Scientific Research Publishing

Advances in Aging Research, 2024, 13, 149-166
https://www.scirp.org/journal/aar
ISSN Online: 2169-0502
ISSN Print: 2169-0499

Efficacy of Metformin in Treating Complications Associated with Polycystic Ovary Syndrome (PCOS)

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How to cite this paper: Alduraywish, R.A. and Al-Sowayan, N.S. (2024) Efficacy of Metformin in Treating Complications Associated with Polycystic Ovary Syndrome (PCOS). *Advances in Aging Research*, **13**, 149-166.
https://doi.org/10.4236/aar.2024.136011

Received: October 21, 2024
Accepted: November 18, 2024
Published: November 21, 2024

Abstract

Polycystic ovarian syndrome (PCOS) is an endocrine disorder that affects women of reproductive age and is characterized by hyperandrogenism, unpredictable ovulation, and a polycystic ovary shape. It is accompanied by metabolic problems, such as obesity and insulin resistance (IR). Factors contributing to PCOS include genetics, epigenetic changes, environmental factors, oxidative stress, chronic inflammation, mitochondrial dysfunction, and metabolic abnormalities. Insulin resistance is the most prevalent classical phenotype, and metformin was the first insulin-sensitizing drug to be used to study its role. Metformin, derived from galegine, inhibits mitochondrial function, activates AMPK, enhances hepatic insulin sensitivity, and lowers the risk of type 2 diabetes in PCOS patients. It also affects tumor growth, with a 30% reduction in the overall tumor onsets in T2DM patients. Metformin treatment has been linked to low rates of multiple pregnancies, increased fetal concentrations of sex hormone-binding globulin, and decreased levels of inflammatory cytokines. It can reduce the risk of endometrial cancer, type 2 diabetes mellitus, cardiovascular disease, dyslipidemia, and hypertension in women with PCOS.

Keywords

Insulin Resistance, AMPK, Ferroptosis, T2DM, IRI, HOMA-IR, Obesity, COVID-19, Androgenism

1. Introduction

The first insulin-sensitizing drug (ISD) was metformin, which was used to study the role of insulin resistance in PCOS pathophysiology [1], and owing to its advantages, which include enhanced menstruation, reduced hyperinsulinemia,

hyperandrogenism, and aberrant metabolism. Metformin may also prevent long-term cardiovascular disease [2]. Three biguanide derivatives, metformin, phenformin, and buformin, were developed because of the abundance of guanides in G. officinalis (French lilac) and the vital components that lower blood glucose levels. Metformin is the most beneficial medication because of its minimal toxicity [3]. Polycystic ovarian syndrome (PCOS) is the most common endocrine ailment affecting women of reproductive age [4] and is characterized by a polycystic ovary shape (abnormal ovarian follicles) [5] hyperandrogenism, and unpredictable ovulation. It is accompanied by metabolic problems, such as obesity and insulin resistance (IR). IR and compensatory hyperinsulinemia are found in 65% - 95% of women with PCOS [5]. PCOS can affect mental health and increase the risk of developing depression. Furthermore, there is a close relationship between PCOS and other mental disorders. PCOS patients are more susceptible to depression, drug-related and bipolar disorders, as well as eating disorders, such as bulimia, anorexia, or non-specific dietary disorders. These issues can significantly affect the quality of life of PCOS patients. In addition, chronic hyperandrogenemia (elevated androgen levels) in PCOS is associated with the development of hormone-dependent tumors, such as endometrial, breast, or ovarian neoplasms [6]. The diagnosis of PCOS over the past 30 years has improved owing to International Guidelines for the assessment and management of the condition. Polycystic ovaries, clinical and/or biochemical indications of hyperandrogenism, and oligo- or anovulation were included, according to the Rotterdam criteria of 2003. Based on these parameters, the prevalence of PCOS has increased [4]. The liver produces less sex hormone-binding globulin (SHBG) when the circulating insulin levels increase. Reduced SHBG levels result in increased circulating testosterone levels in the blood, causing hyperandrogenemia [7]. Androgenic symptoms such as acne, hirsutism, and female-pattern alopecia develop gradually. The Ferriman-Gallwey score can be used to assess hirsutism, but its clinical utility is limited because patients often undergo hair removal [8]. Research indicates that Several factors, including genetics, epigenetic changes, environmental factors, oxidative stress, chronic inflammation, mitochondrial dysfunction, and metabolic abnormalities, may contribute to PCOS, even though the precise etiology of the condition remains unknown [5]. The symptoms of PCOS patients differ between individuals, and not all symptoms appear the same in all patients; therefore, we cannot provide a specific prescription for every patient. Patients who are obese and overweight, adopt healthy eating habits, and engage in physical activity are recommended. Symptoms such as irregular menstrual cycle, hirsutism, and lack of ovulation. International guidelines include recommendations regarding treatment, the most important of which is life modification, such as proper diet, physical exercise, sleep, and stress reduction. If lifestyle modification is ineffective, hormonal therapy can be considered to treat these symptoms. Combined oral contraceptives and medical interventions have proven to be effective. However, it is not suitable for patients with metabolic syndrome or those with severe IR. Pharmaceuticals containing

estrogen-progestin contraceptive preparations are used to regulate the menstrual cycle. Metformin therapy is less effective than COCs; however, it can slightly and moderately improve menstrual regularity. The updated guidelines refer to an innovative approach that combines metformin with COCs in overweight and obese women, yielding satisfactory outcomes [6]. Insulin resistance contributes to PCOS, leading to speculation regarding the use of insulin-sensitizing agents such as metformin to treat these conditions. Early studies and small trials were promising, and metformin was adopted early. Anecdotal observations suggest that it can improve hyperandrogenic symptoms and promote weight loss [9]. To reduce the long-term consequences, it is crucial to acknowledge the impact of insulin sensitivity, accurately test insulin sensitivity, and implement efficient IR interventions [5]. There are now additional alternatives for patients and healthcare professionals to address the endocrine, metabolic, and reproductive problems associated with PCOS because of the safe and effective use of many oral insulin-sensitizing drugs including thiazolidinediones (TZDs), inositols, and berberine. Compared to injectable treatments, these oral insulin sensitizers are more affordable, practical, and convenient [2]. Unfortunately, these two agents have severe adverse effects such as gastrointestinal disturbances and liver injury [10]. Hence, the development of more effective and safer insulin sensitizers for PCOS-IR remains a challenge [10]. In this study, we examined polycystic ovary syndrome and its association with insulin resistance and mentioned that the insulin sensitizer metformin treats polycystic ovary syndrome and its accompanying symptoms. We also mentioned that metformin treats cancerous tumors that arise in patients with PCOS and T2DM. This study aimed to provide an overview and update the current status of research on insulin-sensitizing therapy and to investigate the impact of metformin on PCOS patients.

2. Materials and Methods

A thorough search of PubMed and Google Scholar databases for relevant literature was conducted until January 2024. The terms "PCOS" and "insulin" or "PCOS" and "tissues" or "PCOS" and "insulin" and "pathogenesis" or "PCOS" and "insulin" and "diagnosis" or "PCOS" and "insulin" and "evaluation" or "PCOS" and "insulin" and "therapy" or "METFORMIN" and "PCOS" or "BERBERINE" and "PCOS" and "GENETIC" and "PCOS" were among the keywords and subject terms included. Only research papers written in English were taken into account. Furthermore, articles that highlight the most recent ones (those published since 2014) omit those that lack complete manuscripts.

3. Theories of PCOS

Two theories explain why hyperandrogenism occurs: the altered gonadotropin secretion theory, which suggests that increased GnRH pulse frequency leads to excessive levels of LH and FSH; and the functional ovarian or adrenal hyperandrogenism theory, which suggests that hyperandrogenism originates from dysregulated

steroidogenesis. Hyperinsulinemia has multiple effects including elevated LH, decreased SHBG, increased conversion of androstenedione to testosterone, and reduced LH desensitization (Figure 1). The primary pathophysiological mechanisms underlying PCOS include hyperandrogenism and hyperinsulinemia. Their relationship has been outlined, with each factor reinforcing the other, although the precise mechanisms remain unclear. The two main theories proposed (Figure 1) are elevated gonadotropin-releasing hormone (GnRH) pulse frequency (Theory 1) and functional hyperandrogenism of the ovaries or adrenal glands (Theory 2). Gonadotropin-releasing hormone triggers the anterior pituitary to release follicle stimulating hormone (FSH) and luteinizing hormone (LH). LH stimulates theca cells in the ovary to create androgens, whereas FSH stimulates granulosa cells to produce estrogen. Estrogen inhibits FSH production and both androgens and estrogen promote LH production. The release of androgens from the ovaries can also play a functional role. Hyperandrogenism, along with FSH and LH activation, triggers follicle growth and degeneration, resulting in the typical presence of multiple follicles in ovaries on ultrasound. Excessive production of androgens by adrenal glands can also be stimulated by insulin. Both excess insulin in the blood and excess male hormones are believed to be exacerbated by fat accumulation in the body [8]. Various theories have been proposed regarding PCOS pathogenesis [7].

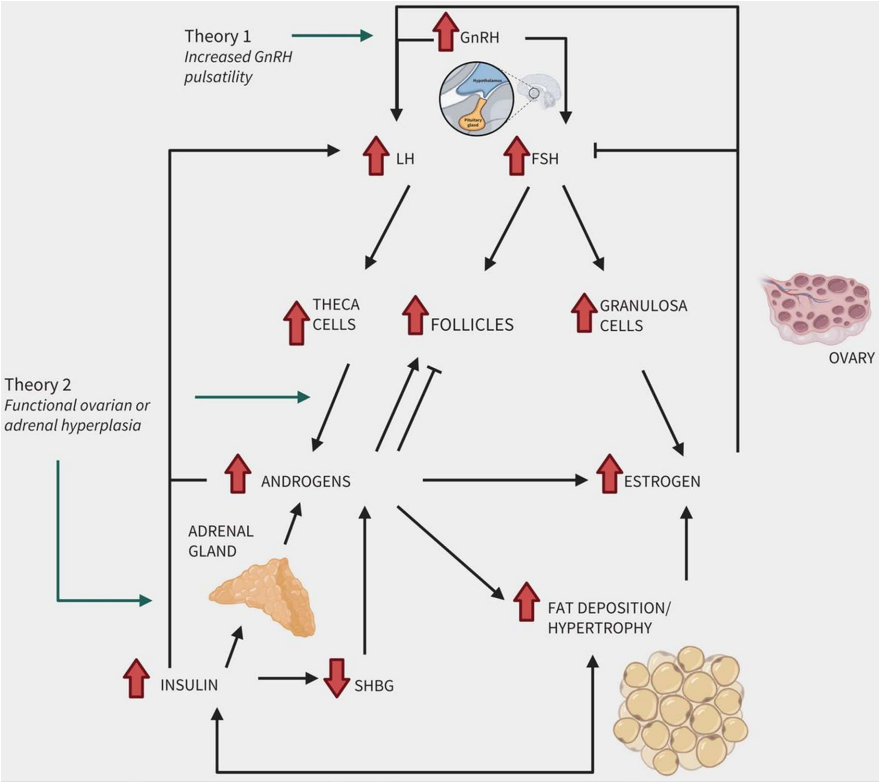

Figure 1. Polycystic ovarian syndrome (PCOS) has two main pathophysiological mechanisms: hyperandrogenism and hyperinsulinemia. The relationship between these two is not fully understood [8].

3.1. Pathogenesis of PCOS

There are no clear or specific reasons for the occurrence of PCOS. A combination of environmental and genetic factors is expected to be associated with this syndrome. During puberty, the hypothalamic-pituitary-ovarian axis matures, leading to increased LH levels in PCOS adolescents. Insulin plays a crucial role in androgen regulation, and resistance to insulin induces compensatory hyperinsulinemia, which drives phenotypic PCOS characteristics. The ovary remains sensitive to insulin activity and subsequent androgen activity in the pathophysiological association between hyperinsulinemia and hyperandrogenaemia [11].

Abnormalities in pituitary gonadotropin secretion, excessive stimulation of IGF-I receptors, excessive activity of 17a-hydroxylase, an enzyme that regulates the conversion of 17-hydroxyprogesterone into androstenedione, and decreased synthesis of insulin-like growth factor binding protein 1 (IGF-BP1) are some of the mechanisms that have been linked to the pathogenesis of insulin resistance in PCOS [12]. Insulin acts through its own receptor on the ovary and combines with gonadotropins to regulate the steroid production. A malfunctioning PI3K signaling cascade is linked to both IR and hyperandrogenism [10].

Oxidative stress is a basic sign of ferroptosis, a mechanism of cell death driven by iron-dependent lipid peroxidation that occurs in cells. The role of ferroptosis in PCOS development has received increasing attention as a novel finding in the pathogenesis of PCOS.

Studies have indicated that ferroptosis is elevated in PCOS patients and that oxidative stress and mitochondrial damage impact the development of PCOS and its related outcomes.

Numerous antioxidant enzymes, including catalase (CAT), exhibit decreased expression and ferroptosis is the outcome of peroxide accumulation. Studies have indicated that GPX4 plays a critical role in controlling iron death in mice and cells. According to their findings, ferroptosis increases and GPX4 expression decreases in the ovaries of mouse models [13].

Recently, genetic factors including CYP19, CYP17A1, HSD17B6, HSD17B5, and CYP21 have been linked to PCOS pathophysiology, affecting reproductive hormone biosynthesis, chronic inflammation, and cell metabolism [11].

3.2. Genetic Basis of PCOS

PCOS has a unique genetic basis that varies between families and within families. The susceptibility to different genes varies among patients belonging to the same family. Intrauterine programming has been hypothesized to be a risk factor for PCOS. Genome screening is unrealistic in complex diseases such as PCOS, and linkage analysis in such families often yields negative results. Case-control and genome-wide association studies (GWAS) are helpful for identifying possible associations [1].

There was a significant genetic association with PCOS (**Figure 2**). Genes such as CAPN10, cytochrome family p450, insulin, AR, FTO, and FSHR.

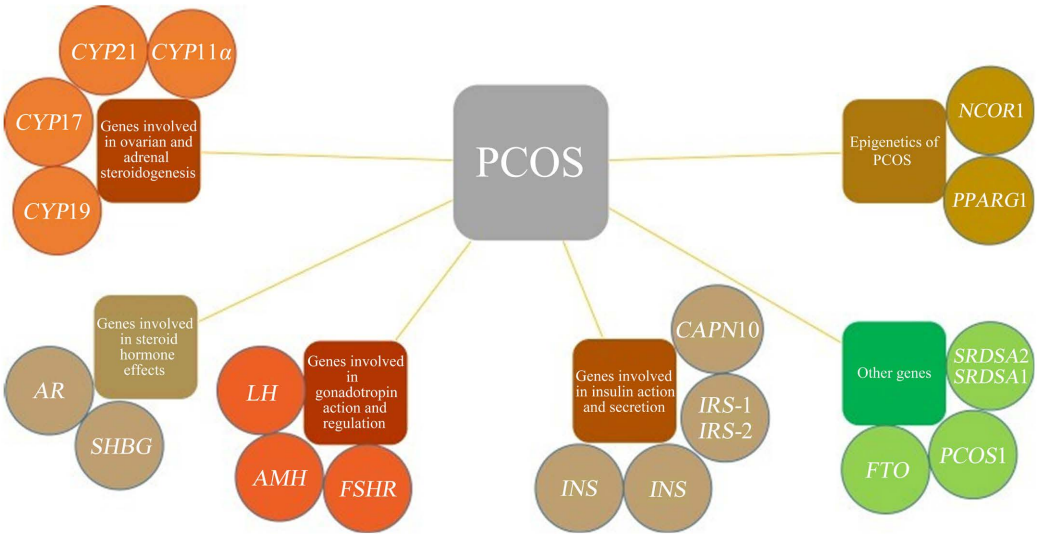

Figure 2. An overview of the genes implicated in PCOS demonstrates the disease's complexity [14].

All genes/mutations associated with PCOS can directly or indirectly affect the ovaries. We discussed the groups of related genes and their roles in PCOS.

Elevated androgen levels are the most common endocrine disorder associated with PCOS, and several genes involved in ovarian and adrenal steroidogenesis, as follows:

An intermediate stage in the conversion of cholesterol to progesterone requires an enzyme encoded by CYP11a. The conversion of 17-hydroxyprogesterone to 11-deoxycortisol in the synthesis of steroid hormones is catalyzed by an enzyme encoded by CYP21.

Enzyme (P450c17*α*) that is encoded by CYP17 catalyzes the conversion of pregnenolone and progesterone into 17-hydroxypregnenolone and 17-hydroxyprogesterone. The CYP19 gene produces aromatase p450, essential for the formation of estrogen.

The androgen receptor gene is involved in the genes that are involved in steroid hormone effects. Mutations and structural disruption of this gene can lead to PCOS.

Sex hormone-binding globulin gene synthesis protein of 373 amino acids controls the levels of sex hormones in the body by binding to androgen, often with estrogen and testosterone. Multiple metabolic factors, such as androgens and insulin, control the synthesis of SHBG.

3.2.1. Genes Responsible for Gonadotropin Action and Regulation

Abnormalities in the lutein hormone (LH) gene and its receptor are known to cause PCOS. High levels of LH promote androgen production. In contrast, elevated LH levels reduce follicle-stimulating hormone (FSH) levels, which can be indirectly responsible for excess testosterone in the ovaries due to the reduced transfer of androgen to estrogen. AMH encodes a protein involved in infertility. The follicle-stimulating hormone receptor (FSHR) gene encodes a protein that is

responsible for gonadal development. Hormonal imbalance may result from mutations in genes that disrupt structural proteins.

3.2.2. Insulin Action and Secretion-Related Genes

The insulin gene has a major impact on androgen synthesis, which occurs through receptors on theca cells. Similar to LH, high insulin levels promote testosterone production.

Research has been conducted to determine whether infertility and PCOS are associated with obesity in women. INSR encodes a turmeric protein.

Insulin and its receptor proteins bind to its receptor substrates. The binding of insulin autophosphorylates and activates the receptor. Arg972 IRS-1 was more prevalent in PCOS-afflicted women.

Calpain 10 gene. This gene encodes a calcium-dependent cysteine protease. This gene has been linked to type 1 diabetes mellitus. The "calpain10" protein obstructs the metabolism and release of insulin.

3.2.3. Other Genes

The Fat Mass Obesity (FTO) gene encodes an enzyme related to obesity and T2DM.PCOS1 gene has been associated with PCOS. SDR5A2 and SRD5A1 have been observed to have high activity in PCOS patients. Later, these genes were tested for susceptibility to PCOS in patients with hirsutism [14].

4. The Mechanism of Action of Metformin

Metformin and phenformin are derived from galegine, a natural product of the Galega officinalis plant, which is used in herbal medicines in medieval Europe. Galegine was tested as a glucose-lowering agent in humans in the 1920s but was found to be toxic. Two synthetic derivatives of galegine, metformin and phenformin, were first synthesized and tested but were not introduced for clinical use until the 1950s [15].

Metformin, a member of the Biguanide family, is a commonly used insulin-sensitizing medication for the treatment of PCOS and Type 2 Diabetes Mellitus (T2DM). Although it improves insulin sensitivity and glucose absorption in peripheral tissues, it has not yet been approved for use in PCOS. Bloating in the abdomen, diarrhea, vomiting, and nausea are typical side effects [16]. The benefits of metformin are likely to be due to various molecular mechanisms. Metformin has beneficial effects on metabolism and inflammation in the liver, kidneys, and intestines [15].

It functions by decreasing blood lipid levels, decreasing the production of glucose by the liver, increasing the amount of glucose absorbed by the skeletal muscles and liver through insulin, and decreasing the use of gluconeogenic substrates [17].

5. Uses of Metformin for PCOS

In 1994, Velazquez *et al.* assessed the effect of metformin administration in 26

obese PCOS patients to study the relationship between insulin resistance and the development of the syndrome. Furthermore, metformin was effective in promoting consistent menstrual and ovulatory cycles in these individuals.

An increasing number of gynecologists and endocrinologists are now prescribing metformin for patients with PCOS and AEPS. It has been proposed as a treatment to manage and prevent the progression to impaired glucose tolerance (IGT) in individuals with PCOS, while the American Association of Clinical Endocrinologists recommends metformin as a primary intervention for overweight and obese patients with this condition. However, metformin has not been approved for this treatment in patients with PCOS in either Europe or the United States. Similar to the Rotterdam conference, a group of experts from around the world was asked to discuss treatment options for patients with PCOS to reach an agreement on the best therapy. The workshop team determined that ISDs should not be the first option for inducing ovulation in women with PCOS, and should only be used in patients with IGT. This is likely because information on how it affects PCOS patients and how it should be administered is still not fully understood [18].

Metformin is the most common glucose-lowering agent; however, it has become the first-line medication for the treatment of type 2 diabetes mellitus in most guidelines over the past 60 years.

In 1949, an influenza epidemic broke out in the Philippines, where metformin was used to treat this disease. It was found to lower blood glucose levels in some patients.

Long-term safety and effectiveness data, low risk of hypoglycemia, cardiovascular and mortality benefits, additive or synergistic effects in combination therapy, affordability, and accessibility are all advantages.

COVID-19 increases the risk of mortality due to low-grade inflammation. Despite increased lactic acidosis, metformin could have protective and therapeutic effects owing to its anti-aging, antiviral, and anti-inflammatory properties. However, the lack of clinical benefit does not support early treatment. Metformin can reduce the incidence of long-term COVID.

Elevated expression and circulation levels of Growth differentiation factor 15 (GDF15) have become novel biomarkers for metformin usage, which is associated with weight loss and reduced appetite. The gastrointestinal tract releases GDF15 in response to metformin [19].

5.1. Molecular Mechanisms for Metformin-Associated AMPK Activation and Its Role in PCOS

Metformin has diverse effects by activation of AMPK on various tissues and organs by different mechanisms (**Figure 3**), including anti-inflammatory effects, increased energy metabolism, protection against glucotoxicity-induced pancreatic β-cell dysfunction, reduction in macrovascular events and diabetes-related mortality, protection against cardiac ischemia-reperfusion injury, and modulation of microbial diversity [20].

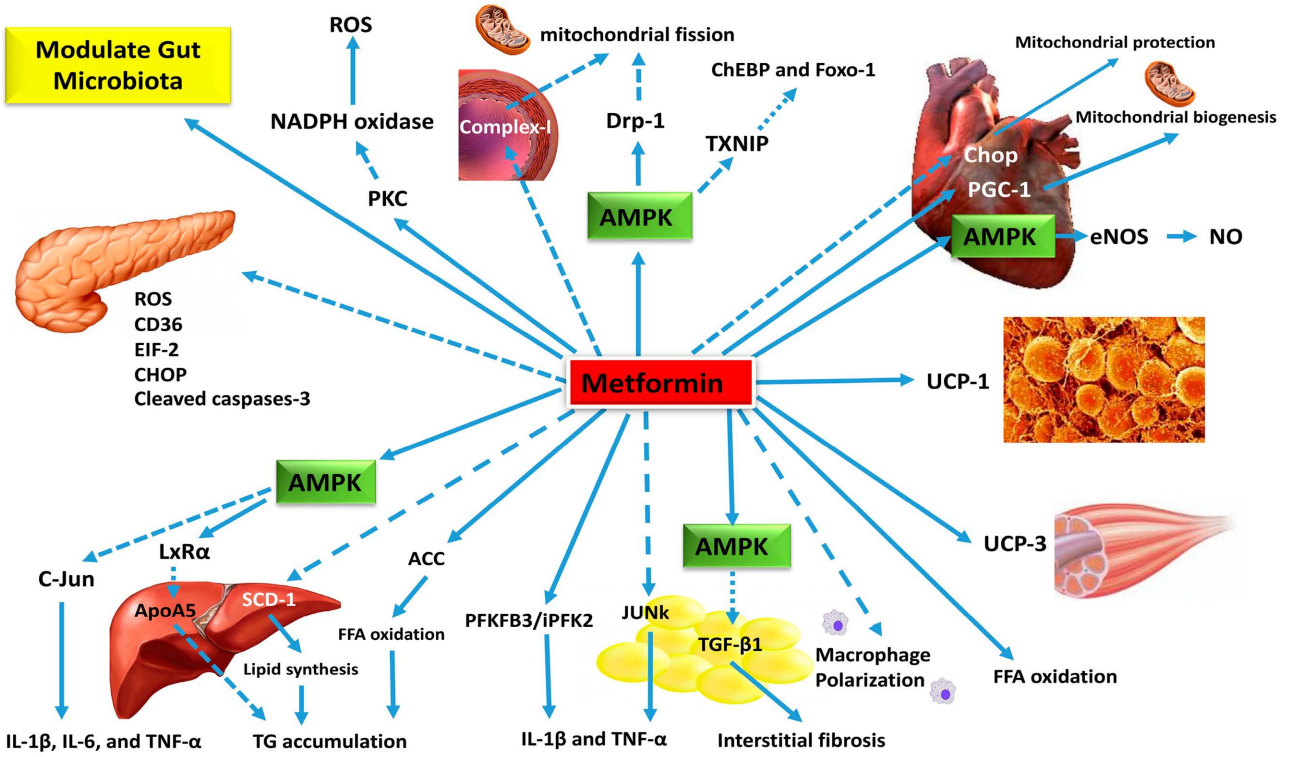

Figure 3. Possible biochemical pathways by which metformin acts on pancreatic β-cell dysfunction, atherosclerosis, oxidative stress, and NAFLD (solid arrows represent activation, dotted arrows represent inhibition [20].

The mechanism of action of metformin in AMPK is still not fully understood [13]. METFORMIN activates the cellular energy sensor AMP-activated protein kinase (AMPK) by inhibiting mitochondrial function that prevents ATP production and restores energy homeostasis by switching on catabolic pathways that generate ATP and cellular processes that consume ATP. Metformin uptake by hepatocytes increased cellular AMP: ATP ratios, consistent with the inhibition of the respiratory chain.

Metformin also inhibits fructose-1,6-bisphosphatase, reducing hepatic lipid stores by directly phosphorylating two isoforms of acetyl-CoA carboxylase (ACC1/ACC2) at comparable serine residues, and enhancing insulin sensitivity. Glucagon-induced increase in cAMP activates cAMP-dependent protein kinase A, causing a switch from glycolysis to gluconeogenesis. Metformin also inhibits the glycerophosphate shuttle, a key component of the shuttle that carries reducing equivalents from the cytoplasm into the mitochondrion for re-oxidation.

Several studies have been conducted on mouse hepatocytes and transgenic mice, suggesting a role for metformin in reducing hepatic gluconeogenesis and/or insulin sensitivity. Metformin improves blood glucose levels by suppressing hepatic glucose production and light effects on peripheral insulin-mediated glucose uptake.

The first pharmacological agent of AMPK was 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), which is phosphorylated to the nucleotide 5-

amino-4-imidazolecarboxamide riboside 5'-monophosphate (ZMP). The long-term insulin-sensitizing effects of metformin appear to be mediated by AMPK [15].

In a recent study, the expression of SIRT3 was restored after treatment with metformin. AMPK phosphorylation of AMPK is regulated by SIRT3. Additionally, activation of AMPK by metformin in rat granulosa cells can reduce steroid production [13].

Physiologically, the effect of metformin is initiated when Met interacts with mitochondria in hepatocytes and inhibits complex I of the respiratory chain, leading to the activation of AMPK, which suppresses ATP production and decreases the production of hepatocyte glucose. This may result in the reduction of blood glucose, enhancing insulin sensitivity, reducing fat synthesis, and activating fat oxidation. In theca cells, metformin significantly reduced androstenedione and testosterone production and reduced hyperandrogenism by its effect on both the ovaries and adrenal gland, suppressing the production of androgen, reducing pituitary LH, and increasing SHBG in the liver. Thus, it improves the appearance of symptoms, such as acne and hirsutism. This induces ovulation in PCOS patients. AMPK activation plays a key role in regulating and enhancing symptoms and management [1].

5.2. In Tumor

Metformin has been shown to have a significant impact on tumor growth, with a 30% reduction in the overall tumor onset in T2DM patients. *In vitro* studies have shown that metformin inhibits tumor cell growth via various mechanisms, including AMPK. LKB1, a key AMPK pathway, is a tumor suppressor required for maximal activation. Metformin induces cytoplasmic translocation of LKB1 and enhances AMPK activation. AMPK targets key tumor-promoting signaling pathways, such as mTOR, which regulates protein translation and cell cycle progression. Metformin activation of the LKB1/AMPK pathway is a useful strategy for tumor treatment [3]. As AMPK and LKB1 insufficiency after therapy lowers serum glucose levels, metformin also has AMPK-independent effects on glucose metabolism. The main effect of metformin is metabolic balance; however, research is currently being conducted to determine whether metformin can be used to treat other cancers.

Epidemiological and clinical evidence has shown that metformin prevents various malignancies; however, its underlying molecular mechanisms remain unclear. Metformin treatment of endometrial cancer cells results in constitutively active K-Ras displacement from the cell membrane and subsequent dissociation of the mitogen-activated protein kinase (MAPK) signaling pathway. Metformin also inhibits endogenous reactive oxygen species and directly scavenges free radicals, which have antiangiogenic properties. Studies have also been conducted on the possible therapeutic and preventive effects of metformin on breast cancer. A retrospective analysis involving 2529 diabetes patients revealed that metformin-

treated diabetic individuals with breast cancer showed greater rates of full response to neoadjuvant chemotherapy than non-metformin treatment. Metformin inhibits the growth of many cancer cells, including breast cancer cells, in cell culture. It also controls epithelial-to-mesenchymal transition (EMT), which is the first step in the ontogeny of breast cancer cells. Overall, metformin may help prevent breast cancer because of its effects on the PI3K/Akt/mTOR signaling pathway, biological cycle, and detrimental effects of insulin on the growth and development of tumors [21].

5.3. In Obesity

Metformin is an oral antihyperglycemic drug. It can decrease androgen synthesis from ovarian theca cells and repress ovarian steroidogenesis, in addition to lowering body weight and re-establishing ovulation. AMP-activated kinase (AMPK), an energy sensor that tracks the levels of cellular AMP and ATP, is activated as part of its mode of action. Longevity factors that reduce inflammatory responses, boost stress tolerance, and lengthen lifespan are targets of AMPK. These factors include SIRT1, p53, and FoxOs. AMPK activation also protects against arachidonic acid + iron-induced OS through mitochondrial impairment and ROS generation.

Gastrointestinal issues, which can be uncomfortable and result in the discontinuation of medication, have been reported in up to 30% of patients. It would be helpful to find substitute tactics that have effects on PCOS, similar to those of metformin. The AMPK-SIRT1 pathway may assist in controlling these negative events. Resveratrol, an antioxidant and anti-inflammatory drug, has positive effects on ovaries [22].

Akt and AS160's simultaneous phosphorylation and deactivation. In a recent study, women with PCOS and control participants showed comparable increases in insulin-stimulated phosphorylation of Akt and PAS in the skeletal muscle. Furthermore, there were no differences in skeletal muscle protein expression levels of the insulin receptor, GLUT4, and HKII. The absence of inhibition of the insulin signaling cascade is consistent with other studies that found that impaired insulin signaling could not account for the lower insulin sensitivity caused by lipid infusion. Contrary to earlier findings in PCOS-affected women, our data indicate that insulin-stimulated activation of IRS-1 ser312 and IRS-1-associated phosphatidylinositol-3-kinase and Akt at Thr308 and Ser473 was reduced. Notably, obese women were found to exhibit this, which could account for inconsistent results. Numerous genes linked to hepatic lipid metabolism, such as CD36 and other proteins involved in lipid handling, trafficking, and lipolysis, have been shown to be regulated by the transcription factor PPARa. Recent studies on blastocysts have demonstrated that AMPK is required for PPARa transcription, which is consistent with the finding in the mouse liver that AMPK activity and PPARa transcription are both activated by adiponectin. In the current study, we found that women with PCOS had low expression and phosphorylation of AMPK, and reduced expression of many proteins involved in FA transport, FA trafficking, and lipolysis (CD36,

FATP1, DGAT1, CGI-58, and HSL). These data suggest that androgens decrease adiponectin levels, which in turn lowers AMPK protein content and activity as well as PPARa transcription of key proteins involved in lipid turnover [23].

5.4. HOMA-IR and IRI

A previous study discovered a limited correlation between the Belfiore index, which is based on the measurement of glucose and insulin concentrations during the OGTT, and the HOMA index, which is based on fasting glucose and insulin levels. The Matsuda and insulin sensitivity index (ISI) showed weak associations. Depending on the approach used, the assessment of insulin levels using HOMA-IR index and IRI produced dramatically different results. For instance, only 26 (38.2%) of the 68 patients with elevated IRI showed increased HOMA-IR at a HOMA-IR cutoff point of 3.46. This difference was far more pronounced when the 3.8 HOMA-IR cut-off value was used. The opposite situation, that is, high HOMA-IR and "normal" IRI, was very uncommon and applied to only five (7.25%) and three (4.3%) subjects, for HOMA-IR cut-offs of 3.46 and 3.8, respectively.

The lack of a strong correlation between the HOMA-IR and IRI methods was expected, as these indices mainly represent hepatic insulin sensitivity rather than peripheral insulin sensitivity. Some studies have raised questions about the presumed strong link between data gathered from these indices and data obtained from the euglycemic clamp technique for both fasting glucose and insulin models, as well as methods utilizing glucose and insulin during the OGTT.

Nevertheless, research on the role of metformin in treating PCOS has mostly focused on how patients' problems with lipid and glucose metabolism improve [12].

5.5. Obesity Resulting from PCOS

According to clinical and epidemiological data, approximately 50% of patients with PCOS are overweight or obese. Overweight PCOS patients experience more severe endocrine and metabolic disorders than non-overweight patients do. According to previous studies, being overweight increases insulin secretion but decreases its metabolism in fat, skeletal muscle, and the liver. Being overweight may also result in lipodystrophy and insulin resistance by lowering the expression of lipid droplet proteins in adipocytes, which can impair their responsiveness to insulin [17].

Obesity occasionally exacerbates the association between PCOS and diabetes. However, women with PCOS are more likely to develop prediabetes or type 2 diabetes mellitus. It is imperative to avoid type 2 diabetes, and metformin has been shown to lower the risk in women who are at a high risk [16].

5.6. Effect of Metformin in Reproduction Characteristics

Pregnancy complications are more likely to occur in PCOS patients. Obesity, IR, hyperandrogenism, and other metabolic alterations may increase the risk of complications during pregnancy and at the newborn stage. Adverse health outcomes

predispose infants born to women with PCOS to adverse health outcomes. Growing data suggest that children of PCOS-affected mothers are more likely to experience preterm birth, perinatal death, congenital defects, and higher hospitalization rates [24]. The prevalence of subclinical hypothyroidism (SCH) in non-pregnant women with PCOS was 16.9% in a cross-sectional study by Novais *et al.* and 6.2% in a non-PCOS group. Additionally, compared to controls, non-pregnant women with PCOS have been reported to have a higher prevalence of autoimmune thyroiditis, and pregnant women with PCOS are more likely to experience complications, such as low birth weight, caesarean delivery, premature birth, gestational diabetes mellitus, and neonatal intensive care unit admission. Similar to overt hypothyroidism (OH), borderline thyroid dysfunction during pregnancy has also been linked to adverse outcomes in newborns, miscarriages, preterm deliveries, low birth weight, preeclampsia (PE), and gestational diabetes mellitus. Miscarriage and preterm delivery are associated with the prevalence of thyroid peroxidase antibodies (TPO-Abs). The potential impact of metformin on thyroid hormone status in pregnant women remains unclear [25].

A previous study showed that the multiple pregnancy rate in the PCOS-IR group was significantly higher than that in the control group, and decreased after metformin treatment. PCOS-IR rats had a lower conception rate, which was improved by the medication. Pregnancy-related metformin use increases the fetal concentration of sex hormone-binding globulin and decreases the *in vitro* release of inflammatory cytokines by trophoblasts. In this study, metformin use was associated with a low rate of multiple pregnancies. According to a Cochrane review, women with PCOS taking metformin had higher rates of ovulation and pregnancy. According to a recent study, metformin treatment from the late first trimester until delivery may lower the risk of late miscarriage and preterm birth in pregnant women with PCOS, with no discernible difference in the incidence of serious adverse events between the mothers and their offspring. There were two instances of placental abruption in the PCOS-IR rats used in this study. According to Palomba *et al.*, PCOS patients have a two-fold increased risk of preterm delivery, a three- to four-fold increase in gestational diabetes, and a three- to four-fold increase in pregnancy-induced hypertension and preeclampsia [24].

A new discovery in a recent investigation was the reduced reduction in fT4 caused by metformin during pregnancy compared to placebo. Haddow *et al.* (2016) previously proposed that in obesity and insulin resistance conditions, low fT4 levels could act as a surrogate marker for elevated peripheral deiodinase activity. T4 to T3 conversion increases as a result, lowering fT4 levels and increasing T3/T4 ratio. Given that women with PCOS should also have elevated peripheral deiodinase activity, we hypothesized that metformin may suppress this, leading to higher fT4 levels in the metformin group than in the placebo group [25].

5.7. Metformin Treating the Complications of PCOS

Reducing insulin resistance and hyperinsulinemia should be the main goal of

treatment efforts because these characteristics are thought to be crucial in the etiology of PCOS. In addition to being a reproductive condition, PCOS is a chronic illness that can lead to long-term problems and adverse outcomes such as metabolic syndrome, CVD, endometrial cancer, hypertension, and type 2 diabetes. The disparity between the relatively low incidence of endometrial cancer and high prevalence of PCOS makes it challenging to establish a causal correlation between the two.

Metformin has been shown to reduce the risk of endometrial cancer; however, it is difficult to justify its prophylactic use in patients with PCOS without firm evidence of its efficacy and cost implications. Type 2 diabetes mellitus is also associated with an increased risk among patients with PCOS; however, the presence of obesity confounds any presumed link between PCOS and T2DM. There is reliable evidence that metformin can reduce the risk of T2DM among high-risk general populations, but this should be considered carefully and on an individual basis, given the current evidence or lack thereof.

Hypertension, dyslipidemia, and cardiovascular diseases are also associated with PCOS. Two notable anomalies that are powerful indicators of cardiovascular disease (CVD) and myocardial infarction are elevated triglyceride and low HDL-C levels. Theoretically, metformin may have a direct effect on dyslipidemia by affecting fatty acid metabolism in the liver or may have an indirect effect by reducing hyperinsulinemia. According to several studies, metformin appears to help women with PCOS and dyslipidemia, although its effectiveness in a polymorphic population such as PCOS is still unknown.

Hypertension in PCOS is a controversial issue, with a 2.5-fold increase in the risk of developing it in menopausal women. Therefore, the use of metformin to prevent long-term disease risk in PCOS patients should be considered carefully and on an individual basis, considering the current evidence or lack thereof [1].

6. Side Effect of Metformin

Participants prescribed metformin often experienced nausea, vomiting, and stomach pain, particularly during the initial 1 - 2 weeks of treatment, with a prevalence of 8% - 21%. Multiple studies have indicated that starting treatment with small doses and consuming food can help reduce gastrointestinal (GI) side effects. Additionally, utilizing controlled and gradual release formulations in treatment could help to reduce GI discomfort. Additionally, metformin can enhance the secretion of GLP1, a hormone that can decelerate digestion and suppress hunger (anorectic effect) in the intestine. Commonly reported side effects include headaches, dizziness, fatigue, itching, and altered taste perception. A rare but feared side effect of metformin treatment is lactic acidosis, which occurs in 1 in 30,000 patients and potentially results in death. In most instances, the reported occurrence occurred in individuals who were administered large amounts of medication and/or had significant liver and kidney damage, advanced age, or alcohol addiction. The likelihood of ADRs is higher in patients undergoing polytherapy

because of interactions between drugs. Long-term metformin use is associated with lower vitamin B12 levels and higher levels of homocysteine and methylmalonic acid, possibly resulting in anemia and diabetic peripheral neuropathy. An RCT was conducted on patients with DM2 to investigate the impact of long-term metformin use. Vitamin B12 levels were notably reduced in the metformin group compared to the untreated group (231 vs. 486 pmol/L; $p < 0.001$), with 18 patients (31%) having a severe deficiency, in contrast to only two subjects (3%) in the control group. The metformin dosage is linked to serum vitamin levels and more severe peripheral neuropathy. This information emphasizes the significance of regularly checking vitamin B12 levels in individuals undergoing long-term metformin treatment, and in those with a higher risk of deficiency, such as vegans, owing to their dietary preferences. A recent clinical trial in Chinese individuals with T2DM confirmed a link between long-term metformin use and peripheral neuropathy, which is dependent on the dosage. Moreover, vitamin B12 deficiency has been linked to the deterioration of several other CNS illnesses, specifically Parkinson's, which may explain the potential connection between metformin therapy and the exacerbation of Parkinson's [26].

7. Other Insulin-Sensitizer

In 2009, an international consensus group recommended limiting metformin use to patients with glucose intolerance, based on a large American multicenter trial that showed clear benefits of clomiphene over metformin [9].

Recent studies have suggested that myo-inositol, a novel insulin-sensitizing agent in PCOS, can improve ovulation and fertility. It supports glucose entry into the cells and enhances oocyte and follicle maturation. Combined with metformin, it may improve ovulation in insulin-resistant PCOS, thereby reducing financial burden and physical risk in women. This approach could potentially improve the efficacy of oral ovulation induction despite the traditional use of metformin alone [27].

8. Conclusions

In conclusion, this study demonstrated that the assessment of insulin resistance in women with PCOS is highly method-dependent, and there is no agreement on the cut-off points that should be used as surrogate measures of insulin resistance. Some clinicians use surrogate measures of insulin resistance to determine the indications for metformin treatment as the current status of metformin treatment in PCOS remains debatable.

More RCTs with rigorous research designs are needed to determine the efficacy of metformin in treating PCOS patients, evaluate risk factors in overweight women, and apply metformin in interventions for non-overweight PCOS patients to prevent or treat PCOS and its complications.

Conflicts of Interest

The authors have declared that no competing interests exist.

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Abbreviation

ISD: insulin-sensitizing drug
PCOS: polycystic ovary syndrome
IR: insulin resistance
SHBG: sex hormone-binding globulin
COCs: combined-oral contraceptives
TZDs: thiazolidinediones
PI3K: Phosphinositide 3-kinases
IGF-1: insulin-like growth factor 1
IGF-BP1: insulin-like growth factor 1 binding protein
CAT: catalase
AMH: Anti-mullerian hormone
IGT: impaired glucose tolerance
IRI: insulin resistance index
HOMA-IR: Homeostatic Assessment for Insulin Resistance
AMPK: AMP-activated protein kinase
T2DM: Type 2 Diabetes Mellitus
NIH: National Institutes of Health
MET: Metformin
FSH: Follicle Stimulating Hormone
LH: Luteinizing Hormone
GnRH: Gonadotropin Releasing Hormone
GPX4: Glutathione Peroxidase 4-gene
GWAS: Genome-Wide association study
AR: Androgen Receptor
FSHR: Follicle Stimulating Hormone Receptor
DHEAS: dehydroepiandrosterone sulfate
CVD: cardiovascular disease
SIRT1: sirtuin 1
NAFLD: nonalcoholic fatty liver disease
IGT: Impaired glucose tolerance
ATP: Adenosine triphosphate
ADP: Adenosine Diphosphate
AMP: Adenosine Monophosphate
ZMP: zero moment point
RCTs: randomized control trial
CNS: central nervous system