ISSN 1948-9358 (online)

World Journal of *Diabetes*

World J Diabetes 2021 May 15; 12(5): 514-684

Published by Baishideng Publishing Group Inc

World Journal of Diabetes

Contents

Monthly Volume 12 Number 5 May 15, 2021

OPINION REVIEW

REVIEW

MINIREVIEWS

World Journal of Diabetes

Monthly Volume 12 Number 5 May 15, 2021

Contents

World Journal of Diabetes

Monthly Volume 12 Number 5 May 15, 2021

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Diabetes*, Fernando Cordido, MD, PhD, Full Professor, Department of Medicine, University A Coruña, A Coruña 15006, Spain. Cordido.Carballido@sergas.es

AIMS AND SCOPE

The primary aim of *World Journal of Diabetes* (*WJD*, *World J Diabetes*) is to provide scholars and readers from various fields of diabetes with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJD mainly publishes articles reporting research results and findings obtained in the field of diabetes and covering a wide range of topics including risk factors for diabetes, diabetes complications, experimental diabetes mellitus, type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes, diabetic angiopathies, diabetic cardiomyopathies, diabetic coma, diabetic ketoacidosis, diabetic nephropathies, diabetic neuropathies, Donohue syndrome, fetal macrosomia, and prediabetic state.

INDEXING/ABSTRACTING

The *WJD* is now abstracted and indexed in Science Citation Index Expanded (SCIE, also known as SciSearch®), Current Contents/Clinical Medicine, Journal Citation Reports/Science Edition, PubMed, and PubMed Central. The 2020 Edition of Journal Citation Reports® cites the 2019 impact factor (IF) for *WJD* as 3.247; IF without journal self cites: 3.222; Ranking: 70 among 143 journals in endocrinology and metabolism; and Quartile category: Q2.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Yu-Jie Ma*; **Production Department Director:** *Xiang Li*; **Editorial Office Director:** *Jia-Ping Yan.*

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
June 15, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Timothy Koch

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-9358/editorialboard.htm

PUBLICATION DATE
May 15, 2021

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 May 15; 12(5): 616-629

DOI: 10.4239/wjd.v12.i5.616

ISSN 1948-9358 (online)

MINIREVIEWS

Role of insulin and insulin resistance in androgen excess disorders

Kursad Unluhizarci, Zuleyha Karaca, Fahrettin Kelestimur

ORCID number: Kursad Unluhizarci 0000-0003-2024-7433; Zuleyha Karaca 0000-0003-3241-2352; Fahrettin Kelestimur 0000-0002-2861-4683.

Author contributions: Unluhizarci K performed the majority of the writing; Karaca Z contributed to the writing of the manuscript and the figures; Kelestimur F approved the final version of the manuscript to be published.

Conflict-of-interest statement: The authors declare that there are no conflicts of interest to disclose.

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Kursad Unluhizarci, Zuleyha Karaca, Department of Endocrinology, Erciyes University Medical School, Kayseri, 38039, Turkey

Fahrettin Kelestimur, Department of Endocrinology, Yeditepe University Medical School, Istanbul, 34755, Turkey

Corresponding author: Kursad Unluhizarci, MD, Professor, Department of Endocrinology, Erciyes University Medical School, Kosk Mahallesi, Turhan Feyzioglu Caddesi, No. 42, Melikgazi, Kayseri 38039, Turkey. kursad@erciyes.edu.tr

Abstract

Insulin has complex effects on cell growth, metabolism and differentiation, and these effects are mediated by a cell-surface bound receptor and eventually a cascade of intracellular signaling events. Among the several metabolic and growth-promoting effects of insulin, insulin resistance is defined as an attenuated effect of insulin on glucose metabolism, primarily the limited export of blood glucose into skeletal muscle and adipose tissue. On the other hand, not all the signaling pathways and insulin-responsive tissues are equally affected, and some effects other than the metabolic actions of insulin are overexpressed. Ovaries and the adrenal glands are two examples of tissues remaining sensitive to insulin actions where insulin may contribute to increased androgen secretion. Polycystic ovary syndrome (PCOS) is the most common form of androgen excess disorder (AED), and its pathogenesis is closely associated with insulin resistance. Patients with idiopathic hirsutism also exhibit insulin resistance, albeit lower than patients with PCOS. Although it is not as evident as in PCOS, patients with congenital adrenal hyperplasia may have insulin resistance, which may be further exacerbated with glucocorticoid overtreatment and obesity. Among patients with severe insulin resistance syndromes, irrespective of the type of disease, hyperinsulinemia promotes ovarian androgen synthesis independently of gonadotropins. It is highly debated in whom and how insulin resistance should be diagnosed and treated among patients with AEDs, including PCOS. It is not suitable to administer an insulin sensitizer relying on only some mathematical models used for estimating insulin resistance. Instead, the treatment decision should be based on the constellation of the signs, symptoms and presence of obesity; acanthosis nigricans; and some laboratory abnormalities such as impaired glucose tolerance and impaired fasting glucose.

Key Words: Insulin; Insulin resistance; Hyperinsulinemia; Hyperandrogenism; Androgen excess

Country/Territory of origin: Turkey

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 26, 2021
Peer-review started: January 26, 2021
First decision: March 1, 2021
Revised: March 13, 2021
Accepted: April 26, 2021
Article in press: April 26, 2021
Published online: May 15, 2021

P-Reviewer: Ding W, Kukongviriyapan V
S-Editor: Liu M
L-Editor: A
P-Editor: Wang LL

Core Tip: In patients with insulin resistance, not all signaling pathways and insulin-responsive tissues are equally affected, and some effects other than the metabolic actions of insulin are overexpressed. Ovaries and the adrenal glands are two examples of tissues remaining sensitive to insulin actions where insulin may contribute to increased androgen secretion leading to androgen excess disorders. Therefore, the role and contribution of hyperinsulinemia triggered by (selective) insulin resistance has paramount importance for elucidating the pathogenesis of these disorders and establishing the right patient for insulin sensitizer therapy.

Citation: Unluhizarci K, Karaca Z, Kelestimur F. Role of insulin and insulin resistance in androgen excess disorders. *World J Diabetes* 2021; 12(5): 616-629
URL: https://www.wjgnet.com/1948-9358/full/v12/i5/616.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i5.616

INTRODUCTION

Androgen excess disorders (AEDs) affect approximately 10% of childbearing women. Most of these patients suffer from hirsutism. Various pathogenetic factors play a role in the evolution of these disorders. Most of these disorders are associated with metabolic abnormalities during the course of the disease. Insulin resistance and hyperinsulinemia are the main contributors to metabolic derangements and are players in the pathogenesis of some of these disorders. In this review, we present the relationship among insulin resistance, hyperinsulinemia and AEDs.

INSULIN AND INSULIN SIGNALING

Insulin is an anabolic hormone and is secreted by the beta cells of the pancreas. Although it has a large number of cellular responses/effects, maintaining glucose homeostasis is considered the main physiological function of insulin. Insulin exerts its effect on muscle cells to promote glucose uptake and protein synthesis; in adipose tissue, insulin promotes fatty acid and glucose uptake and inhibits lipolysis; and in the liver, insulin suppresses glucose production. Insulin has complex effects on cell growth, metabolism and differentiation, which and these effects are mediated by a cell-surface bound receptor and eventually a cascade of intracellular signaling events[1,2] (Figure 1).

The insulin signaling cascade is composed of reversible enzymatic reactions. The cornerstone of insulin signaling is the sequential phosphorylation of downstream targets. Following insulin binding, insulin receptor tyrosine kinase is activated, leading to tyrosine phosphorylation of the insulin receptor. The autophosphorylation of the insulin receptor leads to tyrosine phosphorylation of insulin receptor substrate (IRS) proteins and Src homology 2 domain-containing transforming proteins (SHCs)[3]. There are four isoforms of IRS; however, isoforms 1 and 2 are the main isoforms involved in metabolic actions[4]. In addition to the differences in their functions, these isoforms show different tissue distributions, thus leading to pleiotropic actions of insulin. IRS proteins are adaptor proteins that convert the tyrosine phosphorylation signal into a lipid kinase signal *via* the catalytic subunit of the enzyme phosphatidylinositol-3-kinase (PI3K). During the insulin signaling cascade, tyrosine phosphorylation is activated, while serine/threonine phosphorylation inactivates insulin receptor and IRS proteins[5]. In general, although the effects are site-dependent, the major mechanism of the termination of insulin receptor signaling is serine/threonine phosphorylation.

The phosphorylation of IRS leads to the binding of PI3K and the synthesis of phosphatidylinositol-triphosphate. These intracellular cascades lead to the phosphorylation and activation of serine/threonine-specific protein kinase B (AKT). There are three isoforms of AKT, and AKT2 is the most important isoform for glucose homeostasis[6]. Several substances interact, and *via* the PI3K/AKT pathway, anabolic

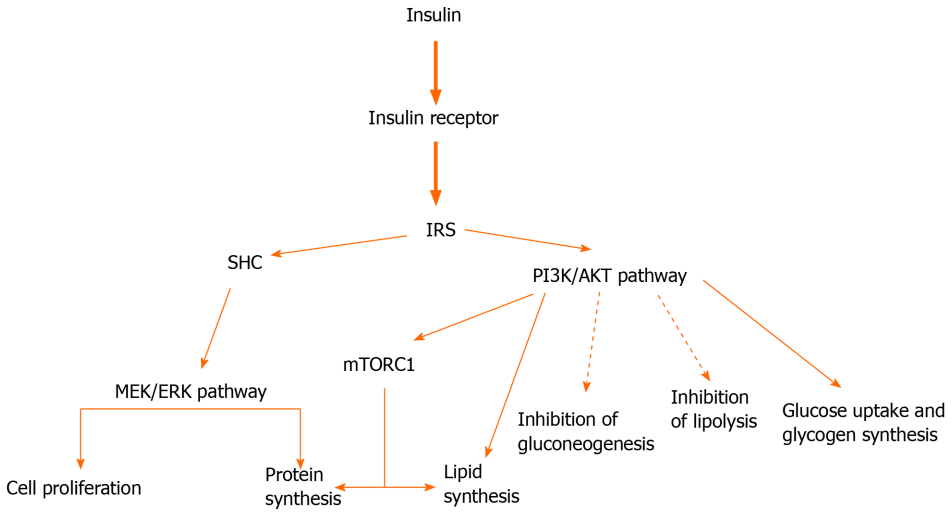

Figure 1 A brief scheme of the insulin signaling pathway under physiological conditions. After insulin binds to its own receptor, several pathways are activated/inactivated, resulting in an anabolic state of insulin. The autophosphorylation of insulin receptor tyrosine kinase is followed by tyrosine phosphorylation of insulin receptor substrate. The phosphatidylinositol-3-kinase/serine/threonine-specific protein kinase B (AKT) signaling pathway promotes glucose uptake and glycogen and lipid synthesis while inhibiting hepatic gluconeogenesis and lipolysis. Moreover, AKT kinases activate mechanistic target of rapamycin complex 1, which promotes de novo synthesis of proteins and lipids. An additional insulin signaling pathway *via* Src homology 2 domain-containing transforming proteins and the mitogen-activated protein kinase/extracellular signal-related kinase pathway promotes cell proliferation and protein synthesis. Dotted lines represent inhibition, and solid lines represent stimulation/activation. IRS: Insulin receptor substrate; SHC: Src homology 2 domain-containing transforming proteins; MEK: Mitogen-activated protein kinase; ERK: Extracellular signal-related kinase; PI3K: Phosphatidylinositol-3-kinase; AKT: Serine/threonine-specific protein kinase B; mTORC: Mechanistic target of rapamycin complex.

effects of insulin, such as glycogen synthesis, glucose uptake and de novo lipid synthesis, occur. This pathway also induces protein synthesis and de novo lipogenesis, which is mediated by mechanistic target of rapamycin complex 1 (mTORC1)[3].

Another insulin receptor-activated pathway is mitogen-activated protein kinase (MEK)-extracellular signal regulated kinase (ERK), which is triggered by the phosphorylation of SHC. Under physiological conditions, the activation of this pathway induces cell proliferation and protein synthesis[3,7]. There are dozens of proteins that are phosphorylated in response to insulin, and it has been shown that tyrosine phosphorylation of insulin receptor and IRS occurs within a minute upon insulin secretion/treatment[8-10]. Other downstream events occur within up to 45 min. Therefore, the occurrence of insulin receptor signaling at different times and specific patterns of phosphorylation may underlie the various responses of each insulin signaling pathway. For recent and detailed reviews on insulin signaling in normal and insulin-resistant individuals, we refer the reader to references 3 and 5.

INSULIN RESISTANCE AND HYPERINSULINEMIA

Among the several metabolic and growth-promoting effects of insulin, insulin resistance is defined as an attenuated effect of insulin on glucose metabolism, primarily the limited export of blood glucose into skeletal muscle and adipose tissue. Beta cells secrete much more insulin to compensate for and overcome insulin resistance. The resultant hyperinsulinemia is the hallmark of insulin resistance, at least at the beginning of the disease. Under physiological conditions, transient elevations in insulin concentrations are adaptive responses to environmental factors such as dietary stimuli[11]. In the case of prolonged hyperinsulinemia, there is less insulin receptor expression on the plasma membrane, which is one of the primary mechanisms of insulin resistance. However, this is not the sole mechanism of insulin resistance, and glucose homeostasis is also maintained by decreased insulin signaling *via* the PI3/AKT pathway for glucose transport from the circulation into tissues. Thus, at early stages, insulin resistance is considered a part of the defense mechanism to avoid hypoglycemia[12]. In the presence of prolonged/chronic insulin resistance and hyperinsulinemia, not all the abovementioned signaling pathways are equally affected, and relatively insulin-sensitive pathways of the insulin signaling cascade result in metabolic, vascular and reproductive dysfunctions (Figure 2).

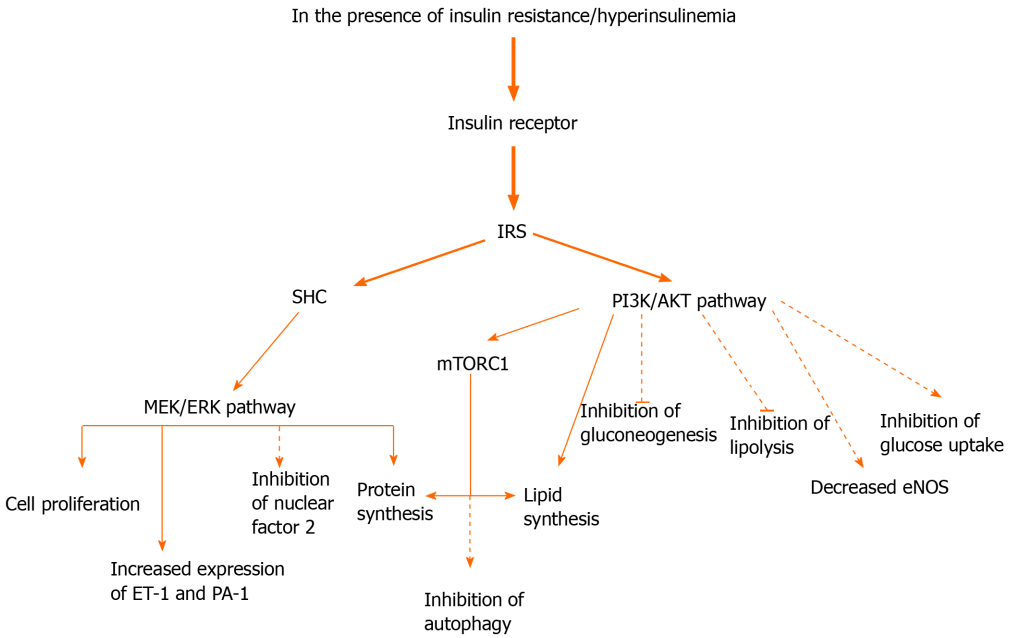

Figure 2 A brief scheme of the insulin signaling pathway in the presence of insulin resistance. Not all insulin signaling pathways are equally affected, and selective insulin resistance is observed. (Partial) resistance in the phosphatidylinositol-3-kinase/serine/threonine-specific protein kinase B pathway results in decreased glucose uptake mediated by insufficient translocation of glucose transporter 4 and decreased inhibition of lipolysis and gluconeogenesis. Additionally, deficient activation of endothelial nitric oxide synthase is also observed. Insulin-resistance-associated hyperinsulinemia promotes anabolic cell activities *via* the mitogen-activated protein kinase (MEK)/extracellular signal-related kinase (ERK) pathway and *via* mechanistic target of rapamycin complex 1. In addition to the anabolic actions of signaling *via* the MEK/ERK pathway, there is also enhanced expression of plasminogen 1 and endothelin 1. The inhibition of nuclear factor 2 compromises cell defense mechanisms against radical stress. Dotted lines represent inhibition, and solid lines represent stimulation/activation. IRS: Insulin receptor substrate; SHC: Src homology 2 domain-containing transforming proteins; MEK: Mitogen-activated protein kinase; ERK: Extracellular signal-related kinase; PI3K: Phosphatidylinositol-3-kinase; AKT: Serine/threonine-specific protein kinase B; mTORC: Mechanistic target of rapamycin complex 1; GLUT4: Glucose transporter 4; ET-1: Endothelin 1; eNOS: endothelial nitric oxide synthase; PAI: Plasminogen activator.

Insulin action *via* the MAP kinase MEK/ERK pathway and partially *via* the PI3K/AKT pathway are relatively less inhibited, and these pathways promote a number of insulin-mediated functions. The activation of mTORC1 results in the suppression of autophagy, leading to a dysfunction of the turnover and removal of lipids and proteins. Insulin resistance suppresses the activation of endothelial nitric oxide synthase by AKT, and endothelial dysfunction is further enhanced by MEK/ERK-dependent expression of plasminogen activator-1 and endothelin-1. Moreover, insulin-resistance-mediated hyperinsulinemia promotes calcium influx into smooth vascular cells, leading to increased contractility and increased sodium reabsorption in renal tubules. In addition to the abovementioned systems, prolonged hyperinsulinemia also triggers functional impairments in the adrenal glands and ovaries, contributing to AEDs.

AEDs

Androgens are steroid hormones synthesized by the adrenal glands and the ovaries of women. Among the several effects on the skin, hirsutism is the main complaint of women with androgen excess. Although using some drugs, such as anabolic steroids, androgens and valproic acid, may lead to hirsutism, in most cases, the underlying cause is an AED[13]. These include polycystic ovary syndrome (PCOS), idiopathic hirsutism, nonclassic congenital adrenal hyperplasia (CAH), syndromes of severe insulin resistance and androgen-secreting tumors[14,15]. Each AED has its own pathogenesis, and different mechanisms are responsible for the increased androgen level or androgen effect. The role of insulin resistance and hyperinsulinemia in the pathogenesis of AED has been implicated for a long time. In this review, we have concentrated on the possible relationships among insulin resistance, hyperinsulinemia and AEDs; thus, the clinical manifestations and diagnostic and therapeutic aspects of AEDs are beyond the scope of this review.

PCOS AND INSULIN RESISTANCE

Theca and granulosa cell functions in normal physiology

Ovarian function is regulated by the changing levels of gonadotropic hormones (FSH/LH) as well as nonsteroidal substances such as inhibin A and B. Ovulation is the ultimate target and is the rupture and release of the dominant follicle from the ovary into the fallopian tube. The stimulation of immature oocytes by FSH results in their maturation into secondary follicles before ovulation. FSH receptors are found in the granulosa cells that surround developing ovarian follicles. Granulosa cells exclusively produce the estrogen needed to mature the developing dominant follicle. Estradiol is the main hormone of the follicle during the follicular phase of the menstrual cycle[16,17].

After the sustained elevation of estrogen levels, the characteristic midcycle LH surge causes the luteinization of granulosa cells. Prior to the LH surge, LH interacts with theca cells that are adjacent to granulosa cells in the ovary. Ovarian theca cells produce androgens that diffuse into granulosa cells and are converted to estrogen for follicular development. Luteinized granulosa cells start to respond to LH and produce progesterone. LH is responsible for inducing ovulation and induces ovarian progesterone production *via* the stimulation of theca cells and luteinized granulosa cells[16,17].

In contrast to the abovementioned physiological conditions, insulin resistance and hyperinsulinemia result in significant disturbances in ovarian functions, such as premature arrest of follicle growth and anovulation. Moreover, hyperinsulinemia has a role in amplifying LH-induced androgen production by theca cells.

Potential mechanisms of insulin resistance

In addition to being a reproductive disorder, PCOS is considered a metabolic disease associated with insulin resistance. The prevalence of insulin resistance among women with PCOS is 60%-70%; however, the detection of insulin resistance among patients with PCOS is dependent on the method used[18-20]. Although lean women with PCOS may also have insulin resistance, obesity further increases insulin resistance in those patients. Several mechanisms have been proposed for the cellular mechanisms of insulin resistance among women with PCOS. *In vitro* studies showed that the mechanisms of insulin resistance among women with PCOS are heterogeneous and involve various steps of insulin signaling. Although altered postreceptor signaling mechanisms are considered the main defect, the insulin receptor beta subunit is described as a novel molecular marker of insulin resistance. Decreased insulin receptor beta subunit has been demonstrated in several tissues, such as skeletal muscle, the liver, adipose tissue and the kidneys, during insulin-resistant states[21]. Some of the studies indicating the cellular mechanisms of insulin resistance in women with PCOS are shown in Table 1[22-24].

The role of insulin resistance and hyperinsulinemia is not limited to ovarian dysfunction. Endometrial physiology is also negatively affected since this tissue is also dependent on the action of steroids and insulin[25]. Lee *et al*[26] found that insulin receptors, IRS proteins and glucose transporters are aberrantly regulated in the endometrium of women with PCOS and are associated with hyperandrogenemia. The authors used human endometrial stromal cells (hESCs) obtained from seven healthy women and 13 women with PCOS. They demonstrated increased phosphorylation of IRS1/IRS2 on Tyr612 in androgen-treated hESCs, suggesting the role of hyperandrogenemia in the insulin signaling pathway of the endometrium. They also found that increased expression of glucose transporter (GLUT) 1 and GLUT12 was inhibited after dihydrotestosterone treatment in decidualizing hESCs. This is the only study evaluating the quantification of a series of GLUTs in the endometria of women with PCOS[26]. In addition, the insulin signaling pathway and endometrial energetic homeostasis are compromised in women with PCOS. Concomitantly, defects in GLUT4 synthesis and its translocation to the cell surface are reduced. The results obtained clearly show that molecular defects in PCOS endometria could partially explain the reproductive problems of these patients[25]. In addition to ovulatory dysfunction, blastocyst implantation and maintenance also contribute to the fertility of women with PCOS[27]. It has been shown that metformin administration to women with PCOS increases GLUT4 endometrial levels and improves the fertility of these patients.

Inflammatory cytokines are also involved in insulin resistance by triggering inhibitory phosphorylation in the insulin signaling cascade. Macrophages infiltrating adipose tissue secrete inflammatory cytokines such as tumor necrosis factor (TNF)-alpha, which act in a paracrine manner and activate serine kinases in adipocytes[28].

Table 1 Studies demonstrating cellular mechanisms of insulin resistance in women with polycystic ovary syndrome

Ref.	Objective	Method(s)	Main result(s)	Conclusion
Dunaif *et al*[22]	To investigate the cellular mechanisms of insulin resistance in PCOS.	Cultured skin fibroblasts from 14 women.	Increased serine phosphorylation and reduced tyrosine phosphorylation of insulin receptor.	One of the mechanisms of insulin resistance at the receptor level was demonstrated. However, 50% of women did not show this abnormality, indicating heterogeneity in the pathogenesis of insulin resistance in PCOS.
Book and Dunaif[23]	To explore the mechanisms of the paradox in metabolic and mitogenic actions of insulin.	Metabolic and mitogenic actions of insulin and IGF-1 were evaluated in cultured skin fibroblasts of 16 PCOS and 11 control women.	No difference in the number and affinity of insulin receptor in either group. Decreased glucose incorporation into glycogen in women with PCOS.Thymidine incorporation was similar between the groups.	Women with PCOS show decreased metabolic action but mitogenic action of insulin signaling was similar between the groups.
Belani *et al*[24]	To unravel insulin and steroidogenic signaling pathways in PCOS.	Insulin receptor beta subunit expression was investigated in luteinized granulosa cells obtained from 30 healthy women and 39 women with PCOS.	Compared to controls, 64% of cells show reduced insulin receptor beta subunit expression.Insulin-resistant women also showed decreased PI3 kinase expression.	Lower viability of luteinized granulosa cells in insulin-resistant women with PCOS.

PCOS: Polycystic ovary syndrome; IGF-1: Insulin-like growth factor-1; PI3 kinase: Phosphatidylinositol-3-kinase.

These kinases exhibit inhibitory phosphorylation of IRS-1, thus causing insulin resistance in adipocytes. Macrophages may constitute 40% of the cells in the adipose tissue of obese individuals. Women with PCOS, particularly obese women, show an increased amount of TNF-alpha in their adipose tissue, which contributes to the development of insulin resistance[29]. It is well known that weight loss is associated with improved insulin sensitivity and metabolic parameters in addition to decreased serum and tissue TNF-alpha levels. Moreover, cytokines such as TNF-alpha may enter the systemic circulation from adipose tissue, resulting in endocrine actions and decreasing insulin sensitivity in insulin target tissues. In cultured adipocytes, TNF-alpha reduced insulin signaling by attenuating the phosphorylation of IRS proteins by insulin receptor tyrosine kinases[30].

The role of insulin in ovarian/adrenal androgen secretion

PCOS is a reproductive and metabolic disease exhibiting an insulin paradox in which ovarian and adrenal tissue remain sensitive to the stimulatory effects of insulin despite resistance to metabolic effects[31-33]. In other words, women with PCOS have a selective defect in insulin action that is characterized by resistance in metabolic signaling pathways but not in mitogenic pathways, which is particularly important in androgen production by the ovaries[33]. Patients with PCOS also have an increased adrenal androgen responsiveness to ACTH stimulation, and adrenal hyperandrogenemia is also a characteristic feature of PCOS. Some of the studies showing the effects of insulin on ovarian/adrenal hormone secretion are shown in Table 2[34-38].

Critical points in the evaluation of insulin resistance in women with PCOS

There are different phenotypes of PCOS that differ not only by the clinical spectrum of the symptoms but also by the presence/absence and the degree of insulin resistance. Euglycemic clamp studies showed that insulin sensitivity is remarkably reduced in PCOS patients who have a classic/complete phenotype, while it is less severe in those with normoandrogenic or ovulatory phenotypes[39]. It is important to note that the accurate estimation of insulin resistance in clinical studies and in outpatient clinics is a matter of debate. Although some surrogate indices of insulin resistance, such as the homeostasis model assessment (HOMA) index, have fair correlations with direct measures of insulin action, it may be misleading to categorize patients as insulin resistant or vice versa according to these parameters. There may be mismatches when using a glucose clamp and surrogate indices[19,40].

In surrogate indices such as the HOMA index, the important player is insulin, and its concentrations depend on both the metabolic clearance rate and the secretion rate from beta cells. Although there is ample evidence for the secretion of insulin, a limited number of studies have investigated the metabolic clearance rate of insulin in women with PCOS. Recently, Tosi *et al*[41] investigated the metabolic clearance rate of insulin and its relationship with the clinical, hormonal and metabolic characteristics in 190

Table 2 Studies showing the effects of insulin on ovarian and adrenal hormone secretion

Ref.	Objective	Method(s)	Main result(s)	Conclusion
Cadagan *et al*[34]	To investigate the effects of insulin and LH on PCOS theca cell CYP17 expression and androgen secretion.	Cells were obtained from three women with PCOS and three healthy women.	PCOS theca cells exhibit increased CYP17 enzyme activity/expression and increased androgen secretion.	There is a defect of steroid biosynthesis in ovarian theca cells, which is further augmented under hyperinsulinemia and increased LH secretion.
Munir *et al*[35]	To define the intracellular signaling pathways that link the insulin receptor to androgen biosynthesis.	Third-passage human ovarian theca cells were used.	Insulin regulation of 17-alpha hydroxylase activity is mediated by PI3 kinase.	Insulin stimulates ovarian androgen production, which is different from the effects on glucose metabolism.
la Marca *et al*[36]	To test the hypothesis of the linkage of hyperinsulinemia and abnormal activity of P450CYP17.	HCG test before and one month after metformin (1500 mg/d) therapy in 11 women with PCOS	After metformin, women with PCOS had significantly lower insulin and testosterone concentrations as well as lower 17-OHP responses.	Metformin leads to a reduction in stimulated ovarian P45017-alpha hydroxylase activity.
Homburg *et al*[37]	To elucidate the relationship and role of IGF-1, IGFBP-1, insulin and LH in the pathogenesis of PCOS.	Serum concentrations of IGF-1, IGFBP-1, insulin and LH in women with PCOS with or without anovulation.	Similar serum IGF-1 levels were found. However, IGFBP-1 levels were decreased in anovulatory PCOS, which is negatively correlated with insulin concentrations.	Hyperinsulinemia and raised LH are independently capable of stimulating ovarian androgen production. Growth factors may have a role in PCOS pathogenesis.
Tosi *et al*[38]	To investigate the role of hyperinsulinemia on adrenal steroidogenesis in women with PCOS.	Hyperinsulinemic clamp and saline infusion tests were performed on separate days in 12 hyperandrogenic women. Concurrent ACTH infusion to evaluate intermediate metabolites of adrenal steroid biosynthesis.	Acute insulin elevation resulted in an increased response of 17 alpha hydroxysteroid intermediates.Increased 17-OHP/androstenedione and 17-OH pregnanolone/DHEA molar ratio suggest relative inhibition of 17-20 lyase activity by insulin.	Acute hyperinsulinemia in a range found in insulin-resistant individuals enhances adrenal response to ACTH stimulation.

PCOS: Polycystic ovary syndrome; LH: Luteinizing hormone; PI3 kinase: Phosphatidylinositol-3-kinase; P450CYP17: Cytochrome 450, 17 hydroxylase; HCG: Human chorionic gonadotropin; 17-OHP: 17-hydroxyprogesterone; IGF-1: Insulin-like growth factor-1; IGFBP-1: Insulin-like growth factor-binding protein-1; ACTH: Adrenocorticotropic hormone; DHEA: Dehydroepiandrosterone.

women with PCOS. It has been shown that insulin clearance is remarkably reduced in women with PCOS compared to healthy women with similar indices[41]. Moreover, in multivariate analysis, body fat, estimates of insulin secretion and levels of serum androgens were all independent predictors of insulin clearance, and they all had negative relationships. The authors revealed that obesity contributes to hyperinsulinemia by both lowering insulin metabolism and increasing insulin secretion in addition to regulating insulin clearance by serum androgens.

Although insulin resistance is associated with PCOS, it is well known that not all women with PCOS have insulin resistance and hyperinsulinemia. Baillargeon *et al*[42] evaluated 100 nonobese women with PCOS with normal indices of insulin sensitivity indicated by normal glucose tolerance, fasting insulin, peak insulin during an OGTT and fasting glucose/insulin ratio. Those women received 850 mg metformin twice daily, 4 mg rosiglitazone, a combination of both drugs or at least one placebo for six months. In comparison to placebo, insulin sensitizers significantly improved ovulation. After treatment, serum testosterone levels also decreased significantly in comparison to the placebo group. The authors suggest that there is a subgroup of women with normal insulin sensitivity, and even those patients may benefit from insulin-sensitizing therapies in terms of the resumption of menses and improvement in hyperandrogenemia[42].

Several studies have demonstrated the role of insulin resistance in the pathogenesis of PCOS, and insulin sensitizers have been used for different clinical indications, such as metabolic effects, aiming to decrease hirsutism, resume menses and increase the ovulatory rate. If we look at the other side of the coin, do insulin sensitizers have a role in the prevention of PCOS? Ibáñez *et al*[43] investigated body composition, lipids, gonadotropins and the progression to PCOS in 24 nonobese postmenarcheal girls with hyperinsulinemic hyperandrogenemia and precocious pubarche. They were randomly assigned to receive metformin (850 mg/d) or no treatment for 12 mo. In comparison to untreated girls, metformin-treated girls had significantly improved parameters (insulin sensitivity, androgens, lipids), and the authors concluded that early metformin treatment helps to prevent the progression of precocious pubarche to PCOS. The

authors also investigated the effects of metformin (1250 mg/d) alone or in combination with an antiandrogen (flutamide, 250 mg/d) in nonobese young women with hyperinsulinemic hyperandrogenism for 9 mo[44]. In comparison to the flutamide alone group, the combination group had greater improvements in serum androgens, insulin resistance and ovulation rates (75% and 92% in the metformin alone and combination groups, respectively, but not in the flutamide alone group). These results suggest that insulin sensitizers, mainly metformin, may have a role in the early stages of PCOS and may be used as additives to other therapies, such as antiandrogens.

Apart from insulin-induced androgen secretion, androgens also contribute to the occurrence of hyperinsulinemia in women with PCOS[41]. Moghetti *et al*[45] previously assessed the effects of androgens on insulin sensitivity in 43 women (13 obese, 30 nonobese) with normal glucose tolerance and hirsutism and compared the results with those of healthy individuals matched for body mass index. Hyperandrogenic women were studied before and 3-4 mo after antiandrogen (spironolactone, flutamide, GnRH agonist buserelin) treatment. Insulin-mediated glucose uptake was lower than that in healthy individuals irrespective of ovarian or nonovarian hyperandrogenism. After antiandrogen therapy, insulin action, determined in both oxidative and nonoxidative metabolism, significantly increased, albeit it remained lower than that of the control groups. This study also showed that androgen excess per se contributes to insulin resistance and that antiandrogen therapy partially reverses peripheral insulin resistance regardless of which antiandrogen was used. These bidirectional relationships between insulin and androgens in the presence of other confounding factors are reminiscent of the relationship of the egg and the chicken.

Idiopathic hirsutism and insulin resistance

Idiopathic hirsutism is the second most common form of hirsutism and is characterized by normal serum androgen levels, normal ovulatory function and normal ovaries[46,47]. Data regarding the presence/absence of insulin resistance in patients with idiopathic hirsutism are limited in comparison to PCOS. In one of the earliest studies in this area, we investigated the presence/absence of insulin resistance in 32 patients (eight of the patients had body mass index higher than 30 kg/m^2) with idiopathic hirsutism by using basal insulin levels, HOMA scores, and OGTT and intravenous insulin tolerance test results. Patients with idiopathic hirsutism had significantly higher basal insulin levels and HOMA scores and a lower plasma glucose disappearance rate than control individuals. Six patients (18.7%) had impaired glucose tolerance (IGT); however, they were more obese than the patients with normal glucose tolerance. It is remarkable that after omitting the patients with IGT, the rest of the patients were still insulin resistant[46]. We have concluded that idiopathic hirsutism is associated with some degree of insulin resistance and an increased tendency for glucose intolerance, particularly in obese patients.

In most of the studies, lean patients with idiopathic hirsutism were also investigated to exclude the effect of obesity. Talaei *et al*[48] also investigated the presence of insulin resistance among nonobese women with PCOS (n = 16), idiopathic hirsutism (n = 30) and healthy individuals (n = 60). All the groups were investigated by using basal insulin levels and HOMA scores. The authors found that patients with idiopathic hirsutism had lower insulin resistance than patients with PCOS, but they had higher insulin resistance than control individuals[48]. Similarly, Sarac *et al*[49] also investigated the presence of insulin resistance among nonobese women with idiopathic hirsutism (n = 20) by using the euglycemic hyperinsulinemic clamp technique and compared the results with those of 20 healthy individuals. Patients with idiopathic hirsutism had lower glucose disposal rates than control individuals[49]. Although most of the studies[46,48-50] showed increased insulin resistance, opposite results have also been reported, albeit rarely. Bonakdaran *et al*[51] investigated insulin resistance in nonobese patients with PCOS (n = 30), idiopathic hirsutism (n = 30) and healthy individuals (n = 30) by using basal insulin levels and HOMA scores. They reported that insulin resistance was no more common than in healthy individuals. In that study, the authors classified the patients as insulin resistant (whose HOMA score > 2.68 based on a previous Iranian study) or insulin sensitive (whose HOMA score < 2.68). When they analyzed their data without classifying the patients, they again did not find any difference in insulin sensitivity between the patients with idiopathic hirsutism and healthy individuals. However, it is notable that they did not find any insulin resistance even in patients with PCOS[51].

Although it has been shown that patients with idiopathic hirsutism may exhibit some degree of insulin resistance, there are not adequate data regarding the (molecular) mechanisms of insulin resistance. Idiopathic hirsutism is considered

among AEDs; patients with idiopathic hirsutism have normal serum androgen levels, and it may be asked, how the patients exhibit similar insulin resistance as their hyperandrogenic counterparts. In that case, an important question arises: are these patients truly defined as normoandrogenic and are they truly idiopathic[52]? Previously, we showed that, although within normal limits, patients with idiopathic hirsutism have relatively higher serum androgen levels than healthy individuals[46]. In other words, those patients are actually hyperandrogenic at the tissue level; however, when we use some cutoff values derived from the reference values of commercial assays, we consider those patients to have (normoandrogenic) idiopathic hirsutism. However, those patients exhibit a lower estradiol/testosterone ratio, which is a function of aromatase activity, leading to relative hyperandrogenemia[46]. Moreover, patients with idiopathic hirsutism also demonstrate metabolic derangements compatible with insulin resistance, such as IGT[46,50].

Insulin resistance and CAH

Deficiencies in the main pathways of steroid biosynthesis lead to CAH, which is a group of disorders characterized by enzymatic defects in cortisol biosynthesis. When any mutation/mutations cause complete or near complete deficiency of the enzymes, the classic form of the disease ensues with severe clinical manifestations, such as the virilization of females or salt wasting in both sexes[53,54]. The milder form of the disease, called the nonclassic form, is typically asymptomatic at birth and is not distinguishable from other hyperandrogenic disorders, such as PCOS. Both forms of the disease differ in terms of the severity of the clinical signs and symptoms, and their treatment modalities are also different[54,55].

In patients with milder forms of CAH, glucocorticoid treatment is rarely indicated since these patients do not exhibit overt glucocorticoid deficiency. Saygili *et al*[56] investigated insulin resistance in 18 patients with untreated nonclassic CAH (NCAH), and the data were compared to those of 26 healthy individuals. Serum basal insulin levels, post glucose loading (2 h) insulin responses and HOMA scores were significantly higher in NCAH patients than in control individuals. The authors also showed a positive correlation between serum androgen and insulin levels[56]. On the other hand, glucocorticoid replacement therapy is the mainstay of therapy in the classic form of the disease, and some patients may be overtreated since the androgen suppressive dose of glucocorticoids is much more than the replacement dose. Recently, Kurnaz *et al*[57] depicted another aspect of the relationship between CAH and insulin resistance. In 56 patients with CAH and 70 healthy individuals, in addition to biochemical and hormonal investigations, the authors measured serum insulin and fetuin-A levels. Fetuin-A is a protein produced in the liver. Insulin and fetuin-A levels were significantly higher in patients with CAH than in controls, and unfavorably high levels of these proteins exhibited a positive correlation with total and free testosterone levels[57]. Since androgen receptors are also expressed in pancreatic and liver cells, high levels of testosterone can result in hyperinsulinemia. Moreover, Fetuin-A is a natural inhibitor of tyrosine kinase, and its overexpression in the liver leads to insulin resistance.

Kroese *et al*[58] investigated insulin resistance and hyperinsulinemia in 12 patients with the classic form of CAH and 12 controls matched for body mass index and age by using a euglycemic clamp. Patients were randomized to treatment with either placebo followed by pioglitazone (45 mg/d) for 16 wk or treatment with pioglitazone for 16 wk followed by placebo in a randomized crossover study design. The results of this study showed that patients with CAH who were treated with glucocorticoids were more insulin resistant than controls, and sixteen weeks of treatment with pioglitazone (45 mg/d) significantly improved insulin resistance[58].

Metformin is an oral hypoglycemic drug that has several other effects and has therefore been used in various clinical conditions[59]. Hirsc *et al*[60] investigated the effects of metformin on adrenal androgen synthesis by using human adrenal NCI-H295R cells. Cells were treated with different doses of metformin for 48 hr and tested for steroid profiles. The authors demonstrated *in vitro* that metformin reduces the activity of two important enzymes in adrenal androgen biosynthesis, 17 alpha hydroxylase/17-20 lyase and 3 beta hydroxysteroid dehydrogenase, in a dose-dependent manner by affecting the mitochondrial respiratory chain[60]. Recently, Parween *et al*[61] investigated the effect of metformin on melanocortin receptor 2 (MCR-2), which plays an important role in ACTH-mediated intracellular signaling. The authors performed the studies in an established adrenal OS3 cell model. They observed a fivefold increase in MCR-2 expression after ACTH stimulation, which was reduced 55% with metformin treatment, indicating that metformin directly affects MCR-2 expression induced by ACTH. These results provide another possible

mechanism of action by which metformin reduces adrenal steroidogenesis, and this mechanism of action may be beneficial in conditions where the hypothalamic-pituitary-adrenal axis is overactivated, such as CAH.

Although it is not as evident as in PCOS, patients with CAH may have insulin resistance, which may be further exacerbated with glucocorticoid overtreatment and obesity. Han *et al*[62] investigated whether the type and dose of glucocorticoid treatment impacts health outcomes, including insulin resistance, in 196 patients with CAH. Increasing the glucocorticoid dose with the aim of reducing serum androgen levels increased blood pressure without a remarkable benefit in disease control. The authors found that compared with those receiving prednisolone or hydrocortisone, patients on dexamethasone had lower serum androgens but greater insulin resistance. Moreover, they reported that using dexamethasone once daily was more likely to induce insulin resistance than using dexamethasone twice daily, which is explained by the higher peak of dexamethasone at night possibly inducing insulin resistance, similar to that seen in patients with primary adrenal failure[62].

On the other hand, inappropriate management of women with CAH mimics an additional PCOS-like phenotype in these women. Lifestyle modifications, changes in glucocorticoid regimens and insulin sensitizers such as metformin and/or pioglitazone may help to overcome this problem.

Syndromes of severe insulin resistance

Syndromes of severe insulin resistance are rare diseases of acquired or genetic origin. There are two main forms of these syndromes: insulin receptor gene mutations cause Type A syndrome, whereas autoantibodies against insulin receptors cause Type B syndrome. The role of insulin and insulin resistance in ovarian functions was established 45 years ago, when Kahn *et al*[63] described patients with acanthosis nigricans, hirsutism and virilization. Today, it is well known that the ovaries express not only insulin receptors but also type 1 and type 2 insulin-like growth factor (IGF) receptors, and the major hyperinsulinemia observed in Type A insulin resistance syndrome mediates its stimulatory effects *via* IGF receptors. Patients with Type A syndrome are mostly nonobese and demonstrate severe hyperinsulinemia, hyperandrogenism and acanthosis nigricans. Most of the patients are incorrectly diagnosed with PCOS. Irrespective of the type of disease, hyperinsulinemia promotes ovarian androgen synthesis independently of gonadotropins[64].

Although it is considered a subtype of PCOS, hyperandrogenism, insulin resistance, and acanthosis nigricans (HAIR-AN) syndrome is also associated with severe insulin resistance. Acanthosis nigricans is a clinical manifestation of insulin resistance characterized by velvety, hyperpigmented skin lesions mostly found on the axillary region and on the back of the neck. Abnormally increased insulin levels cross-react with insulin and IGF receptors on the ovary, leading to androgen overproduction. Metformin and/or pioglitazone may be used and have beneficial effects on serum androgen levels[65]. Recently, in five women with HAIR-AN syndrome, liraglutide improved insulin resistance, serum androgen levels and menstrual abnormalities, with one pregnancy[66].

Links between hyperandrogenism and insulin resistance. How to translate in daily practice

Apart from PCOS and syndromes of severe insulin resistance, the role and contribution of insulin resistance in the pathogenesis of AEDs are a matter of debate, although several molecular and clinical relationships have been given above. Currently, it is highly debated in whom and how insulin resistance should be diagnosed and treated among patients with AEDs, including PCOS.

It is certain that insulin resistance is a common but not universal feature of PCOS. Thus, assuming all patients are insulin resistant is not logical. In daily practice, the most important problem is the correct estimation of insulin resistance. Due to its complex and time-consuming procedures, the gold-standard "euglycemic hyperinsulinemic clamp" technique cannot be applied to all women with PCOS. Instead, some surrogate markers have been suggested for the evaluation of insulin sensitivity. However, surrogate markers such as the basal insulin, glucose/insulin ratio, HOMA index, and quantitative insulin sensitivity check index are all based on basal insulin and fasting glucose and provide similar information[67,68]. Therefore, it is not suitable to administer an insulin sensitizer relying on only some mathematical models. Instead, treatment decisions should be based on the constellation of the signs, symptoms and presence of obesity; acanthosis nigricans; and some laboratory abnormalities such as IGT and impaired fasting glucose. On the other hand, given that metformin is a very

safe drug, in clinical practice, metformin may be used for trial without some sophisticated laboratory investigations if there are no other causes of concern.

Based on several molecular and clinical studies indicating the role of insulin resistance and compensatory hyperinsulinemia in PCOS pathogenesis, many drugs/compounds, including nutraceuticals, have been tested in the treatment of PCOS with the aim of weight reduction and metabolic and reproductive outcomes. Among these, metformin, orlistat, pioglitazone, inositol, glucagon-like peptide-1 agonists, and alpha-lipoic acid were all tested. A recent guideline raised by the International PCOS Network mentioned metformin as the only insulin-sensitizing agent among women with PCOS and suggested its use for weight, hormonal and metabolic outcomes in addition to lifestyle modifications[69]. Metformin has also been suggested alone or in addition to clomiphene citrate for anovulatory infertility. Additionally, the guidelines suggest considering metformin (in addition to lifestyle modification) in adolescents with a clear diagnosis of PCOS or with symptoms of PCOS before the diagnosis is established[69]. Regarding women with lean PCOS, weight maintenance through dietary interventions and obesity avoidance should be a treatment goal. Regular physical exercise has been shown to improve insulin resistance in addition to some other beneficial effects on the symptoms of PCOS[70]. Although the number of patients is limited, Anastasiou *et al*[71] showed that lean or even underweight women with PCOS may benefit from metformin therapy for the resumption of menses and ovulation. In brief, we suggest using the same principles for the decision to treat insulin resistance in patients with AEDs.

CONCLUSION

AEDs are associated with several metabolic and reproductive consequences. Data regarding insulin resistance and its role in AEDs, except for PCOS, are scarce, and current evidence shows that insulin has receptors both on the adrenal glands and the ovaries and stimulates androgen production in several ways. Increased androgens in turn trigger insulin resistance. Furthermore, obesity contributes to established insulin resistance in patients with AEDs in many ways. Future studies are needed to establish the most appropriate time for initiating therapy and candidate patients for prescribing insulin-sensitizing agents.

REFERENCES

1 **Taniguchi CM**, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Biol* 2006; **7**: 85-96 [PMID: 16493415 DOI: 10.1038/nrm1837]

2 **Gehart H**, Kumpf S, Ittner A, Ricci R. MAPK signalling in cellular metabolism: stress or wellness? *EMBO Rep* 2010; **11**: 834-840 [PMID: 20930846 DOI: 10.1038/embor.2010.160]

3 **Haeusler RA**, McGraw TE, Accili D. Biochemical and cellular properties of insulin receptor signalling. *Nat Rev Mol Cell Biol* 2018; **19**: 31-44 [PMID: 28974775 DOI: 10.1038/nrm.2017.89]

4 **Björnholm M**, He AR, Attersand A, Lake S, Liu SC, Lienhard GE, Taylor S, Arner P, Zierath JR. Absence of functional insulin receptor substrate-3 (IRS-3) gene in humans. *Diabetologia* 2002; **45**: 1697-1702 [PMID: 12488959 DOI: 10.1007/s00125-002-0945-z]

5 **Boucher J**, Kleinridders A, Kahn CR. Insulin receptor signaling in normal and insulin-resistant states. *Cold Spring Harb Perspect Biol* 2014; **6**: a009191 [PMID: 24384568 DOI: 10.1101/cshperspect.a009191]

6 **Gonzalez E**, McGraw TE. Insulin-modulated Akt subcellular localization determines Akt isoform-specific signaling. *Proc Natl Acad Sci USA* 2009; **106**: 7004-7009 [PMID: 19372382 DOI: 10.1073/pnas.0901933106]

7 **Ünal EB**, Uhlitz F, Blüthgen N. A compendium of ERK targets. *FEBS Lett* 2017; **591**: 2607-2615 [PMID: 28675784 DOI: 10.1002/1873-3468.12740]

8 **Schmelzle K**, Kane S, Gridley S, Lienhard GE, White FM. Temporal dynamics of tyrosine phosphorylation in insulin signaling. *Diabetes* 2006; **55**: 2171-2179 [PMID: 16873679 DOI: 10.2337/db06-0148]

9 **Krüger M**, Kratchmarova I, Blagoev B, Tseng YH, Kahn CR, Mann M. Dissection of the insulin signaling pathway *via* quantitative phosphoproteomics. *Proc Natl Acad Sci USA* 2008; **105**: 2451-2456 [PMID: 18268350 DOI: 10.1073/pnas.0711713105]

10 **Humphrey SJ**, Yang G, Yang P, Fazakerley DJ, Stöckli J, Yang JY, James DE. Dynamic adipocyte phosphoproteome reveals that Akt directly regulates mTORC2. *Cell Metab* 2013; **17**: 1009-1020 [PMID: 23684622 DOI: 10.1016/j.cmet.2013.04.010]

11 **Corkey BE**. Diabetes: have we got it all wrong? *Diabetes Care* 2012; **35**: 2432-2437 [PMID: 23173132 DOI: 10.2337/dc12-0825]

12 **Nolan CJ**, Ruderman NB, Kahn SE, Pedersen O, Prentki M. Insulin resistance as a physiological defense against metabolic stress: implications for the management of subsets of type 2 diabetes. *Diabetes* 2015; **64**: 673-686 [PMID: 25713189 DOI: 10.2337/db14-0694]

13 **Martin KA**, Anderson RR, Chang RJ, Ehrmann DA, Lobo RA, Murad MH, Pugeat MM, Rosenfield RL. Evaluation and Treatment of Hirsutism in Premenopausal Women: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 2018; **103**: 1233-1257 [PMID: 29522147 DOI: 10.1210/jc.2018-00241]

14 **Unluhizarci K**, Karaca Z, Kelestimur F. Hirsutism - from diagnosis to use of antiandrogens. *Front Horm Res* 2013; **40**: 103-114 [PMID: 24002408 DOI: 10.1159/000341822]

15 **Yilmaz B**, Yildiz BO. Endocrinology of Hirsutism: From Androgens to Androgen Excess Disorders. *Front Horm Res* 2019; **53**: 108-119 [PMID: 31499500 DOI: 10.1159/000494907]

16 **Tajima K**, Orisaka M, Yata H, Goto K, Hosokawa K, Kotsuji F. Role of granulosa and theca cell interactions in ovarian follicular maturation. *Microsc Res Tech* 2006; **69**: 450-458 [PMID: 16718667 DOI: 10.1002/jemt.20304]

17 **Messinis IE**, Messini CI, Dafopoulos K. Novel aspects of the endocrinology of the menstrual cycle. *Reprod Biomed Online* 2014; **28**: 714-722 [PMID: 24745832 DOI: 10.1016/j.rbmo.2014.02.003]

18 **Marshall JC**, Dunaif A. Should all women with PCOS be treated for insulin resistance? *Fertil Steril* 2012; **97**: 18-22 [PMID: 22192137 DOI: 10.1016/j.fertnstert.2011.11.036]

19 **Tosi F**, Bonora E, Moghetti P. Insulin resistance in a large cohort of women with polycystic ovary syndrome: a comparison between euglycaemic-hyperinsulinaemic clamp and surrogate indexes. *Hum Reprod* 2017; **32**: 2515-2521 [PMID: 29040529 DOI: 10.1093/humrep/dex308]

20 **Lewandowski KC**, Skowrońska-Jóźwiak E, Łukasiak K, Gałuszko K, Dukowicz A, Cedro M, Lewiński A. How much insulin resistance in polycystic ovary syndrome? *Arch Med Sci* 2019; **15**: 613-618 [PMID: 31110526 DOI: 10.5114/aoms.2019.82672]

21 **Tiwari S**, Halagappa VK, Riazi S, Hu X, Ecelbarger CA. Reduced expression of insulin receptors in the kidneys of insulin-resistant rats. *J Am Soc Nephrol* 2007; **18**: 2661-2671 [PMID: 17855644 DOI: 10.1681/ASN.2006121410]

22 **Dunaif A**, Xia J, Book CB, Schenker E, Tang Z. Excessive insulin receptor serine phosphorylation in cultured fibroblasts and in skeletal muscle. A potential mechanism for insulin resistance in the polycystic ovary syndrome. *J Clin Invest* 1995; **96**: 801-810 [PMID: 7635975 DOI: 10.1172/JCI118126]

23 **Book CB**, Dunaif A. Selective insulin resistance in the polycystic ovary syndrome. *J Clin Endocrinol Metab* 1999; **84**: 3110-3116 [PMID: 10487672 DOI: 10.1210/jcem.84.9.6010]

24 **Belani M**, Deo A, Shah P, Banker M, Singal P, Gupta S. Differential insulin and steroidogenic signaling in insulin resistant and non-insulin resistant human luteinized granulosa cells-A study in PCOS patients. *J Steroid Biochem Mol Biol* 2018; **178**: 283-292 [PMID: 29339197 DOI: 10.1016/j.jsbmb.2018.01.008]

25 **Oróstica L**, Rosas C, Plaza-Parrochia F, Astorga I, Gabler F, García V, Romero C, Vega M. Altered Steroid Metabolism and Insulin Signaling in PCOS Endometria: Impact in Tissue Function. *Curr Pharm Des* 2016; **22**: 5614-5624 [PMID: 27514712 DOI: 10.2174/1381612822666160810111528]

26 **Lee MH**, Yoon JA, Kim HR, Kim YS, Lyu SW, Lee BS, Song H, Choi DH. Hyperandrogenic Milieu Dysregulates the Expression of Insulin Signaling Factors and Glucose Transporters in the Endometrium of Patients With Polycystic Ovary Syndrome. *Reprod Sci* 2020; **27**: 1637-1647 [PMID: 32430710 DOI: 10.1007/s43032-020-00194-7]

27 **Giudice LC**. Endometrium in PCOS: Implantation and predisposition to endocrine CA. *Best Pract Res Clin Endocrinol Metab* 2006; **20**: 235-244 [PMID: 16772154 DOI: 10.1016/j.beem.2006.03.005]

28 **Osborn O**, Olefsky JM. The cellular and signaling networks linking the immune system and metabolism in disease. *Nat Med* 2012; **18**: 363-374 [PMID: 22395709 DOI: 10.1038/nm.2627]

29 **Weisberg SP**, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003; **112**: 1796-1808 [PMID: 14679176 DOI: 10.1172/JCI19246]

30 **Ueki K**, Kondo T, Kahn CR. Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. *Mol Cell Biol* 2004; **24**: 5434-5446 [PMID: 15169905 DOI: 10.1128/MCB.24.12.5434-5446.2004]

31 **Barber TM**, McCarthy MI, Wass JA, Franks S. Obesity and polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 2006; **65**: 137-145 [PMID: 16886951 DOI: 10.1111/j.1365-2265.2006.02587.x]

32 **Unlühizarci K**, Keleştimur F, Bayram F, Sahin Y, Tutuş A. The effects of metformin on insulin resistance and ovarian steroidogenesis in women with polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 1999; **51**: 231-236 [PMID: 10468995 DOI: 10.1046/j.1365-2265.1999.00786.x]

33 **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 [PMID: 23065822 DOI: 10.1210/er.2011-1034]

34 **Cadagan D**, Khan R, Amer S. Thecal cell sensitivity to luteinizing hormone and insulin in polycystic ovarian syndrome. *Reprod Biol* 2016; **16**: 53-60 [PMID: 26952754 DOI: 10.1016/j.repbio.2015.12.006]

35 **Munir I**, Yen HW, Geller DH, Torbati D, Bierden RM, Weitsman SR, Agarwal SK, Magoffin DA. Insulin augmentation of 17alpha-hydroxylase activity is mediated by phosphatidyl inositol 3-kinase but not extracellular signal-regulated kinase-1/2 in human ovarian theca cells. *Endocrinology* 2004;

145: 175-183 [PMID: 14512432 DOI: 10.1210/en.2003-0329]
36 **la Marca A**, Egbe TO, Morgante G, Paglia T, Cianci A, De Leo V. Metformin treatment reduces ovarian cytochrome P-450c17alpha response to human chorionic gonadotrophin in women with insulin resistance-related polycystic ovary syndrome. *Hum Reprod* 2000; **15**: 21-23 [PMID: 10611182 DOI: 10.1093/humrep/15.1.21]
37 **Homburg R**, Pariente C, Lunenfeld B, Jacobs HS. The role of insulin-like growth factor-1 (IGF-1) and IGF binding protein-1 (IGFBP-1) in the pathogenesis of polycystic ovary syndrome. *Hum Reprod* 1992; **7**: 1379-1383 [PMID: 1283982 DOI: 10.1093/oxfordjournals.humrep.a137577]
38 **Tosi F**, Negri C, Brun E, Castello R, Faccini G, Bonora E, Muggeo M, Toscano V, Moghetti P. Insulin enhances ACTH-stimulated androgen and glucocorticoid metabolism in hyperandrogenic women. *Eur J Endocrinol* 2011; **164**: 197-203 [PMID: 21059865 DOI: 10.1530/EJE-10-0782]
39 **Moghetti P**, Tosi F, Bonin C, Di Sarra D, Fiers T, Kaufman JM, Giagulli VA, Signori C, Zambotti F, Dall'Alda M, Spiazzi G, Zanolin ME, Bonora E. Divergences in insulin resistance between the different phenotypes of the polycystic ovary syndrome. *J Clin Endocrinol Metab* 2013; **98**: E628-E637 [PMID: 23476073 DOI: 10.1210/jc.2012-3908]
40 **Muniyappa R**, Lee S, Chen H, Quon MJ. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. *Am J Physiol Endocrinol Metab* 2008; **294**: E15-E26 [PMID: 17957034 DOI: 10.1152/ajpendo.00645.2007]
41 **Tosi F**, Dal Molin F, Zamboni F, Saggiorato E, Salvagno GL, Fiers T, Kaufman JM, Bonora E, Moghetti P. Serum Androgens Are Independent Predictors of Insulin Clearance but Not of Insulin Secretion in Women With PCOS. *J Clin Endocrinol Metab* 2020; **105**: dgaa095 [PMID: 32119099 DOI: 10.1210/clinem/dgaa095]
42 **Baillargeon JP**, Jakubowicz DJ, Iuorno MJ, Jakubowicz S, Nestler JE. Effects of metformin and rosiglitazone, alone and in combination, in nonobese women with polycystic ovary syndrome and normal indices of insulin sensitivity. *Fertil Steril* 2004; **82**: 893-902 [PMID: 15482765 DOI: 10.1016/j.fertnstert.2004.02.127]
43 **Ibáñez L**, Ferrer A, Ong K, Amin R, Dunger D, de Zegher F. Insulin sensitization early after menarche prevents progression from precocious pubarche to polycystic ovary syndrome. *J Pediatr* 2004; **144**: 23-29 [PMID: 14722514 DOI: 10.1016/j.jpeds.2003.08.015]
44 **Ibáñez L**, Valls C, Ferrer A, Ong K, Dunger DB, De Zegher F. Additive effects of insulin-sensitizing and anti-androgen treatment in young, nonobese women with hyperinsulinism, hyperandrogenism, dyslipidemia, and anovulation. *J Clin Endocrinol Metab* 2002; **87**: 2870-2874 [PMID: 12050266 DOI: 10.1210/jcem.87.6.8568]
45 **Moghetti P**, Tosi F, Castello R, Magnani CM, Negri C, Brun E, Furlani L, Caputo M, Muggeo M. The insulin resistance in women with hyperandrogenism is partially reversed by antiandrogen treatment: evidence that androgens impair insulin action in women. *J Clin Endocrinol Metab* 1996; **81**: 952-960 [PMID: 8772557 DOI: 10.1210/jcem.81.3.8772557]
46 **Unlühizarci K**, Karababa Y, Bayram F, Kelestimur F. The investigation of insulin resistance in patients with idiopathic hirsutism. *J Clin Endocrinol Metab* 2004; **89**: 2741-2744 [PMID: 15181051 DOI: 10.1210/jc.2003-031626]
47 **Unluhizarci K**, Gokce C, Atmaca H, Bayram F, Kelestimur F. A detailed investigation of hirsutism in a Turkish population: idiopathic hyperandrogenemia as a perplexing issue. *Exp Clin Endocrinol Diabetes* 2004; **112**: 504-509 [PMID: 15505757 DOI: 10.1055/s-2004-821307]
48 **Talaei A**, Adgi Z, Mohamadi Kelishadi M. Idiopathic hirsutism and insulin resistance. *Int J Endocrinol* 2013; **2013**: 593197 [PMID: 24228029 DOI: 10.1155/2013/593197]
49 **Sarac F**, Saygili F, Ozgen G, Tuzun M, Yilmaz C, Kabalak T. Assessment of insulin resistance in the idiopathic hirsutism. *Gynecol Obstet Invest* 2007; **63**: 126-131 [PMID: 17057397 DOI: 10.1159/000096434]
50 **Arduc A**, Sarıcam O, Dogan BA, Tuna MM, Tutuncu YA, Isik S, Berker D, Sennaroglu E, Guler S. Should insulin resistance be screened in lean hirsute women? *Gynecol Endocrinol* 2015; **31**: 291-295 [PMID: 25561024 DOI: 10.3109/09513590.2014.994598]
51 **Bonakdaran S**, Kiafar B, Barazandeh Ahmadabadi F. Evaluation of insulin resistance in idiopathic hirsutism compared with polycystic ovary syndrome patients and healthy individuals. *Australas J Dermatol* 2016; **57**: e1-e4 [PMID: 25496462 DOI: 10.1111/ajd.12276]
52 **Taheri S**, Zararsiz G, Karaburgu S, Borlu M, Ozgun MT, Karaca Z, Tanriverdi F, Dundar M, Kelestimur F, Unluhizarci K. Is idiopathic hirsutism (IH) really idiopathic? *Eur J Endocrinol* 2015; **173**: 447-454 [PMID: 26194504 DOI: 10.1530/EJE-15-0460]
53 **Parsa AA**, New MI. Steroid 21-hydroxylase deficiency in congenital adrenal hyperplasia. *J Steroid Biochem Mol Biol* 2017; **165**: 2-11 [PMID: 27380651 DOI: 10.1016/j.jsbmb.2016.06.015]
54 **El-Maouche D**, Arlt W, Merke DP. Congenital adrenal hyperplasia. *Lancet* 2017; **390**: 2194-2210 [PMID: 28576284 DOI: 10.1016/S0140-6736(17)31431-9]
55 **Unluhizarci K**, Kula M, Dundar M, Tanriverdi F, Israel S, Colak R, Dokmetas HS, Atmaca H, Bahceci M, Balci MK, Comlekci A, Bilen H, Akarsu E, Erem C, Kelestimur F. The prevalence of non-classic adrenal hyperplasia among Turkish women with hyperandrogenism. *Gynecol Endocrinol* 2010; **26**: 139-143 [PMID: 19718570 DOI: 10.3109/09513590903215466]
56 **Saygili F**, Oge A, Yilmaz C. Hyperinsulinemia and insulin insensitivity in women with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency: the relationship between serum leptin levels and chronic hyperinsulinemia. *Horm Res* 2005; **63**: 270-274 [PMID: 15956788 DOI: 10.1159/000086363]

57 **Kurnaz E**, Çetinkaya S, Özalkak Ş, Bayramoğlu E, Demirci G, Öztürk HS, Erdeve ŞS, Aycan Z. Serum Fetuin-A and Insulin Levels in Classic Congenital Adrenal Hyperplasia. *Horm Metab Res* 2020; **52**: 654-659 [PMID: 32108931 DOI: 10.1055/a-1116-2173]

58 **Kroese JM**, Mooij CF, van der Graaf M, Hermus AR, Tack CJ. Pioglitazone improves insulin resistance and decreases blood pressure in adult patients with congenital adrenal hyperplasia. *Eur J Endocrinol* 2009; **161**: 887-894 [PMID: 19755409 DOI: 10.1530/EJE-09-0523]

59 **Fujita Y**, Inagaki N. Metformin: New Preparations and Nonglycemic Benefits. *Curr Diab Rep* 2017; **17**: 5 [PMID: 28116648 DOI: 10.1007/s11892-017-0829-8]

60 **Hirsch A**, Hahn D, Kempná P, Hofer G, Nuoffer JM, Mullis PE, Flück CE. Metformin inhibits human androgen production by regulating steroidogenic enzymes HSD3B2 and CYP17A1 and complex I activity of the respiratory chain. *Endocrinology* 2012; **153**: 4354-4366 [PMID: 22778212 DOI: 10.1210/en.2012-1145]

61 **Parween S**, Rihs S, Flück CE. Metformin inhibits the activation of melanocortin receptors 2 and 3 in vitro: A possible mechanism for its anti-androgenic and weight balancing effects in vivo? *J Steroid Biochem Mol Biol* 2020; **200**: 105684 [PMID: 32360359 DOI: 10.1016/j.jsbmb.2020.105684]

62 **Han TS**, Stimson RH, Rees DA, Krone N, Willis DS, Conway GS, Arlt W, Walker BR, Ross RJ; United Kingdom Congenital adrenal Hyperplasia Adult Study Executive (CaHASE). Glucocorticoid treatment regimen and health outcomes in adults with congenital adrenal hyperplasia. *Clin Endocrinol (Oxf)* 2013; **78**: 197-203 [PMID: 22998134 DOI: 10.1111/cen.12045]

63 **Kahn CR**, Flier JS, Bar RS, Archer JA, Gorden P, Martin MM, Roth J. The syndromes of insulin resistance and acanthosis nigricans. Insulin-receptor disorders in man. *N Engl J Med* 1976; **294**: 739-745 [PMID: 176581 DOI: 10.1056/NEJM197604012941401]

64 **Musso C**, Shawker T, Cochran E, Javor ED, Young J, Gorden P. Clinical evidence that hyperinsulinaemia independent of gonadotropins stimulates ovarian growth. *Clin Endocrinol (Oxf)* 2005; **63**: 73-78 [PMID: 15963065 DOI: 10.1111/j.1365-2265.2005.02302.x]

65 **Rager KM**, Omar HA. Androgen excess disorders in women: the severe insulin-resistant hyperandrogenic syndrome, HAIR-AN. *ScientificWorldJournal* 2006; **6**: 116-121 [PMID: 16435040 DOI: 10.1100/tsw.2006.23]

66 **Livadas S**, Androulakis I, Angelopoulos N, Lytras A, Papagiannopoulos F, Kassi G. Liraglutide administration improves hormonal/metabolic profile and reproductive features in women with HAIR-AN syndrome. *Endocrinol Diabetes Metab Case Rep*2020 epub ahead of print [PMID: 32554829 DOI: 10.1530/EDM-19-0150]

67 **Dube S**, Errazuriz I, Cobelli C, Basu R, Basu A. Assessment of insulin action on carbohydrate metabolism: physiological and non-physiological methods. *Diabet Med* 2013; **30**: 664-670 [PMID: 23683103 DOI: 10.1111/dme.12189]

68 **Hücking K**, Watanabe RM, Stefanovski D, Bergman RN. OGTT-derived measures of insulin sensitivity are confounded by factors other than insulin sensitivity itself. *Obesity (Silver Spring)* 2008; **16**: 1938-1945 [PMID: 18670420 DOI: 10.1038/oby.2008.336]

69 **Teede HJ**, Misso ML, Costello MF, Dokras A, Laven J, Moran L, Piltonen T, Norman RJ; International PCOS Network. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Fertil Steril* 2018; **110**: 364-379 [PMID: 30033227 DOI: 10.1016/j.fertnstert.2018.05.004]

70 **Woodward A**, Klonizakis M, Broom D. Exercise and Polycystic Ovary Syndrome. *Adv Exp Med Biol* 2020; **1228**: 123-136 [PMID: 32342454 DOI: 10.1007/978-981-15-1792-1_8]

71 **Anastasiou OE**, Canbay A, Fuhrer D, Reger-Tan S. Metabolic and androgen profile in underweight women with polycystic ovary syndrome. *Arch Gynecol Obstet* 2017; **296**: 363-371 [PMID: 28608050 DOI: 10.1007/s00404-017-4422-9]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com