Treatments for Polycystic Ovarian Syndrome (PCOS)-Associated Insulin Resistance

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I pledge my word of honor that I have abided by the Washington College Honor Code while completing this assignment.

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Abstract
PCOS is a complex syndrome including the dysfunction of hormones and the development of cysts on the ovaries. Insulin resistance is a common side effect of PCOS and is caused by oxidative stress, inflammation, and disruptions to the insulin signaling pathway. This thesis is a summary of research outlining potential treatments for PCOS-associated insulin resistance and how these treatments target specific mechanisms within PCOS pathology that cause insulin resistance. Ellagic acid is an antioxidant found in fruits that has been shown to decrease inflammatory factors that contribute to insulin resistance. Metformin is a drug that regulates insulin in type 2 diabetes that has shown to increase GLUT4 translocation and glucose uptake within the cell by improving signaling of insulin sensitizing proteins. Berberine is a compound found in traditional Chinese medicine that has shown to improve the PI3K signaling pathway activated by insulin.
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Polycystic Ovarian Syndrome and Insulin Resistance

Polycystic ovarian syndrome (PCOS) is a disease of the endocrine system that causes issues such as cyst development on the ovaries and excessive presence of androgens (hyperandrogenism). Approximately 7-12% of women in reproductive ages are affected by this disease (Zhu et al., 2022). PCOS can cause infertility and elevates the risk of long-term issues such as type 2 diabetes, endometrial cancer, and elevated lipid levels in the blood (hyperlipidemia) (Zhao et al., 2022). There is also no definitive cause for PCOS; however, there are many theories (Bednarz et al., 2022).

One theory has to do with luteinizing hormone and its role in increased androgens. PCOS patients have high levels of luteinizing hormone compared to non-PCOS patients, and it plays a role in a regulatory loop (Petrillo et al., 2022). The hypothalamus produces the gonadotropin releasing hormone, which triggers the release of luteinizing hormone and follicle-stimulating hormone. When those levels increase, the gonadotropin releasing hormone levels will decrease. The process is a negative feedback loop. To regulate luteinizing hormone and follicle-stimulating hormone when their levels increase, progesterone and estradiol are called in. In PCOS, the luteinizing hormone is less sensitive to regulation (Hernández-Jiménez et al., 2022). As seen in figure 1, the excess luteinizing hormone in PCOS patients continuously stimulates the ovarian cells, which produces an excess of ovarian androgens (Petrillo et al., 2022).

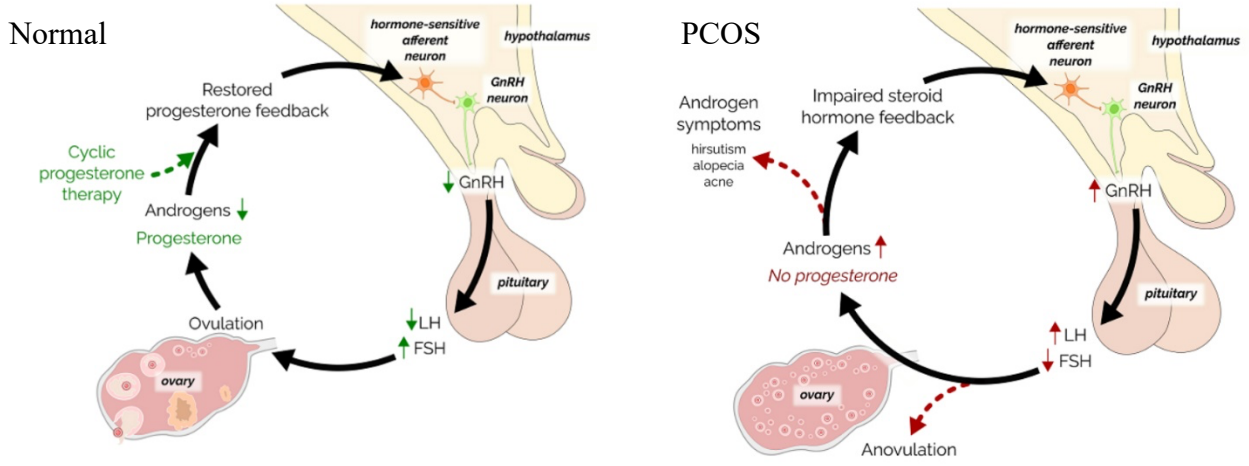

Figure 1. Hormone cycle in normal patients vs PCOS patients. GnRH = gonadotropin releasing hormone, LH = luteinizing hormone, and FSH = follicle-stimulating hormone (Briden, 2022).

PCOS diagnosis is done through two different tools: Rotterdam criteria and Androgen Excess and PCOS (AE-PCOS) Society Assessment. The Rotterdam criteria states that PCOS patients must possess two of the three criteria: irregular or lack of ovulation, hyperandrogenism, and/or multiple cysts on the ovaries measuring 2-9 mm in diameter (Smet & McLennan, 2018). The AE-PCOS Society Assessment is remarkably similar. For a patient to be diagnosed with PCOS, they must meet all the following criteria: hyperandrogenism, ovarian dysfunction (polycystic ovaries and/or irregular or lack of ovulation) and absence of a different disorder that causes androgen excess (Azziz et al., 2009).

The most common symptoms of PCOS include obesity and weight gain, infertility, loss of menstrual cycle, and excessive hair growth. There are also comorbid diseases with PCOS, one being non-alcoholic fatty liver disease (NAFLD). It is a chronic liver disease that is caused by the accumulation of fat in the liver. Patients with PCOS are eight times more likely to have NAFLD due to insulin resistance (Asfari et al., 2020).

Insulin resistance is a major side effect that stems from PCOS. Up to 70% of PCOS patients have insulin resistance (Hernández-Jiménez et al., 2022), and there are many theories as

to why the two are cross linked, such as oxidative stress, inflammation, and issues with microbiota. (Bannigida et al., 2020; Zhao et al., 2022; Zhu et al., 2022). Insulin resistance is usually measured using the homeostasis model assessment for insulin resistance, or HOMA-IR, which equals (fasting plasma insulin x fasting plasma glucose)/22.5 (H.-Q. Qu et al., 2011).

Insulin is a hormone that is secreted by the pancreas and is the main regulator for lipid metabolism, protein synthesis, and cell apoptosis and replication (di Camillo et al., 2016). The most important job of insulin is to aid in glucose uptake and storage using the glucose transporter, GLUT4. Insulin signaling causes GLUT4 to move to the cell membrane for glucose uptake, a process called translocation, even though the exact mechanism is unclear (Lennicke & Cochemé, 2021). Having a resistance to insulin means that glucose is not taken into cells as efficiently because there is a decreased sensitivity to insulin. Since insulin is so important in the body for homeostasis, there are several pathways throughout the body that insulin triggers when it binds to a receptor: PI3k pathway, TSC1.2-mTOR pathway, and MAPK pathways (di Camillo et al., 2016). The two pathways that play a role in insulin resistance are PI3K pathway and MAPK pathway (Davinelli et al., 2020).

The PI3K pathway, also referred to as the PI3K/AKT pathway, is activated when upstream molecules activate PI3k, which then recruits Akt, a signaling protein. Akt has three different isoforms, but Akt2 is found most prevalently in cells that are sensitive to insulin. Akt2 helps regulate glucose in two ways: promoting the translocation of GLUT4 to increase glucose and inhibiting FOX01, a protein that helps increase glucose production from non-carbohydrates, therefore decreasing glucose (Huang et al., 2018). The MAPK pathway has the opposite effect: it down-regulates GLUT4 expression and when it interacts with the PI3K pathway, leading to oxidative stress. When the pathways become dysfunctional, insulin resistance increases (Y.

Zhang et al., 2019). Oxidative stress occurs when the balance of reactive oxygen species and antioxidants is thrown off, allowing oxidation of molecules in the body and alterations within and outside cells by reactive oxygens (Dubey et al., 2021).

There have been correlation studies done to look at the relationship between excess androgens and insulin resistance. One study saw that insulin and luteinizing hormone both increase androgen production in ovarian theca cells. Insulin does this by decreasing the secretion of sex hormone-binding globulin (SHBG), a hormone that binds to testosterone (Soni et al., 2018). Another study showed that if ovarian theca cells were induced to have insulin resistance, there was an increase in androgen activity (J. W. Qu et al., 2009). In PCOS, androgen-forming enzymes are overexpressed due to insulin resistance, and then insulin increases the effect of luteinizing hormone on the theca cells. The different insulin signaling pathways, PI3k and MAPK, also impact androgen synthesis. Because insulin decreases the secretion of SHBG, one would think that because of a resistance to insulin, PCOS patients would have decreased androgen levels. However, the liver is the primary location for SHBG production, and when there is excess glucose or excess androgens in the blood, it decreases production of SHBG. This process creates a positive feedback loop: decreased insulin » increased glucose » decreased SHBG » increased androgens » increased insulin resistance, and the cycle starts over (Petrillo et al., 2022).

Due to the complexity of insulin resistance in PCOS, there are few treatment options other than a change in diet. However, lifestyle changes can be difficult to achieve at best and impossible at worst. Simply put, not everyone can make a lifestyle change of that size. With so many people dealing with this issue and the long-term consequences, discovering a treatment that can help alleviate insulin resistance in PCOS is critical. This thesis will go into the research

and mechanisms behind three different treatment options to manage the complexity of PCOS-associated insulin resistance.

Ellagic acid treatment

Kazemi et al. (2021), in their article, "Randomized double blind clinical trial evaluating the Ellagic acid effects on insulin resistance, oxidative stress and sex hormones levels in women with polycystic ovarian syndrome," discussed the efficacy of ellagic acid on treating insulin resistance in PCOS. According to the authors' research, 5-10% of premenopausal women will be affected by PCOS, but the percentages vary from 2-26% by country. Insulin resistance is a common incidence in PCOS, and research shows it causes oxidative stress. This stress can increase androgen production, further damaging ovarian tissue in PCOS patients. When oxidative stress is increased, the blood antioxidant levels are decreased; therefore, an interest in the use of antioxidants in insulin resistance regulation has been sparked.

Ellagic acid is an antioxidant polyphenol compound (Figure 2) found in strawberries, grapes, pomegranates, and raspberries. It regulates inflammatory signaling cells and suppresses oxidative stress. Ellagic acid has been able to reduce symptoms in NAFLD, hyperlipidemia, type 2 diabetes, and insulin resistance in type 2 diabetes. Despite ellagic acid's reported effects, there is still uncertainty about how ellagic acid functions in the body and a lack of human studies. Due to this, the authors investigated the effect of ellagic acid in blood sugar, insulin resistance, oxidative stress, inflammatory factors, cholesterols, and lipoproteins in women with PCOS.

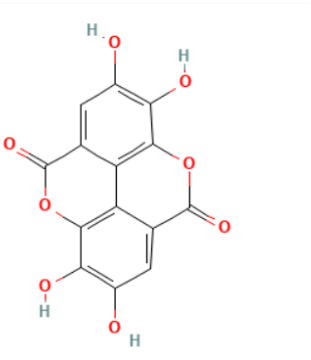

Figure 2. Chemical structure of ellagic acid (*Ellagic Acid*, n.d.)

Women selected for the experiment were between the ages of 18 and 45, and a total of sixty women participated. The participants must have met two of the 3 Rotterdam criteria for PCOS and were separated at random into two groups. There was no statistical difference between the two groups' average height, weight, or BMI. The two groups had thirty participants each, one being the control group with the placebo supplement and the other being the experimental group with the ellagic acid. The participants were given 200mg capsules of their respective treatments daily for 8 weeks. Participants were asked to maintain the same diet and exercise plan they had in their daily life and to avoid foods that were naturally high in ellagic acid. In order to eliminate bias, both the researchers and the patients did not know which group was receiving the placebo.

Fasting blood sugar, plasma insulin, insulin resistance, lipid profile, total antioxidant capacity, oxidative stress markers, and sex hormone levels were used to test the effectiveness of ellagic acid, via blood sample. The HOMA-IR assessment was used to measure insulin resistance. The blood sample was also used to measure the lipid profile, consisting of total cholesterol, triglycerides, low-density lipoproteins, and high-density lipoproteins. A reproductive hormone assay was completed for the sex hormones: testosterone, prolactin, luteinizing hormone, follicle-stimulating hormone, and anti-mullerian hormone. Of the sixty participants, one from the experimental group and two participants from the control group dropped out of the study for unrelated reasons. There were no side effects reported by the participants receiving ellagic acid capsules.

The authors found that ellagic acid significantly decreased fasting blood sugar levels, insulin, and insulin resistance in the experimental group, as can be seen in Table 1. They also found that ellagic acid significantly reduced the total cholesterol, triglycerides, and low-density

lipoproteins in the experimental group, but it did not have a significant effect on high-density lipoproteins. Ellagic acid did decrease the serum levels of malondialdehyde (MDA), c-reactive protein (CRP), and tumor necrosis factors alpha (TNF-α), which are all inflammatory factors and signs of oxidative stress, which can be seen in Table 2. Total antioxidant capacity levels, also shown in Table 2, significantly increased as a result of ellagic acid treatment. There was a significant decrease in total sex hormones of testosterone, prolactin, and anti-mullerian hormone levels. However, there was no significant decrease in luteinizing hormone or follicle stimulating hormone.

Table 1. Effect of ellagic acid on insulin resistance (Table 3 in original article). Data are expressed as means ± SD (Kazemi et al., 2021).

Variables		**Mean ± SD Placebo($n = 28$)**	**Mean ± SD Ellagic acid ($n = 29$)**	**P1**
FBS (mg/dL)	Baseline	107.61 ± 20.13	111.17 ± 18.04	0.3
	End	106.17 ± 21.09	94.29 ± 17.43	0.043
	P2	0.622	0.035	
	Mean Changes	-1.44 ± 0.96	-16.88 ± 0.61	0.04
Insulin (μU/ml)	Baseline	14.98 ± 3.07	15.41 ± 3.24	0.327
	End	14.04 ± 2.19	9.63 ± 1.31	0.03
	P2	0.541	0.027	
	Mean Changes	-0.94 ± 0.88	-5.78 ± 1.93	0.041
HOMA-IR	Baseline	3.98 ± 0.85	4.22 ± 1.14	0.272
	End	3.68 ± 0.41	2.24 ± 0.5	0.04
	P2	0.158	0.031	
	Mean Changes	-0.3 ± 0.44	-1.98 ± 0.64	0.043

Table 2. Effects of ellagic acid on oxidative stress and inflammatory biomarkers (Table 5 in original article). Data are expressed as means ± SD (Kazemi et al., 2021).

Variables		Mean ± SD Placebo($n = 28$)	Mean ± SD Ellagic acid ($n = 29$)	P1
TAC (mg/dL)	Baseline	1.04 ± 0.03	1.03 ± 0.02	0.41
	End	1.03 ± 0.03	1.91 ± 0.07	0.032
	P2	0.358	0.028	
	Mean Changes	-0.01 ± 0.00	0.88 ± 0.05	0.041
MDA (mg/dL)	Baseline	1.47 ± 0.06	1.51 ± 0.09	0.466
	End	1.45 ± 0.07	0.8 ± 0.03	0.039
	P2	0.611	0.032	
	Mean Changes	-0.02 ± 0.01	-0.71 ± 0.06	0.044
TNF-α (pg/ml)	Baseline	17.01 ± 3.78	16.89 ± 4.02	0.41
	End	16.91 ± 4.04	13.5 ± 3.61	0.033
	P2	0.292	0.03	
	Mean Changes	-0.1 ± 0.26	-3.39 ± 0.41	0.038
CRP(ng/ml)	Baseline	9.37 ± 3.19	9.51 ± 3.37	0.359
	End	9.23 ± 2.87	7.01 ± 2.2	0.045
	P2	0.256	0.033	
	Mean Changes	-0.14 ± 0.32	-2.5 ± 1.17	0.048

The authors discussed the additional effects of ellagic acid as a dietary supplement. Despite it only being studied within fruits and not as an isolated compound, research has been conducted around ellagic acid using sources like pomegranate juice. One study showed that with daily consumption of pomegranate juice, triglycerides, total cholesterol, low-density and high-density lipoproteins levels all decreased. Due to ellagic acid's effect on decreased oxidative stress, it actually increased beneficial bacteria in the gastrointestinal tract and decreased excess plasma fat.

Since insulin resistance and oxidative stress are worsened by inflammation, the benefits of ellagic acid can help reverse some damage. With findings showing that ellagic acid can decrease inflammatory factors and oxidative stress indicators, it is implied that ellagic acid is also helping with insulin resistance. Oxidative stress is caused by the presence of reactive oxygen species and can trigger harmful genetic changes that worsen PCOS. Additionally, ellagic acid helped decrease testosterone, prolactin, and anti-müllerian hormone, which are present during hyperandrogenism that is linked to increased insulin. While it needs more research, there

can be a case made for increased insulin sensitivity reestablishing ovulation, which can be achieved using ellagic acid.

Metformin treatment

Oróstica et al. (2022), in their article, "Metformin Treatment Regulates the Expression of Molecules Involved in Adiponectin and Insulin Signaling Pathways in Endometria from Women with Obesity-Associated Insulin Resistance and PCOS," discussed the impact metformin has on regulating the insulin signaling pathways in women with PCOS and obesity-related insulin resistance. As stated in their article, approximately 80% of patients with PCOS also have some form of metabolic pathology, such as obesity or hyperinsulinemia. The reproductive pathology is also thought to be caused in part by the metabolic alterations in the endometrial tissues (the tissue lining the uterus). The GLUT4 transporters have decreased expression in the endometrial tissues, meaning there is a dysfunction in glucose uptake. The TNF-α signaling pathway also has markers indicating its increase in women with PCOS. This is an issue because TNF-α decreases the effect of insulin and the signaling of the insulin sensitizer proteins, like adiponectin. In cases of insulin resistance, adiponectin levels are decreased. There is a common protein between the two pathways: APPL1.

APPL1 is an adapter protein that binds to both insulin and adiponectin receptors, and when the receptors are bound by their molecules (insulin and adiponectin, respectively), it triggers APPL1 bonding. When this bonding occurs, the insulin signaling pathway is activated. Due to this, APPL1 can increase the uptake of glucose by a cell and promotes the interaction of the insulin receptors and insulin receptor substrate 1/2 (IRS1/2). APPL2 works directly opposite to APPL1 by decreasing glucose uptake. When APPL2 binds to APPL1, it stops adiponectin signaling, thus stopping glucose from being transported into the cell. APPL1 already has a

decreased ability to bind to insulin receptors in obese conditions and is expressed less overall. APPL2 levels are increased in obese conditions, allowing it to block APPL1 more effectively.

Metformin (Figure 3) is a widely used drug that treats high blood sugar in type 2 diabetes patients. One of the ways it completes this is through increasing the translocation of GLUT1 and GLUT4 to the cell membrane. It is unknown whether metformin can enhance insulin sensitivity by normalizing levels of APPL1 and APPL2, which is what this study aimed to research.

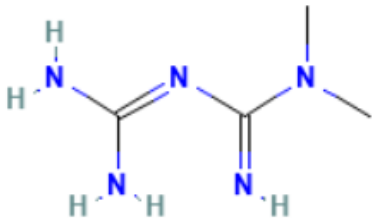

Figure 3. Chemical structure of metformin (*Metformin*, n.d.).

The participants of this study were women between the ages of 21 and 32. They donated endometrial tissue samples that were retrieved via voluntary hysterectomy. The participants were separated into 5 groups: normal weight, obese + insulin resistance, obese + insulin resistance + PCOS, obese + insulin resistance + metformin, and obese + insulin resistance + PCOS + metformin. PCOS prognosis was established using the AE-PCOS Society Assessment and insulin resistance was diagnosed using the HOMA-IR. Treatment groups took 850 mg of metformin for at least 12 weeks, twice daily, and no participants had taken any medications or contraceptives for 3 months before the study was conducted. From the donated tissue, cell cultures were treated with TNF-α or TNF-α + AdipoRon, and the control cells were in serum-free medium.

The author's previous experiment showed that proteins within insulin signaling pathways are decreased in patients with PCOS and that GLUT4 was increased after metformin treatment. Metformin had the strongest impact on GLUT4 levels, being able to elevate the protein to higher levels than in the control group. As can be seen in figure 4, metformin significantly increased GLUT4 levels, even higher than in the control group. The photomicrographs also show significant results in a qualitative way. The metformin treatment groups look more similar to the control group than the untreated groups.

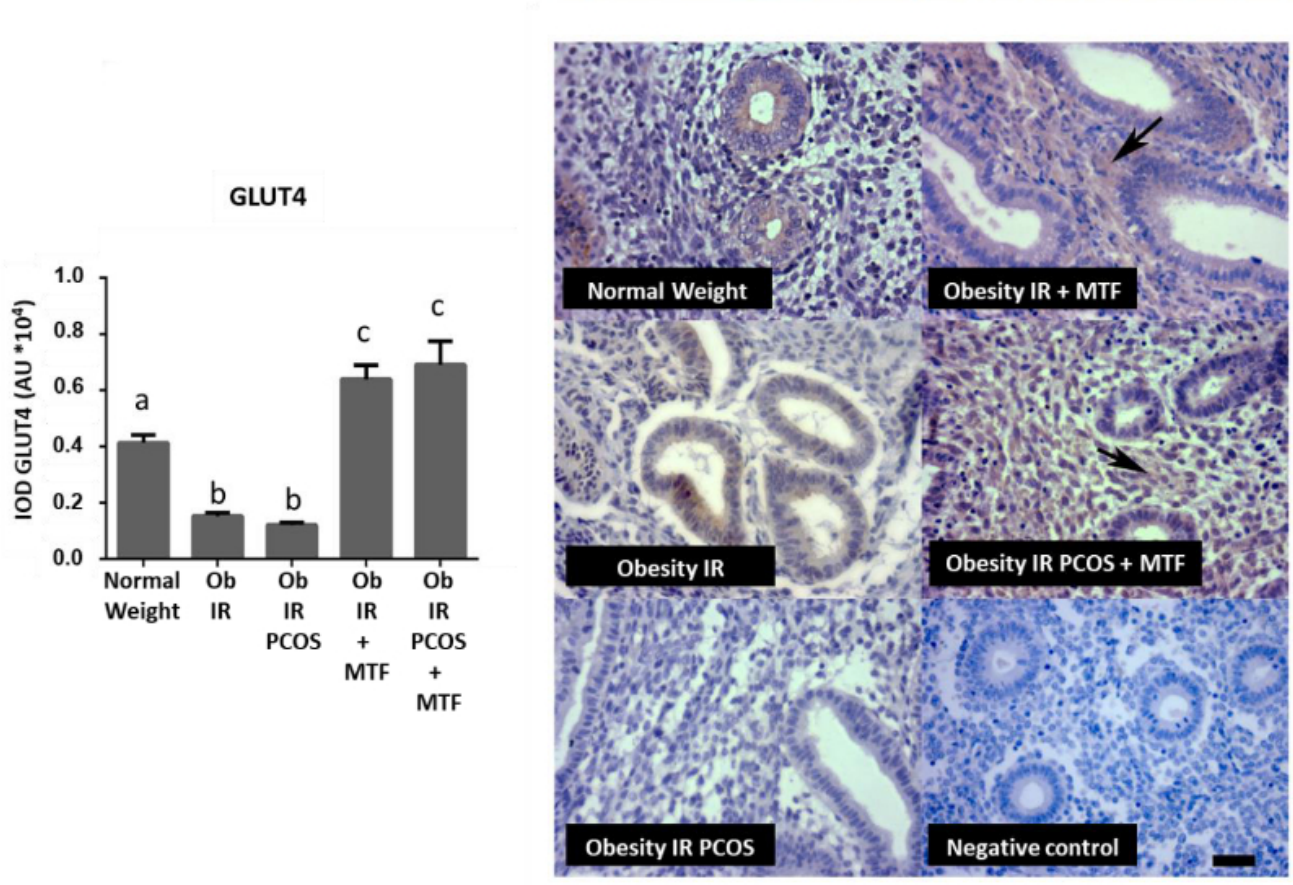

Figure 4. Effects of metformin on GLUT4 expression levels in endometrial tissue (n=7). Photomicrographs show the location of GLUT4 by immune-positive brown color staining, labelled with black arrows (Figure 1B in original article). In the graph, a ≠ b ≠ c indicates a statistically significant difference with a p-value < 0.05. IOD=integrated optical density (Oróstica et al., 2022).

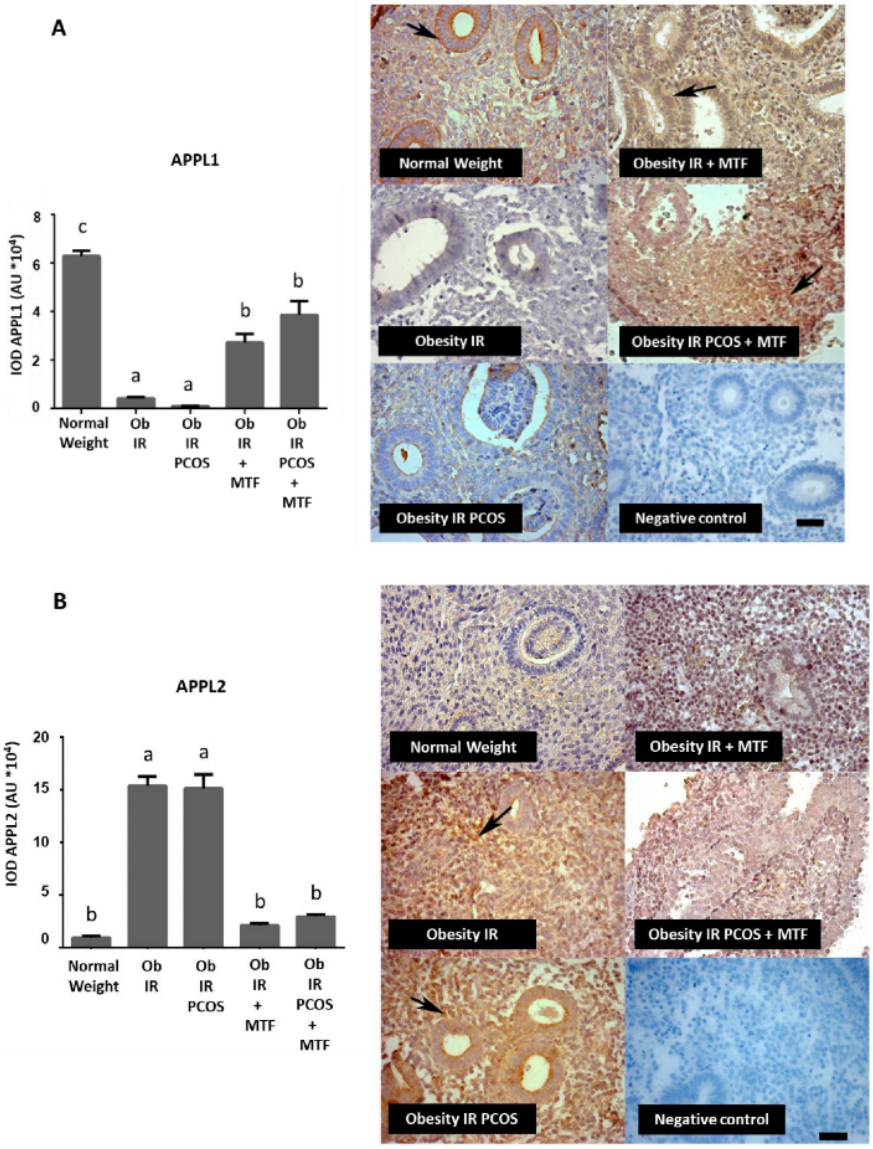

Figure 5. Effect of metformin on APPL1 and APPL2 expression in endometrial tissue (n= 7). Photomicrographs show the location of APPL1 and APPL2 by immune-positive brown color staining, labelled with black arrows (Figure 2 in original article). In the graph, a ≠ b ≠ c indicates a statistically significant difference with a p-value < 0.05. IOD=integrated optical density (Oróstica et al., 2022).

Additionally, in women with PCOS, adiponectin signaling is decreased, especially in the endometria. Metformin both increased APPL1 and decreased APPL2 a significant amount compared to the control and untreated groups (Figure 5). The photomicrographs also show the endometrial tissue samples with APPL1 and APPL2 stained in brown. It is very clear that APPL1

levels were increased in the tissue, as there is more brown staining in the images of tissues treated with metformin, looking similar to the control tissue. The opposite can be said for the APPL2 staining, as there are less proteins in the metformin groups. Metformin was also able to re-establish levels of APPL1 after it was decreased by TNF-α. As mentioned previously, inflammation and the molecules associated with it are increased in PCOS and insulin resistance. Metformin decreased NF-κB and MAPK levels, two inflammation markers. Another test was conducted to test adiponectin as an insulin-sensitizer and yielded an improvement in inulin uptake.

The authors recalled that in a previous experiment, they showed that the proteins in adiponectin signaling had decreased levels in PCOS. In the current study, they saw that the issues with the molecules were present due to insulin resistance, regardless of PCOS status. Metformin was still capable of returning the proteins to normal levels, meaning it influences insulin signaling. There are many debated mechanisms as to why. One suggestion is that it is because of metformin's ability to increase adiponectin and decrease TNF-α and CRP, another inflammation marker. Different studies imply it is because metformin increases the availability and activation of adiponectin signaling. How this works is simple: Low APPL1 expression is linked to high oxidation of fat and low rates of GLUT4 translocation and glucose uptake. APPL2 has the inverse effect.

The results show an increase in APPL1 and a decrease in APPL2 regardless of PCOS status, showing that metformin has an effect on the insulin sensitizing pathway via adiponectin. This process has been shown to prevent interaction between APPL1 and APPL2 in other studies. PCOS is also known to be a pro-inflammatory condition, meaning it has an increase in cytokines, which are inflammatory cell signaling proteins. One prominent example is TNF-α. TNF-α

stimulated cells under increased androgen and insulin conditions are shown to have decreased glucose uptake ability and decreased expression of GLUT4 protein. TNF-α does not work alone; NF-κB and p-38-MAPK proteins also decrease insulin signaling. NF-κB activation by TNF-α has seen decreases in adiponectin level in endometrial cells and p38-MAPK has shown an increase in mitosis in TNF-α. The results indicate that metformin can decrease NF-κB and p38-MAPK, thus improving insulin signaling by increasing proteins in said pathway, and metformin counteracts the negative effects of TNF-α.

Berberine treatment

N. Zhang et al. (2020), in their article, "Berberine decreases insulin resistance in PCOS rats by improving GLUT4: Dual regulation of the PI3K/AKT and MAPK pathways," identify the dysfunction of GLUT4 proteins as one of the causes that negatively affects endometrial tissue in PCOS patients. However, the exact mechanisms are unclear, and there is some questioning if altering the insulin receptor signaling could directly alter GLUT4 expression on PCOS. Insulin receptor signaling is the pathway that triggers GLUT4 expression and translocation to the cell membrane, and the most common one is the PI3K pathway.

An isoquinoline compound called berberine (Figure 6), found in many plants in traditional Chinese medicine, has been shown to treat metabolic conditions, and there has been recent research to suggest its ability to treat insulin resistance in PCOS. Previous research found berberine treatments increased GLUT4 expression in rats with high blood sugar and high cholesterol. While the mechanism is uncertain, it is thought to interact with GLUT4. This article aimed to identify what berberine's effect on GLUT4 expression is and its effects on the PI3K and MAPK signaling pathways to determine if berberine can be used to treat PCOS-associated insulin resistance.

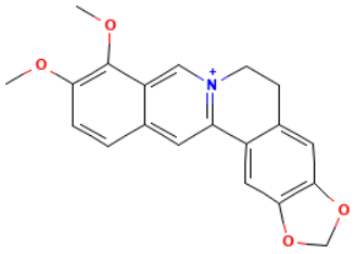

Figure 6. Chemical structure of berberine (*Berberine*, n.d.).

The models for the study were sixty female rats that had equal access to necessary resources, like food, water, etc. They were then separated into 5 groups with 12 rats each: control, PCOS control, PCOS + 100 mg/kg berberine, PCOS + 200 mg/kg berberine, and PCOS + 400 mg/kg berberine. All groups except the control received letrozole to mimic PCOS conditions and the control group received a sodium salt compound. To identify insulin resistance in the models, fasting blood sugar and fasting insulin levels were measured, and insulin resistance was diagnosed using the HOMA-IR. Insulin sensitivity index values were also calculated. The rats were treated for 28 days, and western blotting and real-time quantitative PCR were used to measure the levels of GLUT4, insulin receptor substrate protein (p-IRS), p-PI3K, p-AKT, and the MAPK signaling proteins (p-ERK, p-JNK, and p-P38) in ovarian tissues.

The authors found that berberine was effective in altering insulin resistance levels. At the start of the study, PCOS models had increased HOMA-IR values compared to the control group. Shown in figure 7, berberine helped decrease the HOMA-IR value in rats receiving 200 mg/kg and 400 mg/kg but did not make a significant difference in rats receiving only 100 mg/kg of berberine. Similar effects can be seen for the ISI values. Rats receiving 100 mg/kg of berberine did not show a significant increase in sensitivity to insulin, but the 200 and 400 mg/kg rats did, as indicated in figure 7. GLUT4 mRNA is decreased in PCOS rat models, meaning a decrease in the protein levels as well. Shown in figure 8, berberine increased GLUT4 mRNA and protein

levels in PCOS models but fitting the established pattern of the results; rats receiving 100 mg/kg of berberine did not see this effect. Only the rats that received 200 and 400 mg/kg saw this increase. When looking at how berberine affected the pathway, the researchers found decreased phosphorylated IRS, PI3K, and AKT proteins in PCOS models and that 400 mg/kg of berberine showed a 46%, 30%, and 40% increase, respectively. This implies that berberine helps with activating the PI3K signal pathway or restores activation. The authors also found that berberine reduces the protein expression of MAPK signaling proteins: P38, ERK, and JNK. Reducing these proteins is evidence of berberine capability to inhibit the MAPK pathway.

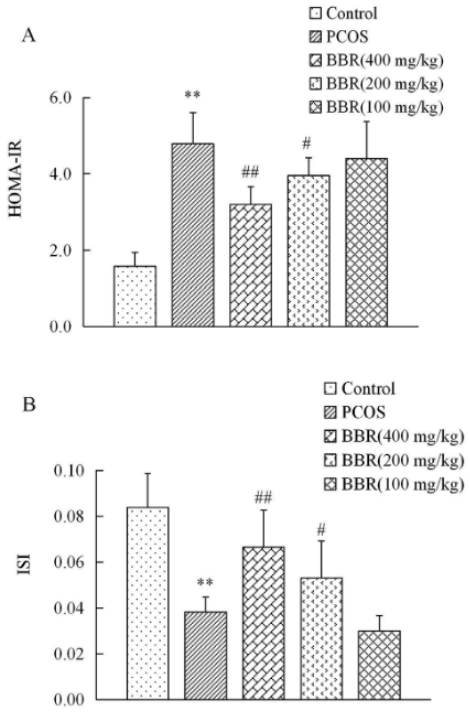

Figure 7. Effects of berberine on HOMA-IR and insulin sensitivity index (ISI). Berberine treatment decreased insulin resistance and increased insulin sensitivity (Figure 1 in original article). **p < 0.01 vs control, ##p < 0.01 vs PCOS control, #p < 0.05 vs PCOS control. BBR=berberine (N. Zhang et al., 2020).

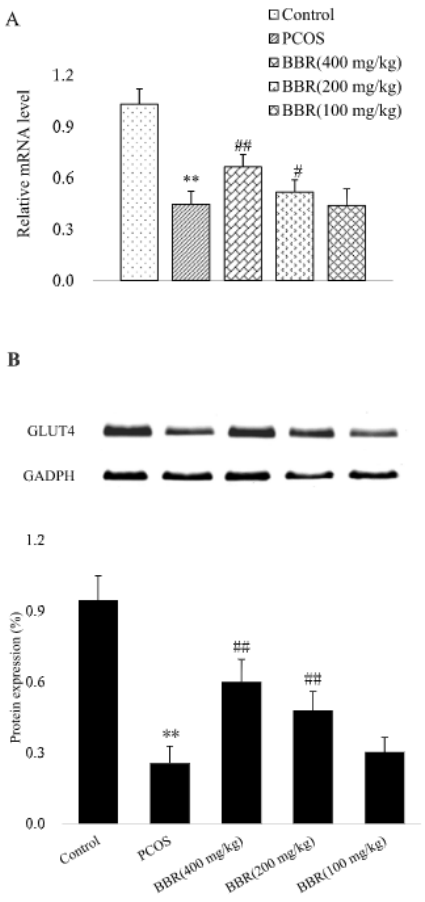

Figure 8. Effects of berberine on GLUT4 expression. Berberine treatment increased GLUT4 mRNA levels and protein expression (Figure 4 in original article). **p < 0.01 vs control, ##p < 0.01 vs PCOS control, #p < 0.05 vs PCOS control. BBR=berberine (N. Zhang et al., 2020).

The authors discuss previous studies conducted with berberine and the results. Berberine has many different benefits, such as decreasing cholesterol and blood sugar and defending against pathogens. Studies have also shown berberine to help insulin resistance in PCOS in vivo, but never identified the process of how it works. The most prominent theory looks at the relationship berberine has on GLUT4 expression and translocation, which was further cemented in this study. Not only did berberine restore GLUT4 expression, but it also alleviated the symptoms of insulin resistance. The insulin signaling pathway regulates GLUT4 expression and translocation, meaning disruptions to this pathway impact a cell's ability to uptake glucose. Some alterations that affect the pathway are down regulating the PI3K signaling or activating the

MAPK pathway. PI3K pathway is a channel of the insulin signaling pathway, so when this is suppressed, GLUT4 cannot be transported into the cell. If the MAPK pathway is upregulated, it increases androgen production in PCOS patients, which is linked to insulin resistance. MAPK signaling also decreases GLUT4 expression and uptake. PI3k signaling down regulation can also trigger MAPK signaling upregulation. In the present study, berberine's effect on the two pathways was researched. Berberine showed an increase in the PI3K pathway proteins and a decrease in the MAPK pathway proteins, meaning it helped reduce insulin resistance by affecting these pathways and increasing GLUT4 expression.

Conclusions

The results of the study conducted by Kazemi et al. (2021) showed that when patients with PCOS and insulin resistance were given 200mg ellagic acid every day over the course of 8 weeks, there was a significant difference in insulin resistance, markers of oxidative stress, lipid profiles, and sex hormone levels. How this was accomplished can be implied by the other effects ellagic acid had, with a focus specifically on the oxidative stress markers and sex hormones. For example, TNF-α significantly decreased over the course of the study, as well as testosterone. As detailed above, the increase of testosterone also causes an increase in oxidative stress; thus, it makes sense when testosterone decreases, so does oxidative stress markers and insulin resistance.

Ellagic acid has been found to reduce insulin resistance and blood sugar in patients with type 2 diabetes (Ghadimi et al., 2021), a common disease that correlates with patients with PCOS-associated insulin resistance (Xie et al., 2021). In the study done by Kazemi et al. (2021), it showed that ellagic acid decreased insulin resistance and fasting blood sugar. Another study done in 2018 also shows promising results that indicate ellagic acid could be beneficial in the liver. The authors found that ellagic acid significantly helped decrease the fat accumulation

around the liver and helped improve insulin signaling in the liver tissues. The development of NAFLD has been linked with increased inflammation within the liver (Polce et al., 2018), and it is known that NAFLD is a comorbid condition with PCOS (Asfari et al., 2020).

Ellagic acid has been shown to reshape the gut microbiota (Yang et al., 2020), another potential theory as to why insulin resistance occurs (Zhao et al., 2022). When ellagic acid is broken down in the gut, the product is a metabolite called urolithin A. Urolithin A helps to improve the microbiota by remodeling its structure (Jin et al., 2022). This metabolite has been shown to potentially decrease the fasting blood sugar levels in the liver by increasing adiponectin signaling. The combined effects of ellagic acid on decreasing inflammatory factors, insulin resistance, and sex hormones makes the polyphenol a possible treatment option for PCOS and PCOS-associated insulin resistance.

The results of the study done by Oróstica et al. (2022) outlined one of the potential mechanisms metformin uses to improve insulin resistance in PCOS patients. The patients were on metformin for a minimum of 12 weeks, and this resulted in a decrease in insulin resistance. Metformin also helped increase APPL1 and decrease APPL2 levels within the adiponectin signaling pathways. APPL2 has been shown to block AKT action, thus stopping glucose uptake into a cell. Additionally, this article showed that metformin helped decrease insulin resistance in obese models, regardless of PCOS presence.

Metformin is a drug that is known to increase insulin signaling and prevent the conversion of non-carbohydrates into glucose in the liver and kidneys. Insulin resistance in PCOS has issues within the PI3K signaling pathway that metformin restored (Hu et al., 2020). Metformin is also able to improve insulin resistance in offspring of mice models. PCOS can affect pregnancy in a variety of ways, since there is the presence of increased androgens, insulin

resistance, and a higher chance of obesity. All these effects can cause issues in pregnancy and in off-spring, but metformin was able to improve these issues in the offspring, such as increasing insulin receptor protein expression (Xie et al., 2021). There is also a way to see how effective metformin could be in PCOS patients. Garzia et al. (2022) looked at the features of PCOS and compared it to the effects metformin had. The authors found that infrequency with menstrual cycle and elevated levels of androgens were the best predictors of the efficacy metformin could have in a patient with PCOS. Since metformin is already used to treat insulin resistance in type 2 diabetes and the correlations between the two diseases (Xie et al., 2021), using the drug to treat insulin resistance in PCOS is not a difficult connection to make. Metformin is actually occasionally used for treatment, but not often, as it is not effective for everyone. However, with research detailing the best predictors for efficacy, metformin can be prescribed to more patients that meet the criterion to help improve their insulin resistance before it progresses into type 2 diabetes.

The results of the experiment done by N. Zhang et al. (2020) showed that berberine treatment can help minimize insulin resistance in PCOS models. Berberine created a significant increase in p-IRS, p-PI3K, and p-AKT, all proteins found within the PI3K pathway. The PI3K signaling pathway is important in insulin signaling and was suppressed in PCOS models. The MAPK pathway has shown to block the PI3K pathway and was suppressed during berberine treatment. This was measured using proteins typically found within the pathway: p-ERK, p-JNK, and p-P38. Berberine was able to activate the PI3K pathway and downregulate the MAPK pathway in rat models to increase the GLUT4 translocation into the cell.

Berberine is a naturally occurring compound from traditional Chinese medicine that has been found to decrease inflammation and decrease blood sugar in non-diabetic patients. A recent

study showed that berberine decreased inflammatory signaling cells and increased glucose uptake, but it also decreased macrophages, immune cells that breakdown tissues, cells, etc. (Lin et al., 2019). Berberine also decreases insulin resistance and helps increase the glucose storage in the liver. This is done through the activation of the sirtuin1 signaling pathway, which plays a role in regulating the oxidative stress levels in the liver. Sirtuin1 regulates the FOXO1 gene and protein expression, which is linked to increased oxidative stress (Sui et al., 2021).

Another study found that berberine was able to help decrease insulin resistance in fructose fed mice. Fructose intake is related to increased hunger and appetite and is commonly found in a lot of processed foods in the USA. Leptin is a hormone that regulates appetite and was found to be increased as a result of berberine treatments (Li et al., 2020). Given berberine's effects on inflammation, oxidative stress, glucose uptake, and insulin resistance in both glucose and fructose, there is a clear connection to how berberine could be used as a treatment for insulin-resistance in PCOS.

PCOS is a complex syndrome that creates many different issues within the body, with the most common issue being insulin resistance. Insulin resistance, or a decreased sensitivity to insulin, usually leads to issues such as high blood sugar and eventually type 2 diabetes (Hernández-Jiménez et al., 2022). Since PCOS causes additional complications in insulin signaling, the mechanism of insulin resistance in PCOS is different than in other metabolic conditions (Hu et al., 2020). There is no treatment for PCOS-associated insulin resistance at the moment, but there have been promising results in recent years. Ellagic acid, metformin, and berberine are three compounds that can help alleviate insulin resistance in PCOS because of their unique approaches to PCOS specific pathologies, such as increased oxidative stress, errors in the

PI3K signaling pathway, and increases in the MAPK pathway (Kazemi et al., 2021; Oróstica et al., 2022; N. Zhang et al., 2020).

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