



Compendium of polycystic ovarian syndrome and its relevance in glycation and diabetes

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Received: 03.06.2021

Accepted/Published Online: 26.07.2021

Final Version: 01.01.2022

Abstract

Polycystic ovarian syndrome (PCOS) is one of the most common endocrine and reproductive disorders observed in women. Its pathophysiology indicates its involvement in plenitude of diseases and/ or disorders. Although the absolute cause of PCOS has not been established, its prevalence in diabetic patients is noteworthy. Glycation and insulin resistance being key factors in diabetes, the present review tries to study their correlation with PCOS. Amongst the available drugs, four commonly prescribed drugs namely, metformin, inositol, thiazolidinediones, and spironolactone have been discussed. The current review also provides insights about usage of *M. oleifera* and *S. platensis* as a potential therapeutic agent for symptomatic relief of PCOS.

Keywords: altered steroidogenesis, glycation, insulin resistance, PCOS, *spirulina*, T2DM

1. Introduction

Polycystic Ovarian Syndrome (PCOS) most often characterized as an endocrine/hormonal disorder, which typically commences during the onset of puberty. It is one of the chief causes of oligomenorrhea (irregular periods), amenorrhea (no periods) or prolonged periods causing anovulatory infertility in women. This is believed to be due to the excess amount of androgen produced known as Hyperandrogenism (1). Hyperandrogenism in PCOS is due to a disturbed Hypothalamic-pituitary-ovarian axis (HPOA) affecting the ratio between luteinizing hormone (LH) and follicle stimulating hormone (FSH) (2). There are several factors that are responsible for the intricate pathophysiology so far identified in PCOS. Although the absolute cause of PCOS is not well comprehended, genetic, and environmental factors are believed to be responsible for the prevalence of the disorder (3). Therefore, it is also classified as an oligogenic disorder (disease inherited is not due to a simple single-gene mutation) implying interlinkage between various genetic and environmental factors causing the characteristic symptoms of the disease (4).

More often PCOS shows a constellation of symptoms and is simply responsible for the onset or cause of other metabolic disorders such as Type 2 Diabetes Mellitus (T2DM) (5). Most women diagnosed with PCOS, are more susceptible to develop Diabetes or prediabetes (borderline diabetes). Another life-threatening symptom which is commonly observed, includes cardiovascular disease (6). Prevalence of

cardiovascular disease may be due to disturbed carbohydrate metabolism in PCOS (7). The chain of symptoms in PCOS is quite complex because of which the ultimate interlinkage between hormonal and metabolic disorder cannot be distinguished. However, in both the cases Insulin resistance (IR) plays a key role in giving rise to the disorders commonly observed in PCOS. Distress in Phosphatidylinositol-3-Kinase (PI-3K) enzyme is primarily held responsible for IR. Due to IR women with PCOS are diagnosed with hyperinsulinemia causing obesity in women. Both hyperinsulinemia and insulin resistance are observed in obese and non-obese women. IR is most possibly considered for the endothelial damage which can aggravate the cardiovascular disease or worsen the situation in some cases (8).

Various foregoing studies have associated defective insulin receptor-mediated signal transduction in PCOS (9, 10). With further investigation on the defective insulin receptor pathway, aberration in PI-3K insulin mediated glucose uptake has been observed in PCOS women (11). Function of PI-3K can be disturbed either by serine phosphorylation of insulin receptor substrate (IRS) or advanced glycation end products (AGEs). AGEs are protein products formed because of glycation (non-enzymatic glycosylation). PI-3K endocytotically uptake of AGE by their receptor (RAGE), regulating their degradation and elimination process (12). Although elimination of AGE can be mediated via the insulin receptor pathway (13).

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AGEs are also associated with Diabetes and their formation increases free radicals' activity (14). This facilitates biomolecular damage increasing the complication in the disorder increasing IR (15). AGEs are responsible for the onset of IR in PCOS and diabetes through insulin receptor-mediated pathways and free radicals, respectively, worsening the pathophysiology of the disorder. Reducing glycation and AGEs in the tissue by subsequently controlling the AGE-RAGE interaction might avert the complication raised due to IR in PCOS and diabetes (15).

2. Insulin Resistance (IR)

Understanding insulin resistance at the molecular level would be the first step in deciphering the abnormalities that occur. Insulin hormone plays a crucial role in glucose homeostasis. Any disturbance in insulin secretion directly initiates certain conditions, such as hypoglycemia, hyperglycemia and T2DM. Insulin transduction pathway consists of several enzymes, receptors and hormones which facilitates the proper functioning of insulin in the body. The signal transduction pathway of insulin generates a cascade of signals in the cell in response to its environment. Impairment in the insulin signal transduction pathway leading to cellular inflammatory pathway is a prime cause of IR (16). Albeit a decreased strength of IRS/PI-3K signaling pathway is the supreme culprit, the authenticity of which is still enigmatic. Two distinct but interdependent mechanisms confer a potential explanation for reduced strength in the IRS/PI-3K pathway, which includes increased expression of p85 α and serine phosphorylation of insulin receptor substrate (IRS)-1 (16).

Apart from being a crucial component of the signally pathway, PI-3K also helps in maintaining insulin sensitivity in the liver. It is composed of two subunits, namely a regulator subunit (p85) which is tightly associated with a catalytic subunit (p110) forming a p85-p110 heterodimer or complex (16). The regulatory subunit (p85) is responsible for regulating the activity of PI-3K by binding to the active site of IRS-1. Over or under expression of p85 α competes with the p85-p110 heterodimer on the binding site of IRS-1 leading to either increase or decrease in PI-3K activity, (17, 18) P85 α an isomer of p85, competes with the p85-p110 heterodimer reducing PI-3K activity (19). However, other growth hormones, steroids, overfeeding, (20) and T2DM subsequently increases the activity of p85 α (21).

In response to insulin binding to insulin receptors. IRS-1 is phosphorylated by tyrosine kinase activating PI-3K enzyme (22). PI-3k binds to a specific motif on IRS-1 which consist of phosphorylated tyrosine residue resulting to which PI-3K is activated initiating a cascade of events causing phosphorylation and activation of Akt (kinase B), mTOR (mechanistic target of rapamycin) and p70S6 kinase (ribosomal protein S6 kinase beta-1) (23). Akt activation is crucial for glucose transport across the cell, however, mTOR and p70S6 activation assist in protein synthesis. Although in

case of IR hyperactivation of mTOR lead by a certain stretch of amino acids, Akt or hyperinsulinemia follows serine phosphorylation of IRS-1 by p70S6 kinase eventually giving rise to a decrease strength of IRS-1/PI 3-kinase signaling (16).

IR is also widely associated with lipids. Irregularities in insulin signaling and lower insulin-stimulated glucose transport are some of the major imputations resulting from Lipid-induced insulin resistance in skeletal muscle (24, 25). Activation of PKC (isoform PKC θ) is linked with muscle lipid accumulation hence altering the insulin intracellular signaling. Lipid induced IR, mediated by diacylglycerol (DAG) for the activation of PKC θ leading to impairment in insulin signaling (26). Although impaired Akt activation limits the translocation of GLUT4-containing storage vesicles to the plasma membrane, (permits the entry of glucose into the cell, and promotes glycogen synthesis via glycogen synthase (GS).), resulting in impaired glucose uptake therefore decreasing insulin-mediated Glycogen synthesis (27). Convincing evidence between DAG-mediated activation of PKC and muscle insulin resistance has been confirmed in human studies (23). Another study demonstrated hepatic insulin resistance relating to intrahepatic lipid content. Therefore, demonstrating that ectopic lipid accumulation of lipids within the liver leads to hepatic insulin resistance (28).

An alternate string signaling towards hepatic IR with non-alcoholic fatty liver disease (NAFLD) involves activation of unfolded protein residues (UPR). The initiation of UPR takes place in the endoplasmic reticulum (ER) lumen due to accumulation of unfolded protein, therefore known as ER stress. Unfolding of protein in the ER may occur due to environmental stress or genetic mutations. Accumulation of unfolded protein induces UPR in a eukaryotic cell (29). Studies noted a reduced activity of PKC in individuals provided with lipid infusion, shows reduction in insulin-related glucose transport, strongly suggesting a defect in the insulin signal mechanism (30). Although the relation between PKC and lipids is not yet clear.

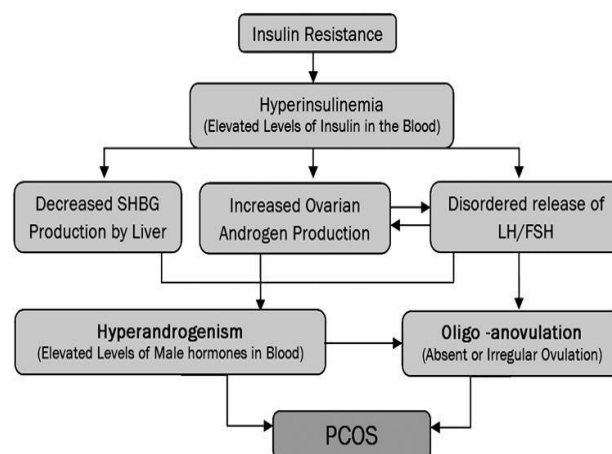


Fig. 1. Intricate linkage between Insulin resistance and PCOS

2.1. Glycation

Glycation is widely defined as a non-enzymatic reaction between reducing sugar, proteins, lipid and nucleic acid (15). The process of glycation leads to the formation of AGE's formed by a complicated molecular process also known as Maillard reaction (31). The electrophilic group of sugar molecules reacting with the free amino group of amino acids commences with the formation of the Schiff base (32). With further intramolecular rearrangement the Schiff base is reversibly converted into a more stable form known as Amadori product (ketoamine). Further engagement of Schiff base and Amadori products with protein or amino acid residue giving rise to protein adducts or protein crosslinks (33). After the formation of Amadori product, they undergo a slow oxidation process yielding dicarbonyl compound such as glyoxal, methylglyoxal (MG), 3-deoxyglucosone (3-DG), and deoxyglucosone in the span of 3 to 30 days (32). The final stage of the glycation reaction includes oxidation, dehydration, polymerization and oxidative breakdown advances in the formation of AGE's (34). Accumulation of AGEs leads to physiological and pathological changes, therefore AGEs are associated with T2DM, Alzheimer's disease, aging, etc. (35) AGE's characterized into three categories, namely pentosidine (fluorescent product; forms protein-protein crosslinks), carboxymethyl-lysine (CML), and glucosepane (a non-fluorescent protein adduct) (36).

yield carbonyl compound, for example, methylglyoxal, undergoing a series of reactions with protein to yield AGEs (38). Although PCOS women are often diagnosed with an abnormal lipid profile, AGEs play a negative role in dyslipidemia (39). Studies show a positive correlation between serum MG level and triglyceride concentration in diabetic patients (40). Accumulation of AGEs intracellularly is mediated through specific AGE receptors (RAGEs) (41). Binding the ligand to RAGE activates signaling pathways such as mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinases (ERK-1/2), PI-3K etc. RAGE stimulation by AGE activates nuclear factor kappa-B (NFκB) (42). Activation of NFκB further increases the expression of RAGE, which in turn stimulates NFκB leading to a cycle of perpetuating proinflammatory signals (43). It also decreases cellular antioxidant defense, such as glutathione (GSH) by inducing oxidative stress. Decrease in GSH causes decreased activity of Glyoxalase-1 (Glo-1) which plays a key role in cellular defense against methylglyoxal (MG) leading to further formation of AGEs (44).

RAGE interaction with amyloid-β peptide (Aβ), β-sheet fibrils etc. involving RAGE in multiple cellular functions via distinct signal transduction pathway (43). Activation of MAPK activates the expression of proinflammatory cytokines which are believed to be associated with IR (45). Generation of ROS due to AGEs is a common link discussed in diabetes pathology. Physical and functional characteristics of stable protein products are altered when interacted with extracellular matrix membrane (ECM) leading to diabetes altering the basement membrane and microvascular system due to excessive AGE formed (46).

2.2. PCOS and IR

The main culprit in the prevalence of PCOS is contemplated as HPOA and hyperinsulinemia (47). Both factors are believed to be interconnected, as disrupted HPOA can cause loss in GnRH pulses resulting in an increasing amount of LH (48, 49). In the menstrual cycle, the role of increased spike of LH is to bring about ovulation and oocyte development and forming corpus luteum in a healthy woman with the help of another hormone progesterone, which suppresses the LH therefore maintaining the LH/FSH ratio (50). More than the required amount of LH secreted due to increased GnRH can inhibit the whole cycle and change the morphology of the ovary (48, 49). Also, increased LH leads to a substantial decrease of FSH in the ovary causing decreased conception and miscarriage in woman (47). LH is also responsible for provoking an increased ovarian androgen. Production of androgen occurs in ovarian cells (theca interna cells) and adrenal cortex (zona fasciculata cells) (51). Increased amounts of androgen or hyperandrogenism (HA) occur due to altered steroidogenesis.

Change in the ovarian morphology or presence of polycystic ovaries is the prime site of endocrine abnormality

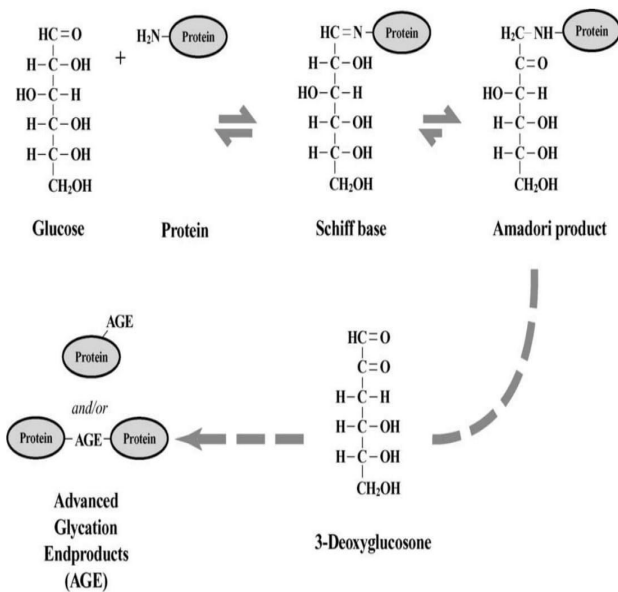


Fig. 2. Steps of protein glycation and formation of AGE (adapted from 15)

The formation of AGEs can take place through three distinct pathways. Apart from Maillard reaction, lipid peroxidation and glycolysis pathway are the alternative means through which AGEs are formed. In the case of lipid peroxidation, reactive oxygen species lead to the formation of reactive carbonyl compounds which then ultimately form AGEs or Advanced lipid end products (ALE) (37). Similarly, in the case of glycolysis pathways, oxidation of glucose to

causing hyperandrogenism. Affected P450c17a enzyme activity plays a principal role in excess ovarian androgen production (52). Serine phosphorylation also increases activity of P450c17 in both the ovary and adrenal gland, thus promoting androgen synthesis (53). The initial substrate, cholesterol, is used to produce androgens in both the organs. Both of which are strongly under endocrine control of LH and adrenocorticotropic (ACTH) in the ovary and adrenal cortex respectively (51). Androgen synthesis takes place through distinct steroid biosynthesis pathways, namely, D5-steroid pathway and D4-steroid pathway occurring parallelly and simultaneously (54). Although intraovarian androgen is crucial for the follicular development, excess of which can cause abnormalities in the follicular growth therefore poor follicular maturation. Both androgen and estrogen are the positive modulators of LH however Insulin-like growth factor-1 (IGF-1) is recognized as a positive modulator of LH. Increased LH stimulated androgen production also takes place via insulin or IGF-1 (52). Insulin and IGF-1 stimulate the production of estrogen mediating the action of FSH on granulosa cells for healthy follicle growth. Androgen production is moreover inhibited by corticotropin releasing hormone, transforming growth factor- β (TGF- β), epidermal growth factor (EGF), tumor necrosis factor (TNF) and cytokines. TNF and EGF inhibit the activity of aromatase, which determines androgen production. Action of activin on the granulosa cells increases estrogen secretion and therefore inhibits thecal androgen secretion (52).

PCOS also increases the prevalence of T2DM and IR. The role of insulin in androgen production is quite crucial and important. Due to affected endocrine signaling other metabolic pathways are also affected hence giving rise to T2DM. IR is considered as one of the pathogenic factors associated with an increased metabolic disturbance which also explains HA, irregular menstrual cycle (5, 55). A positive correlation between hyperinsulinemia and PCOS were recorded showing notable correlation between insulin, testosterone, and androstenedione (56). Elevated insulin level in PCOS concurrently elevating LH levels, suppression of follicle growth hence no ovulation (57). Hyperinsulinemia also affects the secretion of GnRH pulses which leads in the arrest of Sex Hormone Binding Globulin (SHBG) and escalating ovarian androgen production in PCOS (58-61).

Elevated levels of AGE along with their receptor RAGE was observed in the ovarian tissue. Potentially, accumulation of AGE's could alter steroidogenesis in PCOS, although the prime pathway or its potential role in steroidogenesis yet remains in scrutiny. Toxic AGE (TAGE), CML and pentosidine in follicular fluid and serum-TAGE (S-TAGE) show a negative correlation with estradiol (E2) in PCOS women. Earlier studies have reported an increased level of serum AGE's in PCOS women by examining the association of serum AGE's level and RAGE expression with testosterone (62). A different study showed a substantial

association between AGE's and HA (62, 63). To understand the signaling mechanism of AGE-RAGE behind the altered steroidogenesis in PCOS Diamanti-Kandarakis and team (2013) pointed out that AGEs lead to inappropriate activation of ERK1/2 in KGN granulosa-like tumor cells alter steroidogenesis in granulosa cells (5). A vital role of glyoxalase in the ovary has been confirmed by dietary glycotoxins and hyperandrogenic decrease GLO1 (Glyoxalase 1) activity in the ovaries, contributing to enhanced accumulation of AGE in granulosa cells (5).

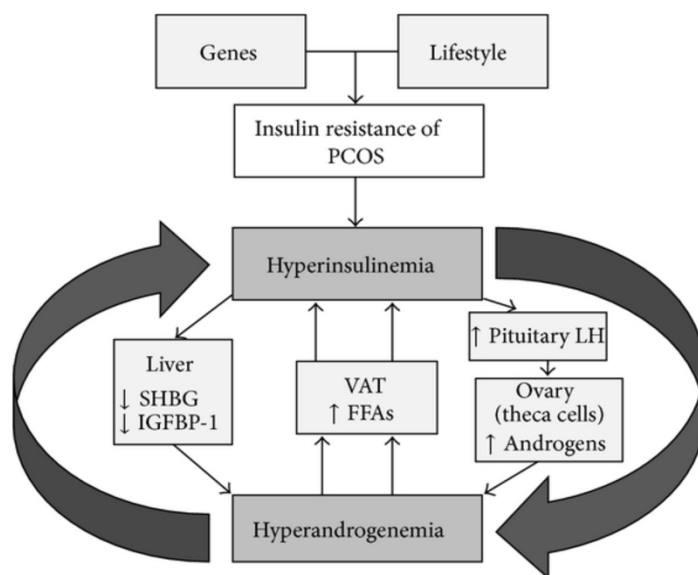


Fig. 3. Cycle between hyperinsulinemia and hyperandrogenemia (136)

2.3. PCOS and diabetes

Diabetes mellitus, a disorder which is often characterized due to elevated levels of blood sugar due to insufficient amounts of insulin produced by the body. Due to its characteristics, T2DM is often associated with hyperinsulinemia. As mentioned earlier, PCOS leads to a collection of syndromes which leads to reproductive disturbance and glucose metabolism disorder (64). HA and IR are identified features of PCOS which lead to hyperinsulinemia causing IR (5). IR arises due to an intrinsic defect in post-receptor insulin signaling in its ideal target tissue, such as muscle cells and adipocytes (47). Protein glycation (dicarbonyl stress) and MG combined, shows a chronic side effect in hyperglycemic diabetic patients which leads to the pathogenesis of various diseases associated with altered redox homeostasis (65).

Even though the molecular basis of AGE in the pathogenesis of T2DM is not well comprehended, certain study shows a strong relation between AGE-induced oxidative stress as well as inflammation (40). Some hypotheses also suggest direct impairment of insulin secretion in pancreatic cells due to AGE activating proinflammatory cytokines provoking inflammation in islet β cells. Inflammation in the pancreatic cells due to increased levels of IL-1 results in decreased cell proliferation, increased cell

death in β cells therefore giving rise to impaired secretion of insulin (40). Inhibition of cyt C oxidase and ATP production due to AGE, leads to the commencement of impaired glucose stimulated insulin secretion potentially through membrane depolarization (40, 65).

High amount of dietary AGE intake is strongly associated with T2DM. T2DM is also associated with weight gain or obesity, controlling dietary AGE is a common strategy used to control the Many studies conducted to understand the link between the PCOS and T2DM have associated overweight and obesity as the primary cause of IR (67). Other Meta-Analysis studies have confirmed a positive association of PCOS with a high prevalence of prediabetes and T2DM (68). However, the ultimate relationship between rapid weight gain, HA, prediabetes and T2DM shown by numerous studies is not convincing. Also, the prevalence of prediabetes, T2DM in PCOS is moreover independent of the BMI, (69-71) on contrary other studies have associated an elevated risk linked with obesity (72). Some studies have also associated women with normal body weight PCOS having a higher chance of progressing T2DM (73).

2.4. Drugs

Inter-relation between PCOS and diabetes reveals that insulin-sensitizing drugs and dietary or lifestyle modifications improve hyperandrogenism in patients suffering from PCOS (74, 5). It is observed that Insulin resistance increases hyperandrogenemia (75). Leptin, a hormone which is used as an insulin-sensitizing agent, is believed to decrease the androgen levels, and induces menstruation in lean women suffering from PCOS (75). This is considered as one of the remedial treatments for PCOS, which pointed out the consideration of insulin-sensitizing agents as part of the management of the disease. These insulin-sensitizing agents include metformin, MYO supplements, and thiazolidinedione. In previous studies, a few biomarkers have been used to detect insulin resistance in PCOS women. Insulin happens to inhibit the release of SHBG secreted from the liver and the production of insulin-like growth factor-binding protein 1 (IGFBP-1) (76). It is also believed to affect the homeostatic model assessment (HOMA-IR) and levels of insulin (77). Various markers have distinct sensitivities as well as specificities in testing for insulin resistance (78).

3. Treatment

The management of PCOS targets the symptoms present, such as anovulation, infertility, hirsutism, or acne.

3.1. Lifestyle changes

Therapy including exercise and calorie-restricted diet perform a crucial part in the management of obesity in women with PCOS. Lifestyle modifications are considered as a cost-effective first-line treatment and as a necessary adjunct to medication (79). Excessive weight is associated with adverse metabolic and reproductive health outcomes in women with PCOS. Female fertility significantly decreases with a BMI

>30–32 kg/m² (81). Studies show that a decrease in the body weight of about 5% regulates the MC, reduces insulin and testosterone levels, improves fertility, reduces acne and hirsutism, and increases psychological well-being (82-85). However, no specific diet or exercise schedule is superior to another in the management of PCOS.

3.2. Medical treatment

If lifestyle changes are not enough to resolve symptoms, medical treatment is added for better management of the patient's complaints.

3.3. Oral contraceptive pills (OCP)

For an exceptionally extended period, OCP was commonly used as a first line of medicinal treatment for hyperandrogenism and MC irregularities as a treatment of PCOS, (79, 80). OCP suppresses the hypothalamic-pituitary-ovarian axis, it also decreases LH secretion and increases SHBG; decreases free testosterone level (86). This shows an improvement in hyperandrogenism-mediated symptoms like acne and hirsutism (86), also maintains the MC and provides positive contraception (87). Albeit the guidelines do not specify the use of one OCP over another, therefore, a low dose which contains anti-androgenic or neutral (88) of OCP is prescribed for symptomatic treatment.

The use of OCP in PCOS patients shows an extended risk of insulin resistance (74, 80). There are adverse impacts of OCP on cardiovascular risk in PCOS women (89). Clomiphene citrate is used for the primary treatment of infertility due to anovulatory (89). Administration with gonadotropins and ovarian diathermy is a secondary line of treatment (90) when other drugs fail to achieve fertility.

3.4. Metformin

Metformin, also known as Glucophage, is a biguanide anti-diabetic drug (Fig. 4), acts by preventing liver glucose production and increasing the peripheral insulin sensitivity (91, 92). Metformin was first studied in 1994 by Velazquez with his co-workers and showed a 35% decline in insulin and a 31% reduction in insulin to glucose ratio (89). Few studies also claim that metformin does not promote insulin resistance itself, it promotes glucose effectiveness, i.e., the ability of glucose to repress endogenous glucose synthesis and stimulate glucose uptake (93). Metformin therapy for obese PCOS adolescents and IGT proved beneficial in enhancing insulin sensitivity and glucose tolerance, in reducing insulinemia, and in elevated androgen levels (94). By contrast, a study done by Tang et al. showed no remarkable shift in insulin sensitivity in PCOS patients receiving metformin which could be explained due to the elevated level of obesity (BMI>30 Kg/m²) and the limited weight loss of the patients (52). Ehrmann et al. (1997) also showed that metformin does not necessarily improve insulin resistance in PCOS women (95, 96). Açıbay and Gündoğdu, (1996) stated that metformin has no visible effect on insulin resistance in PCOS patients.

Although studies show conflicting results of metformin effect, it is suggested as a primary line of treatment for pregnancy complications in PCOS women. Combination of Metformin with clomiphene citrate to improve fertility in clomiphene resistant patients (89).

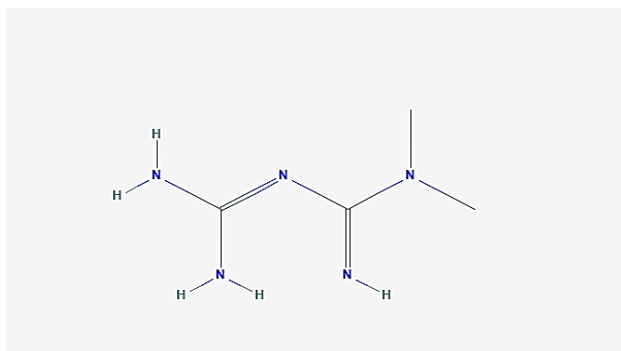


Fig. 4. Structure of Metformin (Source: <https://pubchem.ncbi.nlm.nih.gov/compound/4091>)

metformin alone is given as the primary line treatment of PCOS obese treatment by inhibiting liver glucose absorption, reducing peripheral insulin levels, increasing peripheral glucose uptake, and improving Glucose transporter type 4 (GLUT-4) (97, 98). Metformin affects the endothelium and adipose tissue independent of its action on insulin and glucose levels (99). Metformin does not help to body weight loss; it may help to distribute adipose tissue (100).

3.5. Thiazolidinediones

Thiazolidinediones (TZD), a class of drugs (insulin sensitizer) used in T2DM treatment. They activate the γ isoform of an adipocyte transcription factor, known as peroxisome proliferator-activated receptor (PPAR- γ) (101). The use of pioglitazone (glitazones) was studied in PCOS patients and data showed a decline in insulin resistance and fasting serum insulin levels (Hayek et al., 2016). However, pioglitazone showed an increased chances of bladder cancer (102, 103), therefore it has not been prescribed use or the use of other TZDs (namely rosiglitazone and troglitazone) in PCOS treatment due to its ill effects (89, 80).

3.6. Inositol

Different drugs are identified as a unique treatment for PCOS which are preferred due to their lack of side effects. D-chiro-inositol (C₆H₁₂O₆) (DCI) as well as myoinositol (MYO), which is an insulin-sensitizing molecule (Fig. 5). Metabolic alteration of Inositol Phosphoglycan (IPG) secondary messengers and its mediators or due to a tissue defect, might induce insulin resistance (74). In PCOS women, use of MYO shows to increase insulin resistance (89). Increase in levels of fasting plasma insulin is observed to be positively correlating with a decline in insulin resistance, this supports the role of Inositol as a modulator of the insulin-mediator metabolic pathway (104).

Combining Inositol with monacolin K (Lovastatin) and lipoic acid shows dose-dependent changes in HA associated symptoms (105). Folic acid combined with MYO showed a

decrease in ovarian hyperstimulation syndrome (OHSS) to a greater extent as compared to the use of folic acid alone in PCOS women (106). MYO along with α -lipoic acid also improved reproductive issues in women undergoing an IVF treatment (107, 108). Most importantly, MYO combined with DCI in a blood plasma shows a decreased risk of developing metabolic syndrome in obese PCOS women (107, 109). Another study shows notable improvement in PCOS symptoms, such as MC regularity, decreased insulin resistance, healthier lipid profile, and fewer acne, upon the use of MYO-DCI combination (89). Therefore, MYO and DCI in combination as a therapeutic approach can be used for the PCOS treatment (104).

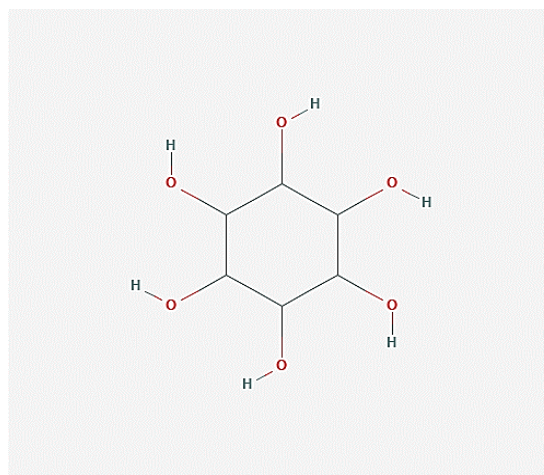


Fig. 5. Structure of Inositol (Source: <https://pubchem.ncbi.nlm.nih.gov/compound/892>)

3.7. Spironolactone

Spironolactone, a chemical steroid related to the aldosterone (Fig. 6) (mineralocorticoid), was observed to enhance insulin sensitivity; it was also suggested its use for HA associated symptoms such as hirsutism and acne (89, 110). Combination of Spironolactone with Metformin often shows an increase in menstrual frequency. However, Spironolactone is also known to induce menstrual irregularities when consumed at higher doses. Although, it is primarily in the management of hirsutism, acne, and androgenic alopecia (110).

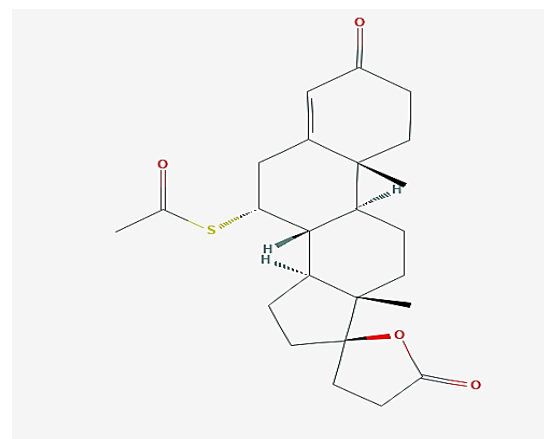


Fig. 6. Structure of Spironolactone (Source: https://pubchem.ncbi.nlm.nih.gov/compound/5833_

A clinical trial with two sub-groups including women with PCOS, lean and overweight, were subjected to spironolactone treatment. Women affected with oligomenorrhea, and amenorrhea were observed with a regular menstrual cycle. Other symptoms include acne development in lean (31%) and overweight women (33%). Spironolactone shows positive antiandrogenic properties. In some cases, use of spironolactone may cause Polyuria, Polydipsia during the initial days of treatment. Patients may also witness some minor to rare symptoms which includes headaches, increased appetite leading to weight gain, breast enlargement or tenderness & dizziness (111).

4. Treatment in adolescents

The aid of treatment for adolescents suffering from PCOS is OCPs, administered as an effective treatment for HA and contraception (112). OCPs bring menstruation in the regular cycle and reduce hirsutism and acne (113). Change in Lifestyle and weight loss are also considered as part of the primary line of treatment, especially in obese adolescents. Nevertheless, uncertainty prevails concerning the best OCPs and their duration of usage in adolescents (89, 114). Alternatively, metformin shows to improve irregular MC, insulin resistance, and HA in PCOS adolescents (89). Finally, adolescents PCOS treatment is recommended with OCPs which may reduce the chance of hyperandrogenism in adults (89) and early changes in lifestyle and metformin treatment show preventative results (89).

4.1. Natural products

Other synthetic drugs may show some side effects, which in turn would lead to some other disorder. Synthetically designed drugs may not work as efficiently/effectively in each patient. Use of synthetic drugs shows some mild symptoms and development of endometrial cancer in some adverse cases. Natural products, which are obtained from plants or organisms, minimize the risk of such side effects. The extract obtained from a natural product, such as moringa leaves, has shown significant results in lowering the blood insulin level. A decrease in insulin level leads to a subsequent decrease in androgen, promoting folliculogenesis. Due to its positive result and lowering the risk of side effects on PCOS women it is essential to switch more towards the utilization of natural products as compared to synthetic drugs.

5. Potential sources of natural drugs

5.1. Moringa

Moringa oleifera or Moringa (family- Moringaceae), extensively spotted in tropical climates. It emerged as a natural therapy as one of the alternatives in PCOS treatments, a food plant which originates from India, which grows in regions with temperatures around 25°C-35°C. Due to the side effect neutralizing the effect of food plants they are safe as compared to synthetic drugs (115). It is appreciated to be rich in various active medicinal chemicals. Recent studies on *Moringa oleifera* have potential as antioxidants, anticancer,

antidiabetic, anti-inflammatory, and as antimicrobial agents (116). Flavanol quercetin was found with high concentrations in *Moringa oleifera* leaves (116). *Moringa oleifera* leaf is composed of Quercetin also known as 3,3',4',5,7-pentahydroxyflavone (117). Quercetin is a flavonoid which portrays a strong bioactive element with free radicals' effects, it also acts as a potential anti-inflammation, antihyperlipidemic, anticancer, and antiplatelet compound (118). Quercetin is also found to produce PI3K inhibin (119). The expression of CYP17A1 gene in the ovarian thecal cells is inhibited by the same quercetin P13K inhibin leading to which the activity of 17 α -hydroxylase is decreased. 17 α -hydroxylase is crucial to produce sex hormones and glucocorticoids therefore a decrease in its activity pay a crucial role in PCOS (120). The action of Quercetin in maintaining the levels of insulin, LH and testosterone and lipid profile have been profoundly noted. The influence of Quercetin is also associated with a decreased ovarian and uterine mass and enhancing the level of follicles and corpus luteum in PCOS (120).

Moringa leaf extract could increase folliculogenesis in the ovary with insulin resistance. Excess amounts of androgens directly inhibit the insulin action in the liver periphery, and indirectly influences the insulin sensitivity in the body through fat metabolism (121). Due to decreased effectiveness of Androgens and the impaired signaling of GLUT-4 insulin resistance is caused. The androgen produced affects the environment of the ovaries, leading to androgen aromatization system disorders into estrogen triggering defective follicular growth. Decrease in insulin resistance decreases LH pulsation and therefore increases the frequency of GnRH secretion hence increases folliculogenesis (early atresia does not occur). Conclusively, *Moringa oleifera* could potentially control the levels of insulin in the blood consequently decreasing androgen and hence folliculogenesis is increased (122).

5.2. Spirulina

Spirulina platensis, now changed to *Arthrospira platensis*, is a cyanobacteria (blue-green algae). It gained notable attention in the healthy food industry as a food supplement for humans, poultry, livestock, and aquaculture diets (123). Typically grown in alkaline water with efficient yield and can be processed easily. It is known to be enriched with macronutrient and micronutrient contents, such as proteins, amino acids, unsaturated fatty acids and a variety of minerals and vitamins (124). It has shown evident therapeutic benefits in a collection of disease conditions, including hypercholesterolemia, hyperglycemia, cardiovascular disorders, inflammatory diseases, cancer, and viral disease (125). The two major species of *Arthrospira* genus are *A. maxima* and *A. platensis*.

Studies including *Spirulina* sp. in a high-cholesterol and high-fat diet showed a notable decline in hepatic lipids and blood lipids (125-127). Blé-Castillo and his team in a 2002

study, reported reduction of total hepatic lipids by almost 40%, decreased TG by 50%, and decreased serum TG by 45%, and there was also a 45% rise in serum HDL cholesterol after feeding fatty liver mice with *Spirulina* sp. for about two weeks (128). *Spirulina* sp. induced by treatment of alloxan was also established in diabetic mice. Lowering fasting blood glucose, GSP, cholesterol, TG levels, averted MDA formation, raised the levels of hepatic glycogen, and maintained TAOC and hepatic glucokinase (GK) expression (126).

Hypolipidemic effects of *Spirulina* sp. were studied with 15 T2DM patients, (129) 2g/day of *Spirulina* consumed for two months shows a notable decrease in LDL, VLDL and serum total cholesterol levels in the blood. Furthermore, a significant decrease in blood sugar and GSP levels were also observed. Subjects in a study group consuming *Spirulina* dosage of 2g/day for 4 weeks, (130) with a decrease in LDL, VLDL, and TG levels, there was a significant decrease in apolipoprotein B level and a parallel increase in apolipoprotein A1 level. These discoveries noted in the early studies were proved in two recent human clinical trials with T2D patients, (131, 132). Another trial involved 60 male patients aged 40 to 60 years consuming 1 or 2 g of *Spirulina* every day for two months (132). A notable decrease in fasting and post-meal blood glucose, serum total cholesterol, triglycerides, LDL, and VLDL cholesterol was observed. Benefits of spirulina on hyperlipidemia were observed in patients with hyperlipidemia and nephrotic syndrome (126; 133). *Spirulina* supplementation at a dose of 1 g/day for four weeks proclaimed a decrease in total serum cholesterol, TG, and LDL fraction by 46 mg/dL, 45 mg/dL, and 33 mg/dL, respectively. The ratios between total cholesterol/HDL and LDL/HDL also decreased significantly. Consumption of spirulina shows a significant decrease in the serum total cholesterol levels, LDL cholesterol, oxidized LDL, and apolipoprotein B levels. On the other hand, total plasma cholesterol and LDL fraction were significantly decreased in females. These data, therefore, largely support that spirulina supplements are helpful mostly in elderly people (134).

Another study has shown a positive action of *Spirulina* on alpha amylase inhibition consisting of antidiabetic properties. Apart from consisting of antidiabetic properties, *Spirulina* also possesses anti-glycation activity, with its action on the formation of ages and dicarbonyl stress (135). A significant increase in serum insulin level was observed due to its antioxidant enzyme activity and RNA expression. A decrease in the level of glycated hemoglobin (HbA1c) and malondialdehyde (MDA) with a proper recovery of the liver cells due its anti-inflammatory and antioxidant property was observed. Lowering levels of glucose eventually improved liver enzyme and increased insulin secretion was observed in the diabetic mice model when ingested with *Spirulina* extract (136).

6. Conclusion

Improper diet and altered steroidogenesis in women lead to the development of PCOS. PCOS is known for its collection of syndromes which affects the reproductive and psychological health of women. As the root cause of PCOS is not yet fully understood, various genetic factors and other environmental factors such as glycation which alter steroidogenesis in the ovary are assumed to be the cause for the pathology. Efforts to control the symptoms of PCOS are undertaken by using various drugs, diet management and lifestyle modification. Many synthetic drugs used for the treatment of PCOS have been recorded with significant side effects. Apart from synthetic drugs, natural products also have reported an improved condition in women with PCOS. Symptoms of PCOS include IR which leads to the development of T2DM. Women with PCOS are also observed with an increased risk of heart disease which may be due to hypercholesterolemia or hyperlipidemia. *Spirulina* has shown effective results in controlling high cholesterol and lipid levels when included in the diet. In recent studies *Spirulina* has also shown some antidiabetic and antiglycation activity. Since T2DM and PCOS are interrelated with the pathology mediated by insulin resistance, use of *Spirulina* in women with PCOS may control high cholesterol level and glycation stress caused in the ovaries due to the glycation of certain ovarian proteins and may function as a therapeutic aid. Thus, inclusion of *Spirulina* in the diet may be an effective alternative for the symptoms of PCOS which may regulate the steroidogenesis and maintain the menstrual cycle improving reproductive health and increase fertility. Further studies and analysis must be done for a conclusive result of the effects of dietary spirulina in PCOS.

Conflict of interest

No conflict of interest was declared by authors.

Acknowledgments

None to declare.

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