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Review article

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THE RELATIONSHIP BETWEEN VITAMIN D_3 AND INSULIN IN POLYCYSTIC OVARY SYNDROME - A CRITICAL REVIEW

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Polycystic ovary syndrome (PCOS) is the most common endocrine disorder affecting women of reproductive age. It is characterized by hormonal, reproductive and metabolic disturbances, including hyperandrogenism, altered gonadotropin level, ovarian cysts and ovulatory dysfunction as well as insulin resistance, hyperinsulinemia and dyslipidemia. It was shown that increased insulin concentration is a plausible factor in the pathogenesis of PCOS. Insulin leads to overstimulation of ovarian theca cells to androgen biosynthesis and contributes to insulin resistance in tissues such as muscle, liver, adipose tissue and ovary of PCOS patients. Noteworthy, recent studies suggested that supplementation with vitamin D_3 may be an alternative therapy increasing insulin sensitivity and thereby improving reproductive parameters in PCOS women. Indeed, various action of vitamin D_3 on the ovarian, hormonal and metabolic features observed in PCOS were presented. Many studies reported therapeutic effects of vitamin D_3, but some research found a lack of influence or contradicted these findings. Therefore, the aim of this review was to summarize the available evidence about vitamin D_3 and insulin interaction in PCOS, and discusses the potential usefulness of VD_3 in PCOS treatment.

Key words: *polycystic ovary syndrome, vitamin D_3, insulin, hyperandrogenism, menstrual cycle, insulin resistance, lipid profile*

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder affecting women of reproductive age. Among the global female population, the prevalence of PCOS is estimated at 5 – 15% (1). PCOS is a multisystem disorder, associated with hormonal, reproductive and metabolic disturbances. The main, but somewhat variable symptoms, are hyperandrogenism, abnormal gonadotropin level, polycystic ovarian morphology (PCOM) and ovulatory dysfunction as well as insulin resistance, hyperinsulinemia, impaired glucose tolerance, type 2 diabetes mellitus (T2DM), dyslipidemia, hypertension and hirsutism (2, 3). Currently, insulin resistance occurs in 75% of PCOS women, both obese and non-obese (4). Diminished sensitivity to insulin leads to compensatory hyperinsulinemia, which contributes to development of hyperandrogenism in women with PCOS, stimulating androgen synthesis in ovarian theca cells (5). A plethora of research has been carried out on drugs that improve insulin sensitivity in patients with PCOS. Many are already widely used, *e.g.* rosiglitazone, pioglitazone and metformin (6). However, there are some concerns about their safety and research is underway to find an alternative treatment of hyperinsulinemia in PCOS. Promising evidence has been provided by recent studies, which suggest that supplementation with vitamin D_3 (VD_3) may be beneficial in increasing insulin sensitivity in PCOS patients and thereby improving reproductive parameters. This review summarizes the available evidence about VD_3 and insulin interaction in PCOS, and discusses the potential usefulness of VD_3 in PCOS treatment.

POLYCYSTIC OVARY SYNDROME - A BRIEF GLANCE

PCOS is a heterogeneous disease making diagnostic difficulties. Diagnosis requires the establishment of key symptoms while excluding other hyperandrogenic or oligoovulatory causes (1). The first PCOS criteria were described in 1990 by National Institutes of Health (NIH) (7) that were further modified in Rotterdam in 2003 in line with European Society for Human Reproduction and Embryology (ESHRE) recommendations (8) and in 2006 by the American Society for Reproductive Medicine (ASRM) (9). Finally, the following two criteria are proposed: 1) clinical and/or biochemical signs of androgen excess, and 2) ovarian dysfunction, including oligo-/anovulation and/or PCOM. PCOS is classified into four separate phenotypes according to the presence or absence of three characteristics: phenotype A - the presence of hyperandrogenism, ovulatory dysfunction and POMC; phenotype B - the presence of hyperandrogenism and oligo-/anovulation; phenotype C - the presence of hyperandrogenism and PCOM; and phenotype D - the presence of oligo-/anovulation and PCOM (1, 10).

The pathophysiology of PCOS is complex and involves many intertwined factors. It is known that hereditary, genetic and environmental factors contribute to PCOS etiology. Features

such as PCOM, maternal PCOS, metabolic syndrome and hyperandrogenism are heritable traits and therefore PCOS risk factors (11). Among genetic factors the most important are polymorphism and differential expression of genes encoding sex hormone-binding globulin (SHBG), steroidogenic enzymes, androgen receptor and gonadotropin receptors (12, 13). Environmental factors in PCOS include unhealthy lifestyle, diet, obesity and environmental toxin (1, 14) as well as intrauterine factors such as prenatal nutrition and androgen exposure (11).

The primary defect in PCOS is a functional ovarian hyperandrogenism (FOH) caused by steroidogenic hyperactivity, which disrupts the intraovarian biosynthesis of androgens and estrogens (11). The activity of hypothalamic-pituitary gonadotropin-releasing hormone (GnRH)/luteinizing hormone (LH) system involves different intermediary mechanisms (15). The primary cause of FOH may be an increased GnRH secretion from the hypothalamus, leading to an excessive release of LH by the pituitary gland. This abnormality results in elevated circulating LH/FSH (follicle-stimulating hormone) ratio and hypersecretion of androgens by ovarian theca cells, which impairs follicular development and increases the number of growing small antral follicles (16). In PCOS women, ovarian follicles are more resistant to FSH and the increased concentration of LH inhibits the proliferation of granulosa cells, causing their premature luteinization (17, 18). Reduced FSH sensitivity of follicles leads to inhibition of cytochrome P450 aromatase (CYP19A1) activity and blocks the conversion of androgens to estrogens that further causes the arrest of normal

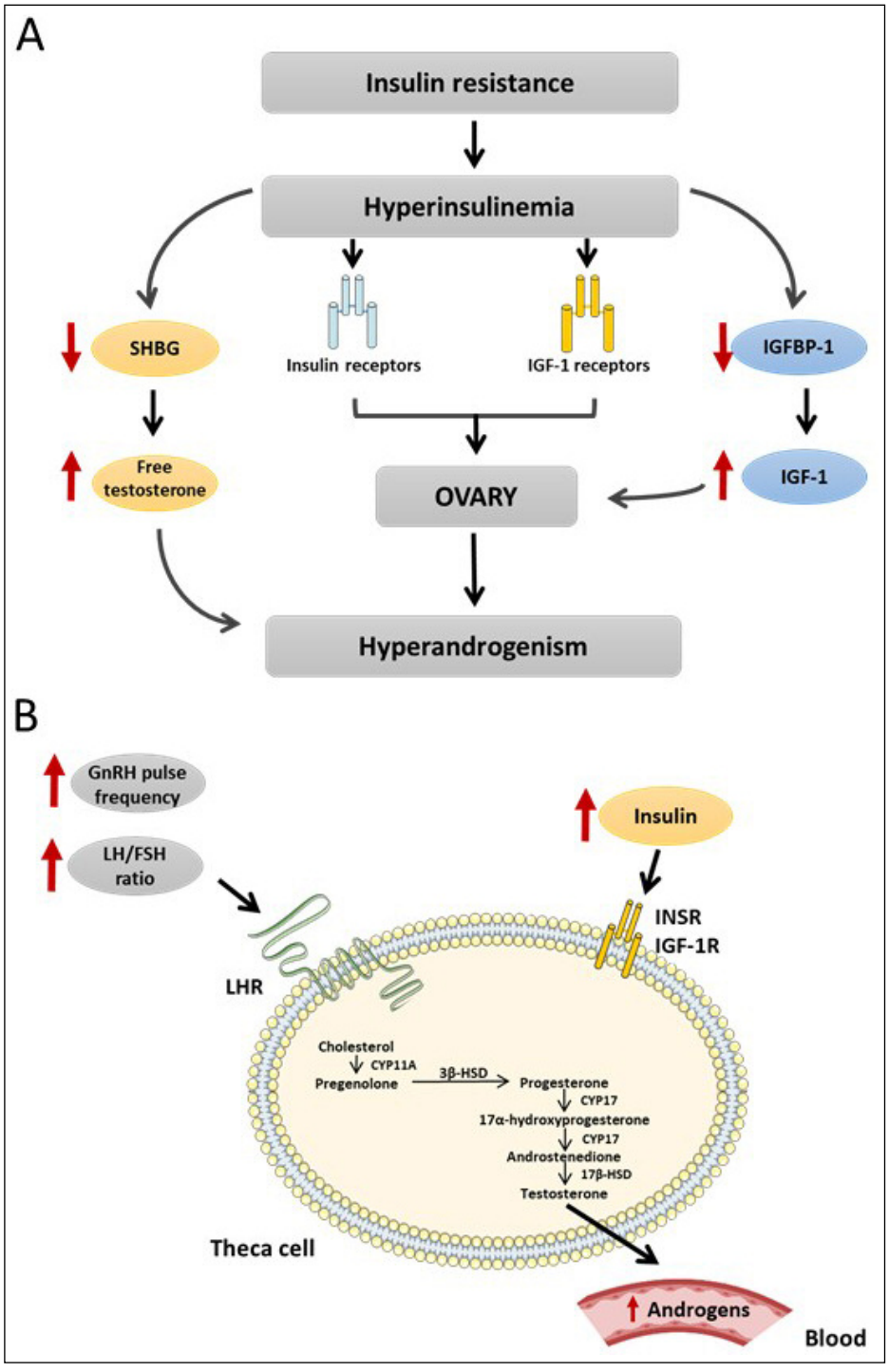

Fig. 1. The link between insulin resistance and hyperandrogenism. (A) Effect of high plasma insulin concentration on the induction of hyperandrogenism. (B) Stimulation of androgens biosynthesis in ovarian theca cells by luteinizing hormone (LH) and insulin. 3β-HSD, 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; CYP11, cholesterol side-chain cleavage enzyme; CYP17, cytochrome P450 17α-hydroxylase/c17-20 lyase; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; IGF-1, insulin-like growth factor 1; IGFBP-1, insulin-like growth factor binding protein 1; IGF-1R, insulin-like growth factor 1 receptor; INSR, insulin receptor; LHR, luteinizing hormone receptor; SHBG, sex hormone-binding globulin.

follicle development. Consequently, dominant follicles are not formed leading to oligo-/anovulation (19, 20). Overstimulation of theca cells by LH is exacerbated by insulin, which acts directly *via* the insulin receptor or indirectly through the insulin-like growth factor 1 (IGF-1) receptor (*Fig. 1*).

INSULIN IN POLYCYSTIC OVARY SYNDROME

Insulin is the most important anabolic peptide hormone that regulates carbohydrate metabolism (21). The predominant stimulus for insulin production and secretion is an increased glucose concentration in the blood. Insulin facilitates glucose uptake and accumulation in the form of glycogen by target tissues such as liver, muscle and adipose tissue, leading to decreased circulating glucose. As blood glucose concentration decreases, glycogen is converted into glucose under the control of glucagon (22). Insulin is also involved in the conversion of carbohydrates into fats or enhances protein synthesis by accelerating the supply of amino acids. It also decreases gluconeogenesis, proteolysis and lipolysis (21).

Insulin synthesis takes place in the pancreas β-cells. In humans, there is a single insulin gene *INS* on chromosome 11 coding the precursor preproinsulin peptide. The peptide is a single chain composed of a signal sequence and peptides B, C and A (23, 24). In order to exert a biological effect in target cells, insulin binds to the specific receptor (INSR) with tyrosine kinase activity (25), which is composed of two extracellular α subunits and two membrane-spanning β subunits (26). Binding of insulin to INSR leads to its dimerization, autophosphorylation and phosphorylation of insulin receptor substrate proteins (IRS) (27) that can activate two main signaling pathways: phosphoinositide-3 kinase (PI3K)-Akt pathway or mitogen-activated protein kinase (MAPK) pathway (28) (*Fig. 2*).

Insulin resistance and hyperinsulinemia are hypothesized to be crucial in the pathogenesis of PCOS. The disrupted molecular mechanism of insulin action in PCOS involves muscle, liver, adipose tissue as well as ovarian tissue. In muscle, constitutive activation of MEK1 and MEK2 (key kinases in the MAPK signal transduction pathway) increases serine phosphorylation of the insulin receptor and IRS1 (29, 30). As a consequence, insulin signaling in the metabolic, but not the mitogenic, pathways is affected (31). Other molecular mechanisms of insulin resistance are observed in adipose tissue of PCOS women (32, 33): the function of adipocytes is disrupted leading to peripheral insulin resistance and inflammation. This is caused by reduced insulin-stimulated glucose transport (34), decreased production of glucose transporter 4 (GLUT4) (35, 36) and reduced insulin-stimulated inhibition of lipolysis (37, 38). Recent data indicates that increased expression of microRNA-93 and microRNA-223 suppressed GLUT4 production and glucose transport in adipocytes of PCOS patients (36, 39). In addition to common insulin-resistant tissues, ovarian cells seem to show decreased insulin sensitivity in PCOS. Insulin alone increases androstenedione synthesis and together with LH stimulates further androgen production by theca cells, contributing to hyperandrogenism (40). This elevated steroidogenesis probably results from cross-talk with LH-induced cyclic adenosine monophosphate (cAMP) accumulation that might, in turn, activate PI3K activity (41). It is notable that ambiguous results were obtained using granulosa cells from polycystic ovary follicles: in follicles less than 8 mm, insulin prematurely enhanced LH action leading to growth arrest, whereas in follicles measuring more than 10 mm, granulosa cells became responsive to LH (41). To explain these discrepancies, experimental data revealed disrupted glucose metabolism and proper steroidogenesis in PCOS granulosa cells, suggesting that the ovarian microenvironment determines the response to insulin (42, 43). It appears that the metabolic pathway of insulin signaling in the ovarian tissues is hampered, while other pathways are active. Overall, the molecular mechanism underlying possible insulin sensitivity of ovarian cells in PCOS requires further study.

VITAMIN D_3 - METABOLISM AND ACTION

Vitamin D_3 and its metabolites belong to the group of fat-soluble organic chemical compounds with the general chemical formula $C_{28}H_{43}OH$. They are classified as secosteroids because

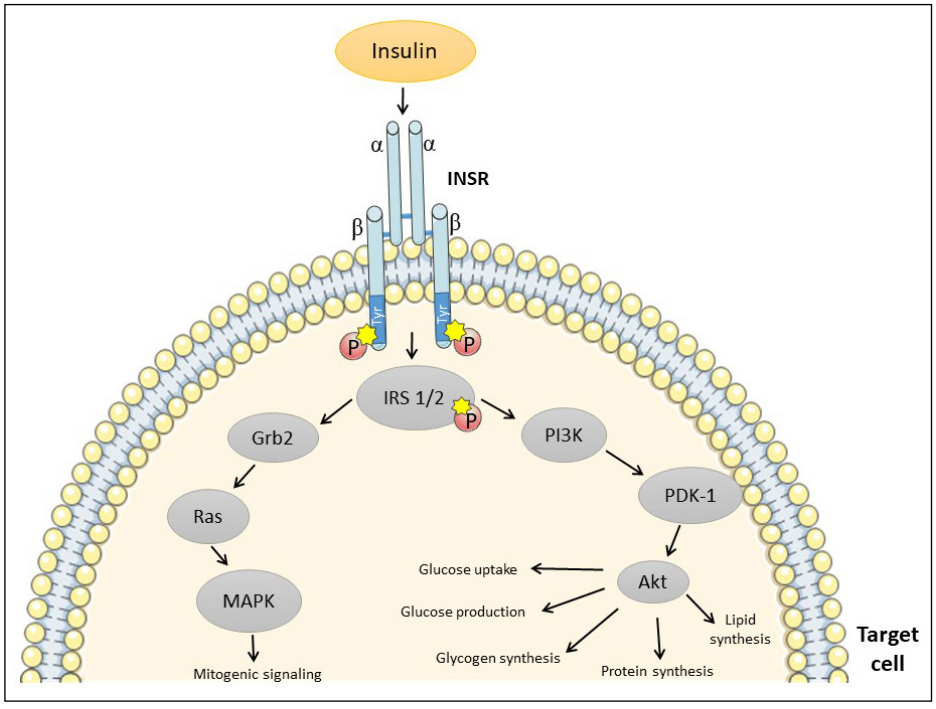

Fig. 2. Intracellular signaling pathways of insulin action in a target cell.
Akt, serine/threonine protein kinase; Grb2, growth factor receptor-bound protein 2; INSR, insulin receptor; IRS1/2, insulin receptor substrate 1/2; MAPK, mitogen-activated protein kinase; P, phosphorylation; PDK-1, phosphoinositide-dependent kinase 1; PI3K, phosphoinositide 3-kinase; Ras, Ras protein.

they are made of four rings A, B, C and D (the B ring is broken) and a side chain. In humans, two major isoforms of vitamin D are vitamin D_2 (VD_2; ergocalciferol) and vitamin D_3 (VD_3; cholecalciferol) (44, 45). Natural sources of VD_2 are plants and fungi, while VD_3 is synthesized endogenously in the organism (80%) or obtained from food (20%) such as fatty fish, fish liver oil, egg yolks, milk, soy milk and butter (46-48).

The process of VD_3 activation is multistage (*Fig. 3*). VD_3 (cholecalciferol) is synthesized from 7-dehydrocholesterol in the skin. Due to the fact that VD_3 itself is not biologically active, the first stage of its bioactivation occurs in the liver, where its hydroxylation to 25-hydroxyvitamin D_3 ($25(OH)D_3$; calcidiol) by 25-hydroxylases (*e.g.* CYP2R1 and CYP27A1) takes place. Calcidiol is transported to the kidney and undergoes final bioactivation *via* hydroxylation to 1α,25-dihydroxyvitamin D_3 ($1\alpha,25(OH)_2D_3$, calcitriol) by 1α-hydroxylase (CYP27B1) (49). Calcitriol, the most biologically active form of VD_3, enters the circulation and exerts biological effects at target tissues. Calcidiol and calcitriol are subjected to a mechanism that tightly regulates their levels in the organism, involving inactivation by 24-hydroxylase (CYP24A1) to, for example calcitroic acid, which is excreted in the bile (49).

Among all VD_3 forms, only calcitriol is biologically active and activates VD_3 receptor (VDR) in target tissues (50). VDR belongs to the superfamily of ligand-activated steroid hormone receptors, acting as a transcription factor (51, 52). The genomic mechanism of action begins with the formation of a calcitriol-VDR complex, which further heterodimerizes with 9-*cis*-retinoic acid receptor (RXR). The heterodimer calcitriol-VDR-RXR translocates into the nucleus and binds to the vitamin D response element (VDRE) inducing recruitment of transcriptional co-activators or co-repressors that regulates the expression of target genes (53). In the non-genomic pathway, calcitriol binds to VDR located in cell membrane cavities or to a membranous receptor identified as a membrane-associated rapid response steroid-binding protein ($1,25D_3$-MARRS) triggering a rapid cell response (51, 54) (*Fig. 3*).

In humans and animals, the main function of VD_3 is to maintain calcium and phosphorus homeostasis through the regulation of their absorption in the intestine, kidney and bone (44). However, recent studies have shown that VD_3 controls many other cellular processes beyond those related to the bone mineralization. Pleiotropic VD_3 actions include effects on the immune, cardiovascular, nervous and reproductive systems as well as interplay with other hormones such as androgens, estrogens and insulin (55-57).

INSULIN AND VITAMIN D_3 - A DIRECT LINK

Results from human and animal diabetic studies showed an association between VD_3 deficiency and abnormal glucose metabolism that was restored after insulin treatment (58, 59). Further studies provided the evidence that pancreatic β-cells express VDR (60) and CYP27B1 enzyme (61), and they are therefore both a target for and a source of VD_3. In addition, the human insulin gene promoter possesses VDRE and VD_3 can upregulate insulin gene transcription and enhance insulin synthesis (62). These molecular background indicates a direct link between VD_3 and insulin action.

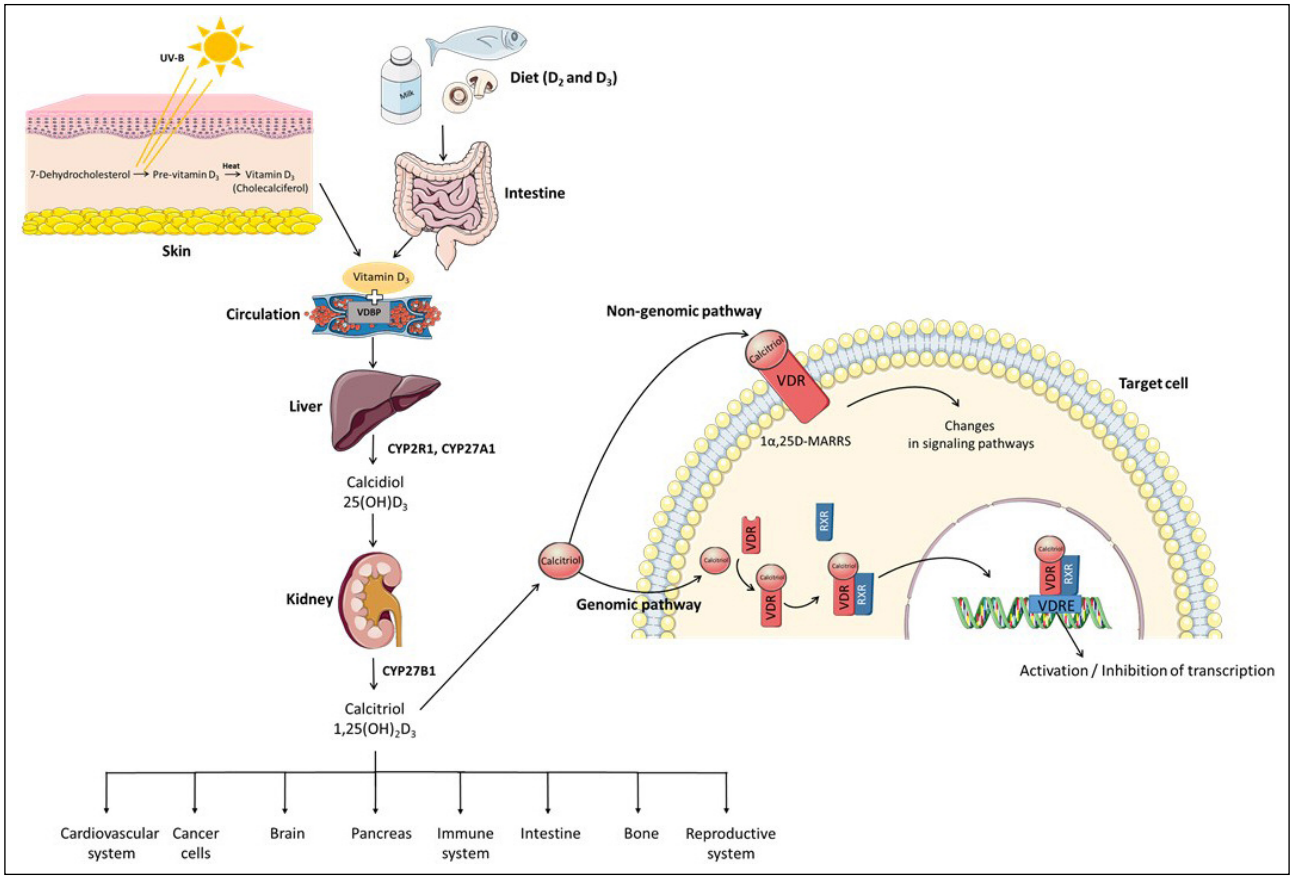

Fig. 3. Vitamin D_3 biosynthesis and mechanism of action in a target cell.
CYP2R1, 25-hydroxylase; CYP27A1, 25-hydroxylase; CYP27B1, 1α-hydroxylase; RXR, 9-*cis*-retinoic acid receptor; UV-B, ultraviolet-B irradiation; VDBP, vitamin D_3 binding protein; VDR, vitamin D_3 receptor; VDRE, vitamin D response element.

Other research suggests additional indirect pathways by which VD_3 may influence insulin secretion. It is proposed that VD_3 regulates cell membrane calcium flux by increasing the ATP/ADP ratio, leading to the closure of ATP-gated channels, cell depolarization and exocytosis of secretory granules containing insulin (63). Furthermore, in pancreatic β-cells, VD3 was found to influence the synthesis and action of calbindin, which is a VD_3-dependent calcium-binding protein (64). Increased intracellular free calcium may also protect against inflammation-induced damage and apoptosis of β-cells (63). Of particular importance, it was observed that VD_3 deficiency enhances insulin resistance in target tissues, such as skeletal muscle and adipose tissue, due to diminished INSR expression or inactivation of peroxisome proliferator-activated receptor delta (PPAR-δ) involved in the mobilization of fatty acids (65). Taking into account disturbed sensitivity of ovarian cells to insulin in PCOS, the question arises of whether VD_3 can improve responsiveness of those tissues.

VITAMIN D_3 IN POLYCYSTIC OVARY SYNDROME TREATMENT

It is generally considered that PCOS is associated with VD_3 deficiency, suggesting its potential role in PCOS pathogenesis (66). Although hypovitaminosis D_3 was found among PCOS patients, it was also associated with obesity (67). Recent data have indicated various effects of VD_3 on the ovarian, hormonal and metabolic features observed in PCOS. Similarly to other factors *e.g.*, D_3 might also reduce negative effects in postmenopausal women (68, 69). Despite many studies showing therapeutic effects of VD_3 in PCOS, some research found a lack of influence or contradicted these findings (66). The results from clinical trials focused on the effects of VD_3 supplementation on hormonal and metabolic parameters in PCOS women and from studies conducted on rodent PCOS models involving effects of VD_3 supplementation on reproductive and metabolic parameters are presented in *Tables 2* and *1*.

Effect on the ovary and menstrual cycle

Considering the importance of VD_3 action at the ovarian level during PCOS, our latest study, conducted on letrozole-induced PCOS rat model, revealed disturbed local ovarian VD_3 metabolism. We found decreased expression of CYP27B1 and reduced VD_3 production in the ovary and periovarian adipose tissue, indicating that the gonad seems to be an additional source of VD_3. Disruption of this biosynthesis might contribute to the peripheral VD_3 deficiency observed in PCOS women (70).

Using rat model of PCOS produced by dehydroepiandrostenedione (DHEA) treatment, VD_3 was found to improve the morphology of the ovary by increasing the number of tertiary follicles and decreasing the number of atretic and cystic ones (71). Likewise, Kuyucu *et al.* (72) found a positive effect of VD_3 on structural changes occurring in cystic follicles, including irregular zona pellucida, disjunction of granulosa cells, attenuated granulosa cell layer and thick theca cell layer, observed by means of electron microscopy. Additionally, they showed that the plasma anti-Muellerian hormone (AMH) concentration, which was higher in the PCOS group than in the control group, decreased following VD_3 treatment (72). AMH is a member of the transforming growth factor β (TGFβ) superfamily, which is produced by the granulosa cells of preantral and early antral follicles. It inhibits the

Table 1. The results of studies involving vitamin D_3 (VD_3) supplementation in rodent models of polycystic ovary syndrome (PCOS) on reproductive and metabolic parameters.

Study/Reference	Species	PCOS inductor	Sample Size	Intervention / Regimen	Duration	Results
Celik *et al.* (71)	Rat	DHEA	N = 24	DHEA+VD_3 group - 6 mg/kg/day DHEA and 120 ng/100 g/week $1,25(OH)_2D_3$ vs. DHEA group (PCOS group) - 6 mg/kg/day DHEA	28 days	Compared to PCOS group, VD_3 supplementation resulted in lowering of FSH, LH levels, LH/FSH ratio, testosterone levels and decrease the number of atretic and cystic follicles.
Kuyucu *et al.* (72)	Rat	DHEA	N = 24	DHEA+VD_3 group – 6 mg/kg/day DHEA and 120 ng/100g $1,25(OH)_2D_3$ vs. DHEA group (PCOS group) - 6 mg/kg/day DHEA	28 days	Compared to PCOS group, VD_3 supplementation resulted in lowering of serum AMH, testosterone, FSH, LH levels, LH/FSH ratios, AMHR2 expression in atretic and premature luteinized antral follicles. Structural changes such as: degenerative changes in developing follicles, cystic follicles and lipid accumulation in the interstitial cells were improved with VD_3 treatment.
Bakhshalizadeh *et al.* (85)	Mouse	DHEA	N = 20	DHEA group (PCOS group) - 6 mg/100 g/day DHEA. Granulosa cells of DHEA-induced PCOS mice were then cultured with and without vitamin D_3 (100 nM)	20 days	Compared to PCOS group, VD_3 supplementation resulted in lowering of mRNA and protein expression levels of steroidogenic enzymes: CYP11A1, StAR, CYP19A1, 3β-HSD and aromatase and 3β-HSD activity that leads to decreased estradiol and progesterone release.
Bakhshalizadeh *et al.* (86)	Mouse	DHEA	N = 40	DHEA group (PCOS group) - 6 mg/100 g/day DHEA. Granulosa cells of DHEA-induced PCOS mice were then cultured with and without vitamin D_3 (100 nM)	20 days	Compared to PCOS group, VD_3 supplementation resulted in lowering of mRNA expression levels of steroidogenic enzymes: StAR, Cyp11a1, Cyp19a1, 3β-HSD and estradiol and progesterone levels. VD_3 could activate AMPK signaling pathway.
Behmanesh *et al.* (90)	Rat	EV	N = 40	EV+VD_3 group - 2 mg/kg/day EV and 1 mg/kg/day VD_3 vs. EV group (PCOS group) - 2 mg/kg/day EV	60 days (induction PCOS) 15 days (suppl. VD_3)	Compared to PCOS group, VD_3 supplementation resulted in an increase in the number of ovarian follicles at the different stages and the number of normal follicles. Testosterone, LH, glucose and insulin concentrations, and insulin resistance decreased, while FSH, estradiol, progesterone concentrations increased after VD_3 treatment.

Abbreviations: AMH, anti-Mullerian hormone; AMHR2, anti-Muellerian hormone type-2 receptor; AMPK, AMP-activated protein kinase; DHEA, dehydroepiandrostenedione; EV, estradiol valerate; FSH, follicle-stimulating hormone; LH, luteinizing hormone; StAR, steroidogenic acute regulatory protein; 3β-HSD, 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase; CYP11A1, cholesterol side-chain cleavage enzyme; CYP19A1, cytochrome P450 aromatase.

recruitment of primordial follicles to growth and the response of antral follicles to FSH (73), contributing to anovulation in PCOS women (74). The presence of VDRE on AMH gene promoter region provides a basis of a direct VD_3 and AMH interaction (75). Indeed, VD_3 supplementation led to significant increase in serum AMH in healthy women (76). On the other hand, studies conducted on an-ovulatory PCOS patients (76) together with above findings by Kuyucu's *et al.* (72) revealed decreased serum AMH level following VD3 treatment. Although these findings seem to be contradictory, the different follicular environment and ovulatory status of PCOS and healthy women should be considered. In PCOS, the high AMH concentration reflects the numerous cohort of arrested small antral follicles, while VD_3 has been shown to improve folliculogenesis and ovulation in these women (75) that could explain diminished AMH level observed in PCOS patients. Another plausible explanation could be genetic variations in PCOS women, indicating the association between polymorphisms Fok1 (rs2228570) and Apa1 (rs7975232) in *VDR* gene and elevated AMH level (77). Noteworthy, PCOS, increased numbers arrested follicles and AMH level have been identified as a risk factors for ovarian hyperstimulation syndrom (OHSS) (78).

There is growing evidence that VD_3 influences the menstrual cycle and may therefore play an important role in female reproductive function. It has been observed that VD_3 deficiency in PCOS is associated with calcium dysregulation: this leads to follicular arrest and menstrual dysfunction (79). Investigation of premenopausal women with chronic anovulation and hyperandrogenism showed that VD_3 and calcium administration during metformin therapy resulted in normalization of the menstrual cycle (80). Jafari-Sfidvajani *et al.* (81) carried out randomized controlled clinical trial on the effect of VD_3 coupled with a low calorie diet in PCOS women with VD_3 insufficiency. Women who received VD_3 reported either a normalization or an improvement in the regularity of their menses (81). Wehr *et al.* (82) also described a positive effect of VD_3 on the menstrual regulation in PCOS women. They observed that VD_3 treatment in women with previous menstrual disorders led to the normalization of menstrual pattern in 50% of patients (82). It is suggested that VD_3 and calcium may influence the conversion of testosterone to estradiol through upregulation of CYP19A1 expression in the granulosa cells of the PCOS ovary, and therefore normalize androgen and estrogen concentrations (83-85). Bakhshalizadeh *et al.* (86), using a mouse model of PCOS,

Table 2. The results of interventional clinical trials focused on the effects of vitamin D_3 (VD_3) supplementation on hormonal and metabolic parameters in women with polycystic ovary syndrome (PCOS).

Study/ Reference	Population	Sample Size	Intervention/ Regiment	Duration	Results
Wehr *et al*. (82)	PCOS patients (Graz, Austria). PCOS diagnosis based on Rotterdam criteria.	N = 57 Non-randomized	PCOS women received 20,000 IU cholecalciferol weekly vs. condition before treatment	24 weeks	VD_3 supplementation resulted in a decrease of fasting and stimulated glucose, C-peptide, triglycerides and estradiol levels, whereas total cholesterol and LDL levels significantly increased. No changes in androgen level were observed.
Selimoglu *et al*. (91)	Obese PCOS patients (Bursa, Turkey). PCOS diagnosis based on Rotterdam criteria.	N = 11 Non-randomized	PCOS women received Devit 3 amp, containing 300,000 IU VD_3 vs. condition before treatment	3 weeks	Administration of the single dose of VD_3 resulted in a non-significant decrease in glucose and insulin levels, but HOMA-IR decreased significantly. No significant alterations were witnessed at the levels of DHEAS, TST, fTST and androstenedione.
Dravecka *et al*. (92)	Obese, insulin resistant and VD_3 deficient PCOS patients (Košice, Slovakia). PCOS diagnosis based on Androgen Excess Society criteria.	N = 39 Randomized	PCOS women received: group 1 - 1 μg alfacalcidiol per day, vs. group 2 - combined therapy 1 μg of alfacalcidiol and metformin in dose 1.700–2.550 mg daily vs. group 3 - metformin 1.700–2.550mg per day vs. condition before treatment	6 months	In the group 2, a significant decrease in TST level and slight but not significant decrease in the group 3 was observed. In all three groups were no significant changes in other parameters (fTST, DHEAS, LH, LH/FSH) or acne and hirsutism. VD_3 administration has no significant effect on androgen levels and clinical features of hyperandrogenism in PCOS women. However, it can potentiate effect of metformin on testosterone levels and LH/FSH ratio but not on clinical hyperandrogenism.
Gupta *et al*. (100)	PCOS patients (New Delhi, India). PCOS diagnosis based on Rotterdam criteria.	N = 50 Randomized	PCOS women received 12,000 IU VD_3 weekly vs. placebo	12 weeks	Compared to placebo, VD_3 supplementation resulted in lowering of HOMA-IR, insulin levels, and also reduction QUICKI.
Dastorani *et al*. (101)	Infertile PCOS patients who were IVF candidates (Kashan, Iran). PCOS diagnosis based on Rotterdam criteria.	N = 40 Randomized	PCOS women received 50,000 IU VD_3 weekly vs. placebo	8 weeks	Compared to placebo, VD_3 supplementation resulted in significantly lower serum AMH, insulin levels, HOMA-IR, total cholesterol and LDL level and improved QUICKI.
Ardabili *et al*. (103)	VD_3 deficient PCOS patients (Tabriz, Iran). PCOS diagnosis based on Rotterdam criteria.	N = 50 Randomized	PCOS women received 3 doses of 50,000 IU VD_3 (1 every 20 days) vs. placebo	2 months	Compared to placebo, VD_3 supplementation resulted in no significant serum insulin and glucose levels, the insulin sensitivity and HOMA-IR.
Garg *et al*. (104)	PCOS patients (New Delhi, India). PCOS diagnosis based on Rotterdam criteria.	N = 36 Randomized	PCOS women received 120,000 IU VD_3 monthly + metformin (1500 mg/day) vs. placebo + metformin (1500 mg/day)	6 months	Compared to placebo, VD_3 supplementation resulted in no significant differences in any of the parameters of IS/IR (area under curve-glucose, area under curve-insulin, insulin:glucose ratio, HOMA-IR, Matsuda index, insulinogenic index, and disposition index), insulin secretion (by insulinogenic index - II30) and cardiovascular risk factors.

Abbreviations: LDL, low-density lipoprotein; (HOMA)-IR, the homeostasis model assessment of insulin resistance; QUICKI, quantitative control of insulin sensitivity; TST, total testosterone; fTST, freetestosterone; DHEAS, dehydroepiandrosterone sulphate; LH, luteinizing hormone; FSH, follicle-stimulating hormone; AMH, anti-Müllerian hormone; IS, insulin sensitivity; IR, insulin resistance.

suggested that VD_3 modulates steroidogenesis in granulosa cells by regulating AMP-activated protein kinase (AMPK). This could be a molecular mechanism underlying the improvement of follicle maturation and ovulation in PCOS following VD_3 treatment.

Effect on hyperandrogenism

The main symptom of PCOS is a hyperandrogenism, which occurs in two forms: a biochemical form characterized by increased concentration of androgens, and a clinical form characterized by hirsutism and/or acne. Some studies indicated that VD_3 deficiency might contribute to the development of hyperandrogenism in PCOS women (87-89). Therefore, it can be hypothesized that VD_3 exerts a therapeutic effect on hyperandrogenism. Research on DHEA-induced PCOS rats showed that VD_3 administration resulted in significant decreases in FSH and LH levels and in the LH/FSH ratio. Plasma testosterone concentration was also lower than in PCOS rats as expected (71, 72). Another study by Behmanesh's *et al.* (90) revealed that VD_3 significantly decreased LH and testosterone concentrations and increased FSH, estradiol and progesterone concentrations in rats with PCOS induced by estradiol valerate.

There are a few randomized, controlled clinical trials in women showing the effect of VD_3 supplementation on hyperandrogenism. Selimoglu *et al.* (91) and Wehr *et al.* (82) detected no changes in testosterone, androstenedione or dehydroepiandrosterone sulfate (DHEAS) following VD3 in PCOS patients, when compared with a control group. On the other hand, Dravecka *et al.* (92) examined the influence of VD_3 supplementation in VD_3 deficient and insulin resistant PCOS women on clinical and biochemical hyperandrogenism, in comparison to metformin or metformin + VD_3 therapy. They found that VD_3 administration did not significantly affect androgen concentration or the clinical features of hyperandrogenism such as acne or hirsutism. Nevertheless, VD_3 may potentiate the effects of metformin on the LH/FSH ratio and testosterone level, but not on clinical hyperandrogenism (92).

Effect on insulin resistance, hyperinsulinemia and lipid profile

PCOS is frequently associated with insulin resistance, hyperinsulinemia, dyslipidemia, and elevated concentrations of total cholesterol, triglycerides and low density lipoproteins (LDL) (93, 94). The role of VD_3 in the insulin sensitivity and insulin secretion has been established by *in vivo* studies. The main methods of measuring insulin sensitivity and the development of diabetes are the homeostasis model assessment of insulin resistance (HOMA-IR) and homeostasis model assessment of β-cell function (HOMA-B) indices (95, 96). These studies have shown that VD_3 deficiency is associated with impaired glucose clearance and insulin secretion (97-99). Thus, VD_3 supplementation might be helpful in the reduction of insulin resistance and metabolic syndrome in PCOS.

Using the estradiol valerate-treated rat model of PCOS, Behmanesh *et al.* (90) found that VD_3 may improve insulin sensitivity and decrease fat mass and obesity. Serum concentrations of glucose and insulin were significantly decreased. HOMA-IR was reduced in the rats following VD_3 administration, and there were decreased LDL and total cholesterol concentrations, and increased high density lipoproteins (HDL) concentration (90).

A number of randomized, controlled clinical trials have demonstrated a therapeutic effect of VD_3 on insulin resistance and lipid profiles in PCOS women. Gupta's *et al.* (100) showed that VD_3 supplementation significantly reduced HOMA-IR and insulin levels, and also found a significant reduction in the quantitative control of insulin sensitivity (QUICKI) (100). Similarly, Wehr *et al.* (82) observed improved glucose metabolism in response to VD_3 supplementation. In another study, infertile women with PCOS, who were candidates for *in vitro* fertilization, showed beneficial effects of VD_3 on insulin metabolism and some lipid profile parameters (101). This study demonstrated that VD_3 supplementation significantly decreased insulin level, HOMA-IR, serum total cholesterol and LDL concentrations, while markedly increasing QUICKI compared with placebo (101). The latest meta-analysis reported that VD_3 reduced insulin resistance and improved the lipid metabolism of PCOS women (102). On the other hand, there are also studies with contradictory findings. Ardabili *et al.* (103) demonstrated no significant changes in serum insulin and glucose concentrations, insulin sensitivity or HOMA-IR following VD_3 supplementation. In another study, treatment with VD_3 had no significant effect on insulin secretion or insulin resistance (104). Noteworthy, significantly decreased VD_3 level was found in obese compared with lean PCOS patients, suggesting that hypovitaminosis D_3 results from obesity (105). However, this relationship is difficult to unequivocal evaluation making a vicious circle. Deeper understanding of vitamin D_3 molecular involvement in processes related to insulin signaling and insulin resistance is needed.

Numerous clinical studies performed on PCOS patients and research conducted on animal PCOS models emphasize a close relationship between VD_3 and insulin in this endocrine disorder. Supplementation with VD_3 has been shown to improve tissue insulin sensitivity and decrease plasma insulin concentration in PCOS women. Although VD_3 deficiency is often an accompanying symptom of PCOS, it is linked predominantly with the obesity that is associated with insulin resistance. Therefore, which is comes first in PCOS: insulin resistance or VD_3 deficiency? Bearing in mind that local ovarian VD_3 synthesis is disrupted in PCOS, a further question is whether VD_3 supplementation could reduce insulin resistance in the ovary. This is a missing link in the complex interaction between insulin and VD_3 in PCOS and is an important challenge for future research.

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REFERENCES

1. Azziz R. Introduction: Determinants of polycystic ovary syndrome. *Fertil Steril* 2016; 106: 4-5.
2. 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: 347-363.
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. doi: 10.1186/1741-7015-8-41
4. Moghetti P, Tosi F. Insulin resistance and PCOS: chicken or egg? *J Endocrinol Invest* 2021; 44: 233-244.
5. Traub M. Assessing and treating insulin resistance in women with polycystic ovarian syndrome. *World J Diabetes* 2011; 2: 33-40.
6. Dupont J, Scaramuzzi RJ. Insulin signalling and glucose transport in the ovary and ovarian function during the ovarian cycle. *Biochem J* 2016; 473: 1483-1501.

7. Zawadski JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Polycystic Ovary Syndrome. Dunaif A, Givens JR, Haseltine F, Merriam GR (eds). Boston, Blackwell Scientific, 1992, pp. 377-384.
8. 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: 41-47.
9. Azziz R, Carmina E, Dewailly D, *et al.* Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guidelines. *J Clin Endocrinol Metab* 2006; 91: 4237-4245.
10. National Institutes of Health. Evidence-based methodology workshop on polycystic ovary syndrome. Executive summary. December 3-5, 2012. Available at: https://prevention.nih.gov/docs/programs/pcos/FinalReport.pdf. Accessed March 1, 2016.
11. Rosenfield RL, Ehrmann DA. The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. *Endocr Rev* 2016; 37: 467-520.
12. Ewens KG, Stewart DR, Ankener W, *et al.* Family-based analysis of candidate genes for polycystic ovary syndrome. *J Clin Endocrinol Metab* 2010; 95: 2306-2315.
13. Zhang T, Liang W, Fang M, Yu J, Ni Y, Li Z. Association of the CAG repeat polymorphisms in androgen receptor gene with polycystic ovary syndrome: a systemic review and meta-analysis. *Gene* 2013; 524: 161-167.
14. Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. *Nat Rev Endocrinol* 2011; 7: 219-231.
15. Ciechanowska M, Kowalczyk M, Lapot M, *et al.* Effect of corticotropin releasing hormone and corticotropin releasing hormone nist on biosynthesis of gonadotropin relasing hormone and gonadotropin relasing hormone receptor in the hypothalamic-pituitary unit of follicular-phase ewes and contribution of kisspeptin. *J Physiol Pharmacol* 2018; 69: 451-461.
16. Azziz R, Carmina E, Chen Z, *et al.* Polycystic ovary syndrome. *Nat Rev Dis Primers* 2016; 2: 16057. doi: 10.1038/nrdp.2016.57
17. Coyle C, Campbell RE. Pathological pulses in PCOS. *Mol Cell Endocrinol* 2019; 498: 110561. doi: 10.1016/j.mce.2019.110561
18. Coutinho EA, Kauffman AS. The role of the brain in the pathogenesis and physiology of polycystic ovary syndrome (PCOS). *Med Sci* (*Basel*) 2019; 7: 84. doi: 10.3390/medsci7080084
19. Pastor CL, Griffin-Korf ML, Aloi JA, Evans WS, Marshall JC. Polycystic ovary syndrome: evidence for reduced sensitivity of the gonadotropin-releasing hormone pulse generator to inhibition by estradiol and progesterone. *J Clin Endocrinol Metab* 1998; 83: 582-590.
20. Watson RE, Bouknight R, Alguire PC. Hirsutism: evaluation and management. *J Gen Intern Med* 1995; 10: 283-292.
21. Ahmad K. Insulin sources and types: a review of insulin in terms of its mode on diabetes mellitus. *J Tradit Chin Med* 2014; 34: 234-237.
22. Tokarz VL, MacDonald PE, Klip A. The cell biology of systemic insulin function. *J Cell Biol* 2018; 217: 2273-2289.
23. Fu Z, Gilbert ER, Liu D. Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes. *Curr Diabetes Rev* 2013; 9: 25-53.
24. Vasiljevic J, Torkko JM, Knoch KP, Solimena M. The making of insulin in health and disease. *Diabetologia* 2020; 63: 1981-1989.
25. Haeusler RA, McGraw TE, Accili D. Biochemical and cellular properties of insulin receptor signaling. *Nat Rev Mol Cell Biol* 2018; 19: 31-44.
26. Hubbard SR. The insulin receptor: both a prototypical and atypical receptor tyrosine kinase. *Cold Spring Harb Perspect Biol* 2013; 5: a008946. doi: 10.1101/cshperspect.a008946
27. Choi K, Kim YB. Molecular mechanism of insulin resistance in obesity and type 2 diabetes. *Korean J Intern Med* 2010; 25: 119-129.
28. Kasuga M. Insulin resistance and pancreatic beta cell failure. *J Clin Invest* 2006; 116: 1756-1760.
29. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. *Endocr Rev* 2012; 33: 981-1030.
30. Rajkhowa M, Brett S, Cuthbertson DJ, *et al.* Insulin resistance in polycystic ovary syndrome is associated with defective regulation of ERK1/2 by insulin in skeletal muscle in vivo. *Biochem J* 2009; 418: 665-671.
31. Book CB, Dunaif A. Selective insulin resistance in the polycystic ovary syndrome. *J Clin Endocrinol Metab* 1999; 84: 3110-3116.
32. Corbould A, Kim YB, Youngren JF, *et al.* Insulin resistance in the skeletal muscle of women with PCOS involves intrinsic and acquired defects in insulin signaling. *Am J Physiol Endocrinol Metab* 2005; 288: E1047-E1054.
33. Ciaraldi TP, Aroda V, Mudaliar S, Chang RJ, Henry RR. Polycystic ovary syndrome is associated with tissue-specific differences in insulin resistance. *J Clin Endocrinol Metab* 2009; 94: 157-163.
34. Ciaraldi TP, el-Roeiy A, Madar Z, Reichart D, Olefsky JM, Yen SS. Cellular mechanisms of insulin resistance in polycystic ovarian syndrome. *J Clin Endocrinol Metab* 1992; 75: 577-583.
35. Rosenbaum D, Haber RS, Dunaif A. Insulin resistance in polycystic ovary syndrome: decreased expression of GLUT-4 glucose transporters in adipocytes. *Am J Physiol* 1993; 264: E197-E202.
36. Chen YH, Heneidi S, Lee JM, *et al.* miRNA-93 inhibits GLUT4 and is overexpressed in adipose tissue of polycystic ovary syndrome patients and women with insulin resistance. *Diabetes* 2013; 62: 2278-2286.
37. Ciaraldi TP. Molecular defects of insulin action in the polycystic ovary syndrome: possible tissue specificity. *J Pediatr Endocrinol Metab* 2000; 13 (Suppl. 5): 1291-1293.
38. Ciaraldi TP, Morales AJ, Hickman MG, Odom-Ford R, Olefsky JM, Yen SS. Cellular insulin resistance in adipocytes from obese polycystic ovary syndrome subjects involves adenosine modulation of insulin sensitivity. *J Clin Endocrinol Metab* 1997; 82: 1421-1425.
39. Chuang TY, Wu HL, Chen CC, *et al.* MicroRNA-223 expression is upregulated in insulin resistant human adipose tissue. *J Diabetes Res* 2015; 2015: 943659. doi: 10.1155/2015/943659
40. Palaniappan M, Menon B, Menon KM. Stimulatory effect of insulin on theca-interstitial cell proliferation and cell cycle regulatory proteins through MTORC1 dependent pathway. *Mol Cell Endocrinol* 2013; 366: 81-89.
41. Diamanti-Kandarakis E, Papavassiliou AG. Molecular mechanisms of insulin resistance in polycystic ovary syndrome. *Trends Mol Med* 2006; 12: 324-332.
42. Rice S, Christoforidis N, Gadd C, *et al.* Impaired insulin-dependent glucose metabolism in granulosa-lutein cells from anovulatory women with polycystic ovaries. *Hum Reprod* 2005; 20: 373-381.

43. Wu XK, Zhou SY, Liu JX, *et al.* Selective ovary resistance to insulin signaling in women with polycystic ovary syndrome. *Fertil Steril* 2003; 80: 954-965.
44. Bikle DD. Vitamin D: newer concepts of its metabolism and function at the basic and clinical level. *J Endocr Soc* 2020; 4: bvz038. doi: 10.1210/jendso/bvz038
45. Japelt RB, Jakobsen J. Vitamin D in plants: a review of occurrence, analysis, and biosynthesis. *Front Plant Sci* 2013; 4: 136. doi: 10.3389/fpls.2013.00136
46. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. *Physiol Rev* 2016; 96: 365-408.
47. Schmid A, Walther B. Natural vitamin D content in animal products. *Adv Nutr* 2013; 4: 453-462.
48. Jeon SM, Shin EA. Exploring vitamin D metabolism and function in cancer. *Exp Mol Med* 2018; 50: 1-14. doi: 10.1038/s12276-018-0038-9
49. Jones G, Prosser DE, Kaufmann M. Cytochrome P450-mediated metabolism of vitamin D. *J Lipid Res* 2014; 55: 13-31.
50. Zmijewski MA, Carlberg C. Vitamin D receptor(s): In the nucleus but also at membranes? *Exp Dermatol* 2020; 29: 876-884.
51. Haussler MR, Jurutka PW, Mizwicki M, Norman AW. Vitamin D receptor (VDR)-mediated actions of 1α,25(OH)$_2$ vitamin D$_3$: genomic and non-genomic mechanisms. *Best Pract Res Clin Endocrinol Metab* 2011; 25: 543-559.
52. Haussler MR, Whitfield GK, Kaneko I, *et al.* Molecular mechanisms of vitamin D action. *Calcif Tissue Int* 2013; 92: 77-98.
53. Pike JW, Meyer MB. Fundamentals of vitamin D hormone-regulated gene expression. *J Steroid Biochem Mol Biol* 2014; 144 (Pt. A): 5-11.
54. Hii CS, Ferrante A. The non-genomic actions of vitamin D. *Nutrients* 2016; 8: 135. doi: 10.3390/nu8030135
55. Lai YH, Fang TC. The pleiotropic effect of vitamin D. ISRN *Nephrol* 2013; 2013: 898125. doi: 10.5402/2013/898125
56. Geleijnse JM. Vitamin D and the prevention of hypertension and cardiovascular diseases: a review of the current evidence. *Am J Hypertens* 2011; 24: 253-262.
57. Grzesiak M. Vitamin D$_3$ action within the ovary - an updated review. *Physiol Res* 2020; 69: 371-378.
58. Schneider LE, Schedl HP, McCain T, Haussler MR. Experimental diabetes reduces circulating 1,25-dihydroxyvitamin D in rat. *Science* 1977; 196: 1452-1454.
59. Scragg R, Holdaway I, Singh V, Metcalf P, Baker J, Dryson E. Serum 25-hydroxyvitamin D$_3$ levels decreased in impaired glucose-tolerance and diabetes-mellitus. *Diabetes Res Clin Pract* 1995; 27: 181-188.
60. Alvarez JA, Ashraf A. Role of vitamin D in insulin secretion and insulin sensitivity for glucose homeostasis. *Int J Endocrinol* 2010; 2010: 351385. doi: 10.1155/2010/351385
61. Bland R, Markovic D, Hills CE, *et al.* Expression of 25-hydroxyvitamin D3-1α-hydroxylase in pancreatic islets. *J Steroid Biochem Mol Biol* 2004; 89-90: 121-125.
62. Maestro B, Davila 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: 223-230.
63. El-Fakhri N, McDevitt H, Shaikh MG, Halsey C, Ahmed SF. Vitamin D and its effects on glucose homeostasis, cardiovascular function and immune function. *Horm Res Paediatr* 2014; 81: 363-378.
64. Lee S, Clark SA, Gill RK, Christakos S. 1,25-Dihydroxyvitamin-D$_3$ and pancreatic β-cell function: vitamin-D receptors, gene expression, and insulin secretion. *Endocrinology* 1994; 134: 1602-1610.
65. Szymczak-Pajor I, Sliwinska A. Analysis of association between vitamin D deficiency and insulin resistance. *Nutrients* 2019; 11: 794. doi: 10.3390/nu11040794
66. 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: 4555-4577.
67. Yildizhan R, Kurdoglu M, Adali E, *et al.* Serum 25-hydroxyvitamin D concentrations in obese and non-obese women with polycystic ovary syndrome. *Arch Gynecol Obstet* 2009; 280: 559-563.
68. Chung YS, Chung DJ, Kang MI, *et al.* Vitamin D repletion in Korean postmenopausal women with osteoporosis. *Yonsei Med J* 2016; 57: 923-927.
69. Chojnacki C, Kaczka A, Gasiorowska A, Fichna J, Chojnacki J, Brzozowski T. The effect of long-term melatonin supplementation on psychosomatic disorders in postmenopausal women. *J Physiol Pharmacol* 2018; 69: 297-304.
70. Grzesiak M, Burzawa G, Kurowska P, *et al.* Altered vitamin D$_3$ metabolism in the ovary and periovarian adipose tissue of rats with letrozole-induced PCOS. *Histochem Cell Biol* 2021; 155: 101-116.
71. Celik LS, Kuyucu Y, Yenilmez ED, Tuli A, Daglıoglu K, Mete UO. Effects of vitamin D on ovary in DHEA-treated PCOS rat model: a light and electron microscopic study. *Ultrastruct Pathol* 2018; 42: 55-64.
72. Kuyucu Y, Sencar L, Tap O, Mete UO. Investigation of the effects of vitamin D treatment on the ovarian AMH receptors in a polycystic ovary syndrome experimental model: an ultrastructural and immunohistochemical study. *Reprod Biol* 2020; 20: 25-32.
73. Moolhuijsen LM, Visser JA. Anti-Mullerian hormone and ovarian reserve: update on assessing ovarian function. *J Clin Endocrinol Metab* 2020; 105: 3361-3373.
74. Garg D, Tal R. The role of AMH in the pathophysiology of polycystic ovarian syndrome. *Reprod Biomed Online* 2016; 33: 15-28.
75. Kalyanaraman R, Lubna P. A narrative review of current understanding of the pathophysiology of polycystic ovary syndrome: focus on plausible relevance of vitamin D. *Int J Mol Sci* 2021; 22: 4905. doi: 10.3390/ijms22094905
76. Moridi I, Chen A, Tal O, Tal R. The association between vitamin D and anti-Mullerian hormone: a systematic review and meta-analysis. *Nutrients* 2020; 12: 1567. doi: 10.3390/nu12061567
77. Szafarowska M, Dziech E, Kaleta B, *et al.* Anti-Mullerian hormone level is associated with vitamin D receptor polymorphisms in women with polycystic ovary syndrome. *J Assist Reprod Genet* 2019; 36: 1281-1289.
78. Issat T, Nowicka MA, Oleksik TP, *et al.* Serum progesterone concentrations on the day of oocyte retrieval above 9.23 ng/ml may predict ovarian hyperstimulation syndrome risk in in vitro fertilized patients. *J Physiol Pharmacol* 2019; 70: 801-806.
79. Thys-Jacobs S, Donovan D, Papadopoulos A, Sarrel P, Bilezikian JP. Vitamin D and calcium dysregulation in the polycystic ovarian syndrome. *Steroids* 1999; 64: 430-435.
80. Kadoura S, Alhalabi M, Nattouf AH. Effect of calcium and vitamin D supplements as an adjuvant therapy to metformin on menstrual cycle abnormalities, hormonal profile, and IGF-1 system in polycystic ovary syndrome patients: a randomized, placebo-controlled clinical trial. *Adv Pharmacol Sci* 2019; 2019: 9680390. doi: 10.1155/2019/9680390
81. 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: 597-607.
82. Wehr E, Pieber T, Obermayer-Pietsch B. Effect of vitamin D_3 treatment on glucose metabolism and menstrual frequency in polycystic ovary syndrome women: a pilot study. *J Endocrinol Invest* 2011; 34: 757-763.
83. Parikh G, Varadinova M, Suwandhi P, *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: 754-757.
84. Anagnostis P, Karras S, Goulis DG. Vitamin D in human reproduction: a narrative review. *Int J Clin Pract* 2013; 67: 225-235.
85. Bakhshalizadeh S, Amidi F, Alleyassin A, Soleimani M, Shirazi R, Shabani Nashtaei M. Modulation of steroidogenesis by vitamin D_3 in granulosa cells of the mouse model of polycystic ovarian syndrome. *Syst Biol Reprod Med* 2017; 63: 150-161.
86. Bakhshalizadeh S, Amidi F, Shirazi R, Shabani Nashtaei M. Vitamin D3 regulates steroidogenesis in granulosa cells through AMP-activated protein kinase (AMPK) activation in a mouse model of polycystic ovary syndrome. *Cell Biochem Funct* 2018; 36: 183-193.
87. Hypponen E, Power C. Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. *Am J Clin Nutr* 2007; 85: 860-868.
88. Belenchia AM, Tosh AK, Hillman LS, Peterson CA. Correcting vitamin D insufficiency improves insulin sensitivity in obese adolescents: a randomized controlled trial. *Am J Clin Nutr* 2013; 97: 774-781.
89. Mahmoudi T, Gourabi H, Ashrafi M, Yazdi RS, Ezabadi Z. Calciotropic hormones, insulin resistance, and the polycystic ovary syndrome. *Fertil Steril* 2010; 93: 1208-1214.
90. Behmanesh N, Abedelahi A, Charoudeh HN, Alihemmati A. Effects of vitamin D supplementation on follicular development, gonadotropins and sex hormone concentrations, and insulin resistance in induced polycystic ovary syndrome. *Turk J Obstet Gynecol* 2019; 16: 143-150.
91. Selimoglu H, Duran C, Kiyici S, *et al.* The effect of vitamin D replacement therapy on insulin resistance and androgen levels in women with polycystic ovary syndrome. *J Endocrinol Invest* 2010; 33: 234-238.
92. Dravecka I, Figurova J, Javorsky M, Petrikova J, Val'kova M, Lazurova I. The effect of alfacalcidiol and metformin on phenotype manifestations in women with polycystic ovary syndrome - a preliminary study. *Physiol Res* 2016; 65: 815-822.
93. Wild RA, Rizzo M, Clifton S, Carmina E. Lipid levels in polycystic ovary syndrome: systematic review and meta-analysis. *Fertil Steril* 2011; 95: 1073-1079.
94. Di Domenico K, Wiltgen D, Nickel FJ, Magalhaes JA, Moraes RS, Spritzer PM. Cardiac autonomic modulation in polycystic ovary syndrome: does the phenotype matter? *Fertil Steril* 2013; 99: 286-292.
95. Katz A, Nambi SS, Mather K, *et al.* Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Meta*b 2000; 85: 2402-2410.
96. Rossner SM, Neovius M, Mattsson A, Marcus C, Norgren S. HOMA-IR and QUICKI Decide on a general standard instead of making further comparisons. *Acta Paediatr* 2010; 99: 1735-1740.
97. Bourlon PM, Billaudel B, Faure-Dussert A. Influence of vitamin D_3 deficiency and 1,25 dihydroxyvitamin D_3 on de novo insulin biosynthesis in the islets of the rat endocrine pancreas. *J Endocrinol* 1999; 160: 87-95.
98. Ortlepp JR, Metrikat J, Albrecht M, von Korff A, Hanrath P, Hoffmann R. The vitamin D receptor gene variant and physical activity predicts fasting glucose levels in healthy young men. *Diabet Med* 2003; 20: 451-454.
99. Zeitz U, Weber K, Soegiarto DW, Wolf E, Balling R, Erben RG. I mpaired insulin secretory capacity in mice lacking a functional vitamin D receptor. *FASEB J* 2003; 17: 509-511.
100. Gupta T, Rawat M, Gupta N, Arora S. Study of effect of vitamin D supplementation on the clinical, hormonal and metabolic profile of the PCOS women. *J Obstet Gynaecol India* 2017; 67: 349-355.
101. Dastorani M, Aghadavod E, Mirhosseini N, *et al.* The effects of vitamin D supplementation on metabolic profiles and gene expression of insulin and lipid metabolism in infertile polycystic ovary syndrome candidates for in vitro fertilization. *Reprod Biol Endocrinol* 2018; 16: 94. doi: 10.1186/s12958-018-0413-3
102. Miao CY, Fang XJ, Chen Y, Zhang Q. Effect of vitamin D supplementation on polycystic ovary syndrome: a meta-analysis. *Exp Ther Med* 2020; 19: 2641-2649.
103. Ardabili HR, Gargari BP, Farzadi L. Vitamin D supplementation has no effect on insulin resistance assessment in women with polycystic ovary syndrome and vitamin D deficiency. *Nutr Res* 2012; 32: 195-201.
104. Garg G, Kachhawa G, Ramot R, *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: 108-116.
105. 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: 4555-4577.

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