementation (e.g., daily vs. weekly vs. monthly supplementation) (174). While the aforementioned RCTs entirely used cholecalciferol for vitamin D supplementation, other studies used ergocalciferol (vitamin D2) for supplementation (198). Additionally, some of the cited studies concomitantly administered e.g. metformin, vitamin K2, or calcium, thus potentially influencing the study outcomes.

Our significant findings regarding secondary study outcomes, i.e. the significant reduction in plasma glucose after 60 minutes during OGTT in the PCOS group and the significant increase in HOMAR-IR and decrease in QUICKI in the group without PCOS, need further discussion. The relevance of these findings should be assessed in light of their nature as secondary outcome parameters; thus, our

study may not have been sufficiently powered to detect differences regarding these values. Furthermore, the clinical relevance of a significant decrease in plasma glucose after 60 minutes remains questionable, especially since there were no significant effects on AUCgluc (174). While increases in HOMA-IR and decreases in QUICKI after vitamin D supplementation were reported previously in different study populations (196,197), our findings in premenopausal women without PCOS certainly should be confirmed in future RCTs. Our findings at the visit after 12 weeks in PCOS patients should also be interpreted with caution, since our study was powered to detect differences in AUCgluc after 24 weeks. Therefore, the significant effect of vitamin D supplementation on AUCgluc after 12 weeks in PCOS patients cannot be uncritically interpreted as a possible short-time effect of vitamin D supplementation (174).

In both women with and without PCOS, vitamin D supplementation lead to a significant increase in 25(OH)D serum concentrations, thus giving proof of the effectiveness of our study intervention. In the PCOS group, vitamin D supplementation furthermore significantly decreased PTH and increased $1,25(OH)_2D$ serum concentrations (174). This fits well with current reports in the literature (199,200), that confirmed these effects after effective vitamin D supplementation. The fact that the effects on PTH and $1,25(OH)_2D$ in the study group without PCOS remained non-significant is probably a consequence of the significantly higher baseline 25(OH)D serum concentrations in this group when compared to the PCOS group. Thus, vitamin D supplementation may lead to less pronounced increases in $1,25(OH)_2D$ (201) and a consequently attenuated decrease in PTH, explaining our non-significant findings.

Baseline differences between the groups with and without PCOS reflect findings from previous investigations: PCOS women were found to have a greater risk for overweight, obesity, and central obesity (202), arterial hypertension (203), glucose intolerance (204), and dyslipidemia (205). Furthermore, PCOS patients are prone to having low 25(OH)D serum concentrations, while the processes involved in the pathogenesis of the syndrome lead to higher serum androgen and LH concentrations as well as lower FSH and estradiol concentrations (159,206). The

baseline differences between the groups underscore the validity of our recruitment process and confirm the characterization of our study groups.

This dissertation and its underlying study have several strengths and limitations that need to be further discussed. A possible limitation is the high drop-out rate in the PCOS group that made the additional recruitment of 30 PCOS patients necessary to match the participant numbers resulting from sample size calculation. However, it needs to noted that the main cause for drop-out was unwillingness to continue study participation due to a variety of personal reasons (e.g., preference to use hormonal contraception or unplanned stays abroad), while other participants had to discontinue study participation due to pregnancy (174). The higher drop-out rate in PCOS patients when compared to the group without PCOS may be explained by recruitment circumstances, as more study participants without PCOS were recruited via promotional material (e.g. postings, internet advertisements), while the majority of PCOS patients was recruited from women referred to our outpatient clinic for evaluation of PCOS. Our findings should be interpreted in view of the possible limitation of multiple testing, as multiple parameters were analyzed at each study visit, thus potentially leading to statistical type one errors (174). Another limitation may be the relatively high baseline 25(OH)D concentrations when compared to some of the previous RCTs. However, the threshold was chosen in light of existing guidelines by the Endocrine Society (98), while the pilot study used for sample size calculation (181) also included participants with 25(OH)D concentrations >75 nmol/L. However, we cannot rule out that vitamin D supplementation in subjects with lower baseline 25(OH)D concentrations might lead to different results (174). As an Austrian monocentric study, our results may not be generalizable to other study populations outside of Austria or Europe (174). A strength of our study is that it was designed to specifically detect vitamin D effects on glucose response during OGTT in women with and without PCOS (174). Furthermore, the study groups were significantly larger than those in previous RCTs, while both groups were well-characterized. The validity of our study results is further underscored by the significant effects of vitamin D supplementation on 25(OH)D in both study groups, and on PTH and $1,25(OH)_2D$ in the PCOS group. Additionally, we used a state of the art method to measure 25(OH)D and TT concentrations (207).

In conclusion, in women with and without PCOS who had 25(OH)D concentrations <75 nmol/L, there was no significant effect of vitamin D supplementation on AUCgluc (174). Participants with and without PCOS showed significant differences regarding several baseline characteristics including anthropometric measures, parameters of glucose homeostasis, and androgen concentrations. Regarding secondary outcome parameters, we found a significant reduction in plasma glucose after 60 minutes during OGTT after vitamin D supplementation in the PCOS group as well as a significant increase in HOMA-IR and a significant decrease in QUICKI in the group without PCOS (174). However, these results need further confirmation in future RCTs.

Bibliography

1. Yildiz BO, Bozdag G, Yapici Z, Esinler I, Yarali H. Prevalence, phenotype and cardiometabolic risk of polycystic ovary syndrome under different diagnostic criteria. Hum Reprod. 2012;27(10):3067–73.
2. Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. Nat Rev Endocrinol. 2011;7(4):219–31.
3. Teede H, Deeks A, Moran L. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. BMC Med. 2010;8:41.
4. Stein IF, Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol. 1935;29(2):181–91.
5. Zawadzki JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif A, Givens JR, Haseltine F, editors. Polycystic Ovary Syndrome. Boston: Blackwell Scientific; 1992. p. 377–384.
6. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19(1):41–7.
7. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. Positions statement: Criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: An Androgen Excess Society guideline. J Clin Endocrinol Metab. 2006;91(11):4237–45.
8. Azziz R. Controversy in clinical endocrinology: diagnosis of polycystic ovarian syndrome: the Rotterdam criteria are premature. J Clin Endocrinol Metab. 2006;91(3):781–5.
9. Chang RJ, Katz SE. Diagnosis of polycystic ovary syndrome. Endocrinol Metab Clin North Am. 1999;28(2):397–408.
10. Diamanti-Kandarakis E, Kouli CR, Bergiele AT, Filandra FA, Tsianateli TC, Spina GG, et al. A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. J Clin Endocrinol Metab. 1999;84(11):4006–11.
11. Slayden SM, Moran C, Sams Jr. WM, Boots LR, Azziz R. Hyperandrogenemia in patients presenting with acne. Fertil Steril. 2001;75(5):889–92.
12. Futterweit W, Dunaif A, Yeh HC, Kingsley P. The prevalence of hyperandrogenism in 109 consecutive female patients with diffuse alopecia. J Am Acad Dermatol. 1988;19(5 Pt 1):831–6.
13. Balen AH, Laven JS, Tan SL, Dewailly D. Ultrasound assessment of the polycystic ovary: international consensus definitions. Hum Reprod Update. 2003;9(6):505–14.

14. Miller KK, Rosner W, Lee H, Hier J, Sesmilo G, Schoenfeld D, et al. Measurement of free testosterone in normal women and women with androgen deficiency: comparison of methods. J Clin Endocrinol Metab. 2004;89(2):525–33.

15. Yildiz BO, Bolour S, Woods K, Moore A, Azziz R. Visually scoring hirsutism. Hum Reprod Update. 2010;16(1):51–64.

16. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertil Steril. 2009;91(2):456–88.

17. Lujan ME, Chizen DR, Pierson RA. Diagnostic criteria for polycystic ovary syndrome: pitfalls and controversies. J Obstet Gynaecol Can. 2008;30(8):671–9.

18. Cela E, Robertson C, Rush K, Kousta E, White DM, Wilson H, et al. Prevalence of polycystic ovaries in women with androgenic alopecia. Eur J Endocrinol. 2003;149(5):439–42.

19. Carmina E, Lobo RA. Polycystic ovaries in hirsute women with normal menses. Am J Med. 2001;111(8):602–6.

20. Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, et al. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2013;98(12):4565–92.

21. National Institutes of Health. Evidence-based Methodology Workshop on Polycystic Ovary Syndrome December 3–5, 2012 [Internet]. 2012 [cited 2016 Jul 5]. Available from: https://prevention.nih.gov/docs/programs/pcos/FinalReport.pdf

22. Ehrmann DA. Polycystic ovary syndrome. N Engl J Med. 2005;352(12):1223–36.

23. Blank SK, McCartney CR, Marshall JC. The origins and sequelae of abnormal neuroendocrine function in polycystic ovary syndrome. Hum Reprod Update. 2006;12(4):351–61.

24. Taylor AE, McCourt B, Martin KA, Anderson EJ, Adams JM, Schoenfeld D, et al. Determinants of abnormal gonadotropin secretion in clinically defined women with polycystic ovary syndrome. J Clin Endocrinol Metab. 1997;82(7):2248–56.

25. Waldstreicher J, Santoro NF, Hall JE, Filicori NE, Crowley Jr. WF. Hyperfunction of the hypothalamic-pituitary axis in women with polycystic ovarian disease: indirect evidence for partial gonadotroph desensitization. J Clin Endocrinol Metab. 1988;66(1):165–72.

26. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev. 2012;33(6):981–1030.

27. DeUgarte CM, Bartolucci AA, Azziz R. Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment.

Fertil Steril. 2005;83(5):1454–60.

28. Kahsar-Miller MD, Nixon C, Boots LR, Go RC, Azziz R. Prevalence of polycystic ovary syndrome (PCOS) in first-degree relatives of patients with PCOS. Fertil Steril. 2001;75(1):53–8.

29. Vink JM, Sadrzadeh S, Lambalk CB, Boomsma DI. Heritability of polycystic ovary syndrome in a Dutch twin-family study. J Clin Endocrinol Metab. 2006;91(6):2100–4.

30. Kosova G, Urbanek M. Genetics of the polycystic ovary syndrome. Mol Cell Endocrinol. 2013;373(1–2):29–38.

31. Piltonen TT, Chen J, Erikson DW, Spitzer TL, Barragan F, Rabban JT, et al. Mesenchymal stem/progenitors and other endometrial cell types from women with polycystic ovary syndrome (PCOS) display inflammatory and oncogenic potential. J Clin Endocrinol Metab. 2013;98(9):3765–75.

32. Li Q, Du J, Feng R, Xu Y, Wang H, Sang Q, et al. A possible new mechanism in the pathophysiology of polycystic ovary syndrome (PCOS): The discovery that leukocyte telomere length is strongly associated with PCOS. J Clin Endocrinol Metab. 2014;99(2):E234-240.

33. Chen ZJ, Zhao H, He L, Shi Y, Qin Y, Shi Y, et al. Genome-wide association study identifies susceptibility loci for polycystic ovary syndrome on chromosome 2p16.3, 2p21 and 9q33.3. Nat Genet. 2011;43(1):55–9.

34. Goodarzi MO, Jones MR, Li X, Chua AK, Garcia OA, Chen YD, et al. Replication of association of DENND1A and THADA variants with polycystic ovary syndrome in European cohorts. J Med Genet. 2012;49(2):90–5.

35. Carmina E, Legro RS, Stamets K, Lowell J, Lobo RA. Difference in body weight between American and Italian women with polycystic ovary syndrome: Influence of the diet. Hum Reprod. 2003;18(11):2289–93.

36. Clark AM, Thornley B, Tomlinson L, Galletley C, Norman RJ. Weight loss in obese infertile women results in improvement in reproductive outcome for all forms of fertility treatment. Hum Reprod. 1998;13(6):1502–5.

37. Guzick DS, Wing R, Smith D, Berga SL, Winters SJ. Endocrine consequences of weight loss in obese, hyperandrogenic, anovulatory women. Fertil Steril. 1994;61(4):598–604.

38. Huber-Buchholz MM, Carey DG, Norman RJ. Restoration of reproductive potential by lifestyle modification in obese polycystic ovary syndrome: role of insulin sensitivity and luteinizing hormone. J Clin Endocrinol Metab. 1999;84(4):1470–4.

39. Pasquali R, Antenucci D, Casimirri F, Venturoli S, Paradisi R, Fabbri R, et al. Clinical and hormonal characteristics of obese amenorrheic hyperandrogenic women before and after weight loss. J Clin Endocrinol Metab. 1989;68(1):173–9.

40. Moran LJ, Pasquali R, Teede HJ, Hoeger KM, Norman RJ. Treatment of obesity in polycystic ovary syndrome: a position statement of the Androgen Excess and Polycystic Ovary Syndrome Society. Fertil Steril. 2009;92(6):1966–82.

41. Zhou W, Liu J, Liao L, Han S, Liu J. Effect of bisphenol A on steroid hormone production in rat ovarian theca-interstitial and granulosa cells. Mol Cell Endocrinol. 2008;283(1–2):12–8.

42. Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A. The estrogenic effect of bisphenol a disrupts pancreatic beta-cell function in vivo and induces insulin resistance. Environ Health Perspect. 2006;114(1):106–12.

43. Martin KA, Chang RJ, Ehrmann DA, Ibanez L, Lobo RA, Rosenfield RL, et al. Evaluation and treatment of hirsutism in premenopausal women: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2008;93(4):1105–20.

44. Rosenfield RL. Clincal Practice. Hirsutism. N Engl J Med. 2005;353(24):2578–88.

45. Hatch R, Rosenfield RL, Kim MH, Tredway D. Hirsutism: implications, etiology, and management. Am J Obstet Gynecol. 1981;140(7):815–30.

46. Lowenstein EJ. Diagnosis and management of the dermatologic manifestations of the polycystic ovary syndrome. Dermatol Ther. 2006;19(4):210–23.

47. Falsetti L, Gambera A, Andrico S, Sartori E. Acne and hirsutism in polycystic ovary syndrome: clinical, endocrine-metabolic and ultrasonographic differences. Gynecol Endocrinol. 2002;16(4):275–84.

48. Toscano V, Balducci R, Bianchi P, Guglielmi R, Mangiantini A, Rossi FG, et al. Two different pathogenetic mechanisms may play a role in acne and hirsutism. Clin Endocrinol (Oxf). 1993;39(5):551–6.

49. Brown SK, Shalita AR. Acne vulgaris. Lancet. 1998;351(9119):1871–6.

50. Fraser IS, Kovacs G. Current recommendations for the diagnostic evaluation and follow-up of patients presenting with symptomatic polycystic ovary syndrome. Best Pract Res Clin Obstet Gynaecol. 2004;18(5):813–23.

51. Legro RS, Kunselman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. J Clin Endocrinol Metab. 1999;84(1):165–9.

52. Ehrmann DA, Liljenquist DR, Kasza K, Azziz R, Legro RS, Ghazzi MN, et al. Prevalence and predictors of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2006;91(1):48–53.

53. Ehrmann DA, Kasza K, Azziz R, Legro RS, Ghazzi MN, PCOS/Troglitazone Study Group. Effects of race and family history of type 2 diabetes on metabolic status of women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2005;90(1):66–71.

54. Palmert MR, Gordon CM, Kartashov AI, Legro RS, Emans SJ, Dunaif A. Screening for abnormal glucose tolerance in adolescents with polycystic ovary syndrome. J Clin Endocrinol Metab. 2002;87(3):1017–23.

55. Moran LJ, Misso ML, Wild RA, Norman RJ. Impaired glucose tolerance, type

2 diabetes and metabolic syndrome in polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update. 2010;16(4):347–63.

56. American Diabetes Association. Standards of medical care in diabetes--2013. Diabetes Care. 2013;36 Suppl 1:S11-66.

57. Legro RS, Gnatuk CL, Kunselman AR, Dunaif A. Changes in glucose tolerance over time in women with polycystic ovary syndrome: a controlled study. J Clin Endocrinol Metab. 2005;90(6):3236–42.

58. Norman RJ, Masters L, Milner CR, Wang JX, Davies MJ. Relative risk of conversion from normoglycaemia to impaired glucose tolerance or non-insulin dependent diabetes mellitus in polycystic ovarian syndrome. Hum Reprod. 2001;16(9):1995–8.

59. Boudreaux MY, Talbott EO, Kip KE, Brooks MM, Witchel SF. Risk of T2DM and impaired fasting glucose among PCOS subjects: results of an 8-year follow-up. Curr Diab Rep. 2006;6(1):77–83.

60. Pesant MH, Baillargeon JP. Clinically useful predictors of conversion to abnormal glucose tolerance in women with polycystic ovary syndrome. Fertil Steril. 2011;95(1):210–5.

61. Lerchbaum E, Schwetz V, Giuliani A, Obermayer-Pietsch B. Assessment of glucose metabolism in polycystic ovary syndrome: HbA1c or fasting glucose compared with the oral glucose tolerance test as a screening method. Hum Reprod. 2013;28(9):2537–44.

62. Wild RA, Carmina E, Diamanti-Kandarakis E, Dokras A, Escobar-Morreale HF, Futterweit W, et al. Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society. J Clin Endocrinol Metab. 2010;95(5):2038–49.

63. Brunzell JD, Ayyobi AF. Dyslipidemia in the metabolic syndrome and type 2 diabetes mellitus. Am J Med. 2003;115 Suppl 8A:24S–28S.

64. Lim SS, Norman RJ, Davies MJ, Moran LJ. The effect of obesity on polycystic ovary syndrome: a systematic review and meta-analysis. Obes Rev. 2013;14(2):95–109.

65. Gambineri A, Pelusi C, Vicennati V, Pagotto U, Pasquali R. Obesity and the polycystic ovary syndrome. Int J Obes Relat Metab Disord. 2002;26(7):883–96.

66. Sathyapalan T, Atkin SL. Recent advances in cardiovascular aspects of polycystic ovary syndrome. Eur J Endocrinol. 2012;166(4):575–83.

67. Wehr E, Gruber HJ, Giuliani A, Möller R, Pieber TR, Obermayer-Pietsch B. The lipid accumulation product is associated with impaired glucose tolerance in PCOS women. J Clin Endocrinol Metab. 2011;96(6):E986-90.

68. Balen AH, Conway GS, Kaltsas G, Techatrasak K, Manning PJ, West C, et al. Polycystic ovary syndrome: the spectrum of the disorder in 1741 patients. Hum Reprod. 1995;10(8):2107–11.

69. Bhattacharya S, Porter M, Amalraj E, Templeton A, Hamilton M, Lee AJ, et al. The epidemiology of infertility in the North East of Scotland. Hum Reprod. 2009;24(12):3096–107.

70. Hull MG. Epidemiology of infertility and polycystic ovarian disease: endocrinological and demographic studies. Gynecol Endocrinol. 1987;1(3):235–45.

71. Laukkanen JA, Lakka TA, Rauramaa R, Kuhanen R, Venäläinen JM, Salonen R, et al. Cardiovascular fitness as a predictor of mortality in men. Arch Intern Med. 2001;161(6):825–31.

72. Moran LJ, Hutchison SK, Norman RJ, Teede HJ. Lifestyle changes in women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2011;(2):CD007506.

73. Kiddy DS, Hamilton-Fairley D, Bush A, Short F, Anyaoku V, Reed MJ, et al. Improvement in endocrine and ovarian function during dietary treatment of obese women with polycystic ovary syndrome. Clin Endocrinol (Oxf). 1992;36(1):105–11.

74. Vrbíková J, Cibula D. Combined oral contraceptives in the treatment of polycystic ovary syndrome. Hum Reprod Update. 2005;11(3):277–91.

75. Lopez LM, Grimes DA, Schulz KF. Steroidal contraceptives: effect on carbohydrate metabolism in women without diabetes mellitus. Cochrane Database Syst Rev. 2007;(2):CD006133.

76. Crook D, Godsland IF, Worthington M, Felton CV, Proudler AJ, Stevenson JC. A comparative metabolic study of two low-estrogen-dose oral contraceptives containing desogestrel or gestodene progestins. Am J Obstet Gynecol. 1993;169(5):1183–9.

77. Skouby SO, Endrikat J, Düsterberg B, Schmidt W, Gerlinger C, Wessel J, et al. A 1-year randomized study to evaluate the effects of a dose reduction in oral contraceptives on lipids and carbohydrate metabolism: 20 µg ethinyl estradiol combined with 100 µg levonorgestrel. Contraception. 2005;71(2):111–7.

78. Kjos SL, Peters RK, Xiang A, Thomas D, Schaefer U, Buchanan TA, et al. Contraception and the risk of type 2 diabetes mellitus in Latina women with prior gestational diabetes mellitus. JAMA. 1998;280(6):533–8.

79. Gallo MF, Lopez LM, Grimes DA, Carayon F, Schulz KF, Helmerhorst FM. Combination contraceptives: effects on weight. Cochrane Database Syst Rev. 2014;(1):CD003987.

80. Coney P, Washenik K, Langley RG, DiGiovanna JJ, Harrison DD. Weight change and adverse event incidence with a low-dose oral contraceptive: two randomized, placebo-controlled trials. Contraception. 2001;63(6):297–302.

81. Tang T, Lord JM, Norman RJ, Yasmin E, Balen AH. Insulin-sensitzing drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhea and subfertility. Cochrane Database Syst Rev. 2010;(1):CD003053.

82. Nieuwenhuis-Ruifrok AE, Kuchenbecker WK, Hoek A, Middleton P, Norman

RJ. Insulin sensitizing drugs for weight loss in women of reproductive age who are overweight or obese: systematic review and meta-analysis. Hum Reprod Update. 2009;15(1):57–68.

83. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002;346(6):393–403.

84. Homburg R, Hendriks ML, König TE, Anderson RA, Balen AH, Brincat M, et al. Clomifene citrate or low-dose FSH for the first-line treatment of infertile women with anovulation associated with polycystic ovary syndrome: a prospective randomized multinational study. Hum Reprod. 2012;27(2):468–73.

85. Morin-Papunen L, Rantala AS, Unkila-Kallio L, Tiitinen A, Hippeläinen M, Perheentupa A, et al. Metformin improves pregnancy and live-birth rates in women with polycystic ovary syndrome (PCOS): a multicenter, double-blind, placebo-controlled randomized trial. J Clin Endocrinol Metab. 2012;97(5):1492–500.

86. Moll E, van der Veen F, van Wely M. The role of metformin in polycystic ovary syndrome: a systematic review. Hum Reprod Update. 2007;13(6):527–37.

87. McCollum E V, Davis M. The necessity of certain lipins in the diet during growth. J Biol Chem. 1913;25:167–231.

88. Mellanby E. An experimental investigation on rickets. Lancet. 1919;1:407–12.

89. DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr. 2004;80(6 Suppl):1689–96.

90. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266–81.

91. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. Am J Physiol Ren Physiol. 2005;289(1):F8-28.

92. Omdahl JL, Gray RW, Boyle IT, Knutson J, DeLuca HF. Regulation of metabolism of 25-hydroxycholecalciferol by kidney tissue in vitro by dietary calcium. Nat New Biol. 1972;237(71):63–64.

93. Trummer C, Schwetz V, Pandis M, Grübler MR, Verheyen N, Gaksch M, et al. Effects of vitamin D supplementation on FGF23: a randomized-controlled trial. Eur J Nutr. 2018;doi: 10.1007/s00394-018-1672-7 [Epub ahead of print].

94. Cooke NE, Haddad JG. Vitamin D binding protein (Gc-globulin). Endocr Rev. 1989;10(3):294–307.

95. Koshiyama H, Sone T, Nakao K. Vitamin-D-receptor-gene polymorphism and bone loss. Lancet. 1995;345(8955):990–1.

96. Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. Gene. 2004;338(2):143–56.

97. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al.

The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab. 2011;96(1):53–8.

98. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911–30.

99. Trummer C, Pandis M, Verheyen N, Grübler MR, Gaksch M, Obermayer-Pietsch B, et al. Beneficial effects of UV-radiation: vitamin D and beyond. Int J Environ Res Public Health. 2016;13(10):pii:E1028.

100. Tripkovic L, Lambert H, Hart K, Smith CP, Bucca G, Penson S, et al. Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. Am J Clin Nutr. 2012;95(6):1357–64.

101. Shab-Bidar S, Bours S, Geusens PP, Kessels AG, van den Bergh JP. Serum 25(OH)D response to vitamin D3 supplementation: a meta-regression analysis. Nutrition. 2014;30(9):975–85.

102. Autier P, Gandini S, Mullie P. A systematic review: influence of vitamin D supplementation on serum 25-hydroxyvitamin D concentration. J Clin Endocrinol Metab. 2012;97(8):2606–13.

103. Zittermann A, Ernst JB, Gummert JF, Börgermann J. Vitamin D supplementation, body weight and human serum 25-hydroxyvitamin D response: a systematic review. Eur J Nutr. 2014;53(2):367–74.

104. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr. 2006;84(1):18–28.

105. Heaney RP, Dowell MS, Hale CA, Bendich A. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. J Am Coll Nutr. 2003;22(2):142–6.

106. Thomas MK, Lloyd- Jones DM, Thadhani RI, Shaw AC, Deraska DJ, Kitch BT, et al. Hypovitaminosis D in medical inpatients. N Engl J Med. 1998;338(12):777–83.

107. Slatopolsky E, Gonzalez E, Martin K. Pathogenesis and treatment of renal osteodystrophy. Blood Purif. 2003;21(4–5):318–26.

108. Boonen S, Bischoff-Ferrari HA, Cooper C, Lips P, Ljunggren O, Meunier PJ, et al. Addressing the musculoskeletal components of fracture risk with calcium and vitamin D: a review of the evidence. Calcif Tissue Int. 2006;78(5):257–70.

109. Larsen ER, Mosekilde L, Foldspang A. Vitamin D and calcium supplementation prevents osteoporotic fractures in elderly community dwelling residents: a pragmatic population-based 3-year intervention study. J Bone Min Res. 2004;19(3):370–8.

110. Bischoff-Ferrari HA, Dawson-Hughes B, Orav EJ, Staehelin HB, Meyer OW, Theiler R, et al. Monthly high-dose vitamin D treatment for the prevention of

functional decline: a randomized clinical trial. JAMA Intern Med. 2016;176(2):175–83.

111. Pilz S, Gaksch M, Hartaigh BÓ, Tomaschitz A, März W. Vitamin D in preventive medicine. Anticancer Res. 2015;35(2):1161–70.

112. Apperly FL. The Relation of Solar Radiation to Cancer Mortality in North America. Cancer Res. 1941;1:191–5.

113. Pilz S, Grübler M, Gaksch M, Schwetz V, Trummer C, Hartaigh BÓ, et al. Vitamin D and mortality. Anticancer Res. 2016;36(3):1379–87.

114. Pilz S, Gaksch M, O'Hartaigh B, Tomaschitz A, März W. The role of vitamin D deficiency in cardiovascular disease: where do we stand in 2013? Arch Toxicol. 2013;87(12):2083–103.

115. Pilz S, Gaksch M, Kienreich K, Grübler M, Verheyen N, Fahrleitner-Pammer A, et al. Effects of vitamin D on blood pressure and cardiovascular risk factors: a randomized controlled trial. Hypertension. 2015;65(6):1195–201.

116. Pilz S, Kienreich K, Rutters F, de Jongh R, van Ballegooijen AJ, Grübler M, et al. Role of vitamin D in the development of insulin resistance and type 2 diabetes. Curr Diab Rep. 2013;13(2):261–70.

117. Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF, et al. Vitamin D and human health: lessons from vitamin D receptor null mice. Endocr Rev. 2008;29(6):726–76.

118. Sung CC, Liao MT, Lu KC, Wu CC. Role of vitamin D in insulin resistance. J Biomed Biotechnol. 2012;2012:634195.

119. Takiishi T, Gysemans C, Bouillon R, Mathieu C. Vitamin D and diabetes. Endocrinol Metab Clin North Am. 2010;39(2):419–46.

120. Lips P, van Schoor NM. The effect of vitamin D on bone and osteoporosis. Best Pract Res Clin Endocrinol Metab. 2011;25(4):585–91.

121. Chang E, Donkin SS, Teegarden D. Parathyroid hormone suppresses insulin signaling in adipocytes. Mol Cell Endocrinol. 2009;307(1–2):77–82.

122. Lindqvist P, Olsson H, Landin-Olsson M. Are active sun exposure habits related to lowering risk of type 2 diabetes mellitus in women, a prospective cohort study? Diabetes Res Clin Pract. 2010;90(1):109–14.

123. Ishii H, Suzuki H, Baba T, Nakamura K, Watanabe T. Seasonal variation of glycemic control in type 2 diabetic patients. Diabetes Care. 2001;24(8):1503.

124. Pittas AG, Nelson J, Mitri J, Hillmann W, Garganta C, Nathan DM, et al. Plasma 25-hydroxyvitamin D and progression to diabetes in patients at risk for diabetes: An ancillary analysis in the Diabetes Prevention Program. Diabetes Care. 2012;35(3):565–73.

125. Liu S, Song Y, Ford E, Manson JE, Buring JE, Ridker PM. Dietary calcium, vitamin D, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. Diabetes Care. 2005;28(12):2926–32.

126. Mitri J, Muraru MD, Pittas AG. Vitamin D and type 2 diabetes: A systematic review. Eur J Clin Nutr. 2011;65(9):1005–15.

127. George PS, Pearson ER, Witham MD. Effect of vitamin D supplementation on glycaemic control and insulin resistance: a systematic review and meta-analysis. Diabet Med. 2012;29(8):142–50.

128. Agic A, Xu H, Altgassen C, Noack F, Wolfler MM, Diedrich K, et al. Relative expression of 1,25-dihydroxyvitamin D3 receptor, vitamin D 1 alpha-hydroxylase, vitamin D 24-hydroxylase, and vitamin D 25-hydroxylase in endometriosis and gynecologic cancers. Reprod Sci. 2007;14(5):486–97.

129. Parikh G, Varadinova M, Suwandhi P, Araki T, Rosenwaks Z, Poretsky L, et al. Vitamin D regulates steroidogenesis and insulin-like growth factor binding protein-1 (IGFBP-1) production in human ovarian cells. Horm Metab Res. 2010;42(10):754–7.

130. Pérez-Fernandez R, Alonso M, Segura C, Muñoz I, García-Caballero T, Diguez C. Vitamin D receptor gene expression in human pituitary gland. Life Sci. 1997;60(1):35–42.

131. Tanamura A, Nomura S, Kurauchi O, Furui T, Mizutani S, Tomoda Y. Purification and characterization of 1,25(OH)2D3 receptor from human placenta. J Obstet Gynaecol (Tokyo 1995). 1995;21(6):631–9.

132. Henry HL, Norman AW. Vitamin D: metabolism and biological actions. Annu Rev Nutr. 1984;4:493–520.

133. Lerchbaum E, Obermayer-Pietsch B. Vitamin D and fertility: a systematic review. Eur J Endocrinol. 2012;166(5):765–78.

134. Johnson JA, Grande JP, Roche PC, Kumar R. Immunohistochemical detection and distribution of the 1,25-dihydrodyvitamin D3 receptor in rat reproductive tissues. Histochem Cell Biol. 1996;105(1):7–15.

135. Merke J, Hügel U, Ritz E. Nuclear testicular 1,25-dihydroxyvitamin D3 receptors in Sertoli cells and seminiferous tubules of adult rodents. Biochem Biophys Res Commun. 1985;127(1):303–9.

136. Corbett ST, Hill O, Nangia AK. Vitamin D receptor found in human sperm. Urology. 2006;68(6):1345–9.

137. Aquila S, Guido C, Perrotta I, Tripepi S, Nastro A, Andò S. Human sperm anatomy: ultrastructural localization of 1alpha,25-dihydroxyvitamin D3 receptor and its possible role in the human male gamete. J Anat. 2008;213(5):555–64.

138. Rojansky N, Brzezinski A, Schenker JG. Seasonality in human reproduction: an update. Hum Reprod. 1992;7(6):735–45.

139. Rojansky N, Benshushan A, Meirsdorf S, Lewin A, Laufer N, Safran A. Seasonal variability in fertilization and embryo quality rates in women undergoing IVF. Fertil Steril. 2000;74(3):476–81.

140. Ozkan S, Jindal S, Greenseid K, Shu J, Zeitlian G, Hickmon C, et al. Replete vitamin D stores predict reproductive success following in vitro fertilization. Fertil Steril. 2010;94(4):1314–9.

141. Anifandis GM, Dafopoulos K, Messini CI, Chalvatzas N, Liakos N, Pournaras S, et al. Prognostic value of follicular fluid 25-OH vitamin D and glucose

levels in the IVF outcome. Reprod Biol Endocrinol. 2010;8:91.

142. Somigliana E, Panina-Bordignon P, Murone S, Di Lucia P, Vercellini P, Vigano P. Vitamin D reserve is higher in women with endometriosis. Hum Reprod. 2007;22(8):2273–8.

143. Hartwell D, Rødbro P, Jensen SB, Thomsen K, Christiansen C. Vitamin D metabolites--relation to age, menopause and endometriosis. Scand J Clin Lab Invest. 1990;50(2):115–21.

144. Yoshida M, Kawano N, Yoshida K. Control of sperm motility and fertility: diverse factors and common mechanisms. Cell Mol Life Sci. 2008;65(21):3446–57.

145. Blomberg Jensen M, Bjerrum PJ, Jessen TE, Nielsen JE, Joensen UN, Olesen IA, et al. Vitamin D is positively associated with sperm motility and increases intracellular calcium in human spermatozoa. Hum Reprod. 2011;26(6):1307–17.

146. Foresta C, Strapazzon G, De Toni L, Perilli L, Di Mambro A, Muciaccia B, et al. Bone mineral density and testicular failure: evidence for a role of vitamin D 25-hydroxylase in human testis. J Clin Endocrinol Metab. 2011;96(4):E646-652.

147. Pilz S, März W, Wellnitz B, Seelhorst U, Fahrleitner-Pammer A, Dimai HP, et al. 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. 2008;93(10):3927–35.

148. Wehr E, Pilz S, Boehm BO, März W, Grammer T, Obermayer-Pietsch B. Low free testosterone is associated with heart failure mortality in older men referred for coronary angiography. Eur J Hear Fail. 2011;13(5):482–8.

149. Somjen D, Katzburg S, Stern N, Kohen F, Sharon O, Limor R, et al. 25 hydroxy-vitamin D(3)-1alpha hydroxylase expression and activity in cultured human osteoblasts and their modulation by parathyroid hormone, estrogenic compounds and dihydrotestosterone. J Steroid Biochem Mol Biol. 2007;107(3–5):238–44.

150. Mordan-McCombs S, Brown T, Wang WL, Gaupel AC, Welsh JE, Tenniswood M. Tumor progression in the LPB-Tag transgenic model of prostate cancer is altered by vitamin D receptor and serum testosterone status. J Steroid Biochem Mol Biol. 2010;121(1–2):368–71.

151. Wehr E, Pilz S, Boehm BO, März W, Obermayer-Pietsch B. Association of vitamin D status with serum androgen levels in men. Clin Endocrinol (Oxf). 2010;73(2):243–8.

152. Lee DM, Tajar A, Pye SR, Boonen S, Vanderschueren D, Bouillon R, et al. Association of hypogonadism with vitamin D status: the European Male Ageing Study. Eur J Endocrinol. 2012;166(1):77–85.

153. Turton CW, Stanley P, Stamp TC, Maxwell JD. Altered vitamin-D metabolism in pregnancy. Lancet. 1977;1(8005):222–5.

154. Looker AC, Pfeiffer CM, Lacher DA, Schleicher RL, Picciano MF, Yetley EA. Serum 25-hydroxyvitamin D status of the US population: 1988–1994

compared with 2000–2004. Am J Clin Nutr. 2008;88(6):1519–27.

155. Zhang C, Qiu C, Hu FB, David RM, van Dam RM, Bralley A, et al. Maternal plasma 25-hydroxyvitamin D concentrations and the risk for gestational diabetes mellitus. PLoS One. 2008;3(11):e3753.

156. Maghbooli Z, Hossein-Nezhad A, Karimi F, Shafaei AR, Larijani B. Correlation between vitamin D3 deficiency and insulin resistance in pregnancy. Diabetes Metab Res Rev. 2008;24(1):27–32.

157. Hollis BW, Johnson D, Hulsey TC, Ebeling M, Wagner CL. Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. J Bone Miner Res. 2011;26(10):2341–57.

158. He C, Lin Z, Robb SW, Ezeamama AE. Serum vitamin D levels and polycystic ovary syndrome: a systematic review and meta-analysis. Nutrients. 2015;7(6):4555–77.

159. Thomson RL, Spedding S, Buckley JD. Vitamin D in the aetiology and management of polycystic ovary syndrome. Clin Endocrinol (Oxf). 2012;77(3):343–50.

160. Li HW, Brereton RE, Anderson RA, Wallace AM, Ho CK. Vitamin D deficiency is common and associated with metabolic risk factors in patients with polycystic ovary syndrome. Metabolism. 2011;60(10):1475–81.

161. Wehr E, Trummer O, Giuliani A, Gruber HJ, Pieber TR, Obermayer-Pietsch B. Vitamin D-associated polymorphisms are related to insulin resistance and vitamin D deficiency in polycystic ovary syndrome. Eur J Endocrinol. 2011;164(5):741–9.

162. Mahmoudi T, Gourabi H, Ashrafi M, Yazdi RS, Ezabadi Z. Calciotropic hormones, insulin resistance, and the polycystic ovary syndrome. Fertil Steril. 2010;93(4):1208–14.

163. Muscogiuri G, Policola C, Prioletta A, Sorice G, Mezza T, Lassandro A, et al. Low levels of 25(OH)D and insulin-resistance: 2 unrelated features or a cause-effect in PCOS? Clin Nutr. 2012;31(4):476–80.

164. Panidis D, Balaris C, Farmakiotis D, Rousso D, Kourtis A, Balaris V, et al. Serum parathyroid hormone concentrations are increased in women with polycystic ovary syndrome. Clin Chem. 2005;51(9):1691–7.

165. Lerchbaum E, Giuliani A, Gruber HJ, Pieber TR, Obermayer-Pietsch B. Adult-type hypolactasia and calcium intake in polycystic ovary syndrome. Clin Endocrinol (Oxf). 2012;77(6):834–43.

166. Sander VA, Hapon MB, Sícaro L, Lombardi EP, Jahn GA, Motta AB. Alterations of folliculogenesis in women with polycystic ovary syndrome. J Steroid Biochem Mol Biol. 2011;124(1–2):58–64.

167. Hahn S, Haselhorst S, Tan S, Quadbeck B, Schmidt M, Roesler S, et al. Low serum 25-hydroxyvitamin D concentrations are associated with insulin resistance and obesity in women with polycystic ovary syndrome. Exp Clin Endocrinol Diabetes. 2006;114(10):577–83.

168. Wehr E, Pilz S, Schweighofer N, Giuliani A, Kopera D, Pieber TR, et al.

Association of hypovitaminosis D with metabolic disturbances in polycystic ovary syndrome. Eur J Endocrinol. 2009;161(4):575–82.

169. Ott J, Wattar L, Kurz C, Seemann R, Huber JC, Mayerhofer K, et al. Parameters for calcium metabolism in women with polycystic ovary syndrome who undergo clomiphene citrate stimulation: a prospective cohort study. Eur J Endocrinol. 2012;166(5):897–902.

170. Jamilian M, Foroozanfard F, Rahmani E, Talebi M, Bahmani F, Asemi Z. Effect of two different doses of vitamin D supplementation on metabolic profiles of insulin-resistant patients with polycystic ovary syndrome. Nutrients. 2017;9(12):pii: E1280.

171. Foroozanfard F, Talebi M, Samimi M, Mehrabi S, Badehnoosh B, Jamilian M, et al. Effect of two different doses of vitamin D supplementation on metabolic profiles in insulin-resistant patients with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Horm Metab Res. 2017;49(8):612–7.

172. Raja-Khan N, Shah J, Stetter CM, Lott ME, Kunselman AR, Dodson WC, et al. High-dose vitamin D supplementation and measures of insulin sensitivity in polycystic ovary syndrome: a randomized, controlled pilot trial. Fertil Steril. 2014;101(6):1740–6.

173. Garg G, Kachhawa G, Ramot R, Khadgawat R, Tandon N, Sreenivas V, et al. Effect of vitamin D supplementation on insulin kinetics and cardiovascular risk factors in polycystic ovarian syndrome: a pilot study. Endocr Connect. 2015;4(2):108–16.

174. Trummer C, Schwetz V, Kollmann M, Wölfler M, Münzker J, Pieber TR, et al. Effects of vitamin D supplementation on metabolic and endocrine parameters in PCOS: a randomized-controlled trial. Eur J Nutr. 2018;doi: 10.1007/s00394-018-1760-8 [Epub ahead of print].

175. Moher D, Hopewell S, Schulz KF, Montori V, Gøtzsche PC, Devereaux PJ, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. BMJ. 2010;340:c869.

176. Büttler RM, Martens F, Fanelli F, Pham HT, Kushnir MM, Janssen MJ, et al. Comparison of 7 published LC-MS/MS methods for the simultaneous measurement of testosterone, androstenedione, and dehydroepiandrosterone in serum. Clin Chem. 2015;61(12):1475–83.

177. Büttler RM, Martens F, Kushnir MM, Ackermans MT, Blankenstein MA, Heijboer AC. Simultaneous measurement of testosterone, androstenedione and dehydroepiandrosterone (DHEA) in serum and plasma using isotope-dilution 2-dimension ultra high performance liquid-chromatography tandem mass spectrometry (ID-LC-MS/MS). Clin Chim Acta. 2015;438:157–9.

178. Dirks NF, Vesper HW, van Herwaarden AE, van den Ouweland JM, Kema IP, Krabbe JG, et al. Various calibration procedures result in optimal standardization of routinely used 25(OH)D ID-LC-MS/MS methods. Clin Chim Acta. 2016;462:49–54.

179. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index : a simple, accurate method for

assessing insulin sensitivity in humans. J Clin Endocrinol Metab. 2000;85(7):2402–10.

180. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab. 1999;84(10):3666–72.

181. Wehr E, Pieber TR, Obermayer-Pietsch B. Effect of vitamin D3 treatment on glucose metabolism and menstrual frequency in polycystic ovary syndrome women: a pilot study. J Endocrinol Invest. 2011;34(10):757–63.

182. Fadda GZ, Akmal M, Lipson LG, Massry SG. Direct effect of parathyroid hormone on insulin secretion from pancreatic islets. Am J Physiol. 1990;258(6 Pt 1):E975-984.

183. Maestro B, Dávila N, Carranza MC, Calle C. Identification of a vitamin D response element in the human insulin receptor gene promoter. J Steroid Biochem Mol Biol. 2003;84(2–3):223–30.

184. Maestro B, Molero S, Bajo S, Dávila N, Calle C. Transcriptional activation of the human insulin receptor gene by 1,25-dihydroxyvitamin D(3). Cell Biochem Funct. 2002;20(3):227–32.

185. Trummer C, Pilz S, Schwetz V, Obermayer-Pietsch B, Lerchbaum E. Vitamin D, PCOS and androgens in men: a systematic review. Endocr Connect. 2018;7(3):R95–113.

186. Weisman Y, Harell A, Edelstein S, David M, Spirer Z, Golander A. 1 alpha, 25-Dihydroxyvitamin D3 and 24,25-dihydroxyvitamin D3 in vitro synthesis by human decidua and placenta. Nature. 1979;281(5729):317–9.

187. Irani M, Merhi Z. Role of vitamin D in ovarian physiology and its implication in reproduction: a systematic review. Fertil Steril. 2014;102(2):460–468.e3.

188. Zec I, Tislaric-Medenjak D, Megla ZB, Kucak I. Anti-Müllerian hormone: a unique biochemical marker of gonadal development and fertility in humans. Biochem Med (Zagreb). 2011;21(3):219–30.

189. Dennis NA, Houghton LA, Jones GT, van Rij AM, Morgan K, McLennan IS. The level of serum anti-Müllerian hormone correlates with vitamin D status in men and women but not in boys. J Clin Endocrinol Metab. 2012;97(7):2450–5.

190. Maktabi M, Chamani M, Asemi Z. The effects of vitamin D supplementation on metabolic status of patients with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Horm Metab Res. 2017;49(7):493–8.

191. Razavi M, Jamilian M, Karamali M, Bahmani F, Aghadavod E, Asemi Z. The effects of vitamin D-K-calcium co-supplementation on endocrine, inflammation, and oxidative stress biomarkers in vitamin D-deficient women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Horm Metab Res. 2016;48(7):446–51.

192. Jafari-Sfidvajani S, Ahangari R, Hozoori M, Mozaffari-Khosravi H, Fallahzadeh H, Nadjarzadeh A. The effect of vitamin D supplementation in combination with low-calorie diet on anthropometric indices and androgen

hormones in women with polycystic ovary syndrome: a double-blind, randomized, placebo-controlled trial. J Endocrinol Invest. 2018;41(5):597–607.

193. Yeow TP, Lim SL, Hor CP, Khir AS, Wan Mohamud WN, Pacini G. Impact of vitamin D replacement on markers of glucose metabolism and cardio-metabolic risk in women with former gestational diabetes - a double-blind, randomized controlled trial. PLoS One. 2015;10(6):e0129017.

194. Salehpour A, Shidfar F, Hosseinpanah F, Vafa M, Razaghi M, Amiri F. Does vitamin D3 supplementation improve glucose homeostasis in overweight or obese women? A double-blind, randomized, placebo-controlled clinical trial. Diabet Med. 2013;30(12):1477–81.

195. Ramly M, Ming MF, Chinna K, Suboh S, Pendek R. Effect of vitamin D supplementation on cardiometabolic risks and health-related quality of life among urban premenopausal women in a tropical country - a randomized controlled trial. PLoS One. 2014;9(10):e110476.

196. Lerchbaum E, Pilz S, Trummer C, Schwetz V, Pachernegg O, Heijboer AC, et al. Vitamin D and testosterone in healthy men: a randomized controlled trial. J Clin Endocrinol Metab. 2017;102(11):4292–302.

197. Heshmat R, Tabatabaei-Malazy O, Abbaszadeh-Ahranjani S, Shahbazi S, Khooshehchin G, Bandarian F, et al. Effect of vitamin D on insulin resistance and anthropometric parameters in type 2 diabetes; a randomized double-blind clinical trial. Daru. 2012;20(1):10.

198. Pal L, Berry A, Coraluzzi L, Kustan E, Danton C, Shaw J, et al. Therapeutic implications of vitamin D and calcium in overweight women with polycystic ovary syndrome. Gynecol Endocrinol. 2012;28(12):965–8.

199. Moslehi N, Shab-Bidar S, Mirmiran P, Hosseinpanah F, Azizi F. Determinants of parathyroid hormone response to vitamin D supplementation: a systematic review and meta-analysis of randomised controlled trials. Br J Nutr. 2015;114(9):1360–74.

200. Trummer C, Schwetz V, Pandis M, Grübler M, Verheyen N, Gaksch M, et al. Effects of vitamin D supplementation on IGF-1 and calcitriol: a randomized-controlled trial. Nutrients. 2017;9(6):pii: E623.

201. Zittermann A, Ernst JB, Birschmann I, Dittrich M. Effect of vitamin D or activated vitamin D on circulating 1,25-dihydroxyvitamin D concentrations: a systematic review and metaanalysis of randomized controlled trials. Clin Chem. 2015;61(12):1484–94.

202. Lim SS, Davies MJ, Norman RJ, Moran LJ. Overweight, obesity and central obesity in women with polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update. 2012;18(6):618–37.

203. Zachurzok-Buczynska A, Szydlowski L, Gawlik A, Wilk K, Malecka-Tendera E. Blood pressure regulation and resting heart rate abnormalities in adolescent girls with polycystic ovary syndrome. Fertil Steril. 2011;96(6):1519–25.

204. Salley KE, Wickham EP, Cheang KI, Essah PA, Karjane NW, Nestler JE.

Glucose intolerance in polycystic ovary syndrome--a position statement of the Androgen Excess Society. J Clin Endocrinol Metab. 2007;92(12):4546–56.

205. Wild RA. Dyslipidemia in PCOS. Steroids. 2012;77(4):295–9.

206. Rosenfield RL, Ehrmann DA. The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocr Rev. 2016;37(5):467–520.

207. Handelsman DJ, Wartofsky L. Requirement for mass spectrometry sex steroid assays in the journal of clinical endocrinology and metabolism. J Clin Endocrinol Metab. 2013;98(10):3971–3.