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Comparative analysis of myo-inositol and metformin in the treatment of insulin-resistant women

PhD thesis

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LIST OF ABBREVIATIONS

AE-PCOS	Androgen Excess and PCOS Society
AMH	anti-Müllerian hormone
AMP	adenosine monophosphate
ASRM	American Society for Reproductive Medicine
BMI	body mass index
CIs	confidence intervals
DCI	D-chiro-inositol
DM	diabetes mellitus
DPP-4	dipeptidyl peptidase-4
ESHRE	European Society for Human Reproduction
FAI	free androgen index
FDA	Food and Drug Administration
FPG	fasting plasma glucose
FPI	fasting plasma insulin
FSH	follicle-stimulating hormone
GIP	glucose-dependent
GLP-1	glucagon-like peptide 1
GnRH	gonadotropin releasing hormone
HCPOS	Hungarian Polycystic Ovarian Syndrome
HDL	high-density lipoprotein
HOMA-IR	homeostasis model assessment for insulin resistance
IDDM	insulin dependent diabetes mellitus
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
IR	insulin resistance

IP	insulinotropic peptide
IPP	inositol phosphoglican
LH	luteinizing hormone
MAP kinase	mitogen activated protein kinase
MET	metformin
MYO	myo-inositol
NIDDM	non-insulin-dependent
NIH	National Institutes of Health
OGTT	oral glucose tolerance test
PCOS	polycistic ovarian syndrome
PI3-kinase	phosphatidylinositol 3-kinase
POMC	proopiomelanocortin
PRL	prolactin
SEM	Standard Error of Mean
SHBG	sex hormone binding globulin
T2DM	type 2 diabetes
VDR	vitamin D receptor

1. INTRODUCTION

1.1. Insulin resistance

A key characteristic of metabolic disorders, which is believed to be the pathogenic cause of many current diseases, is insulin resistance (IR), the primary risk factor for many diseases (1-3). Type 2 diabetes (T2DM), polycystic ovarian syndrome (PCOS), non-alcoholic fatty liver disease, metabolic syndrome, obesity, and impaired glucose tolerance are only a few of the pathological states that are associated with IR, which also produces a wide spectrum of clinical symptoms (2, 4). It is also widely recognized that IR plays a role in the pathophysiology of a wide range of metabolic conditions, atherosclerosis, cardiovascular disease, multiple types of cancer, and neurological diseases (3, 5).

Since insulin serves a variety of purposes in the body, hyperinsulinemia, which can arise from an inability to control the metabolism of carbohydrates, can lead to a wide range of health issues (6). Recent research indicates that IR has a broad effect on working-age individuals and is closely linked to obesity. According to research, between 15.5% - 51.0 percent of individuals in highly industrialized nations suffer from IR (4). Nevertheless, studies have demonstrated that IR can also affect those who appear healthy and do not gain weight (4). Furthermore, lifestyle and genetics are considered to be the main factors influencing IR issues. Because insulin directly affects the liver, white fatty tissue cells, and skeletal muscle—all of which have distinct roles in maintaining metabolic homeostasis—insulin's role in glucose balance is unique (7)

Insulin resistance is characterized by alterations in its intracellular signaling (8), affecting several metabolic abnormalities with or without body mass index (BMI) alterations (9-11). As a prevalent condition that might affect a woman's ability to conceive, insulin resistance is marked by phenotypic heterogeneity (9). For instance, the most prevalent endocrine condition affecting women's fertility is polycystic ovary syndrome (11), and IR appears to be one of the main mechanisms leading to irregular menstruation, anovulation, and infertility (12). Between 50 - 90 % of women with PCOS have shown insulin resistance (13). Due to different cellular reactions to insulin, such as resistance to its metabolic effects and simultaneous increased steroidogenesis, which may present as hyperandrogenism, metabolic malfunction, and reproductive

issues (14). BMI, physical activity, and cardiorespiratory fitness alter the rhythmicity of insulin sensitivity and related metabolites throughout the menstrual cycle (15).

1.1.1. Diagnosis of insulin resistance (IR)

Many models have been established to diagnose insulin resistance (16-18). Compensatory hyperinsulinemia as a consequence of IR (19) and preserved, actual beta-cell function are easiest assessed in daily medical diagnosis by the homeostasis model assessment formula for insulin resistance (HOMA-IR) developed by Matthews et al (20).

In assessing the dynamics of carbohydrate metabolism glucose tolerance test mostly performed with 75g glucose and in parallel with insulin measurements is also a fundamental element of diagnostics (21, 22). Following menstrual cycle alterations and assessing hormonal profiles in both the follicular and the lutheal phase of the menstrual cycle, are important tools for evaluating the effectivity of therapy during treatment influenced by the compliance of the patients (23). The effectiveness of the applied treatment can be followed by repeating the diagnostic tests measurement of insulin sensitivity and characterisation of insulin response.

The most commonly used technique is the previously described oral glucose tolerance test (OGTT) (24) supplemented by insulin measurements (25) as a 3 point (26), as a 5 point (27) or as a 6 point (28) diagnostic tool. However, the same amount of glucose can result is significantly different insulin response in the very same individual due to numerous external and internal circumstances, which is likely to cause diagnostic and follow-up difficulties in case of OGTT curves (29). Several parameters have been considered (28, 30, 31) that can function as standardized and reproducible variants correlating closely with diurnal serum insulin changes, allowing longitudinal detection of changes in carbohydrate metabolism. In case of human liver cells estrogens and thyroid hormones enhance sex hormone binding globulin (SHBG) synthesis. Prolactin and insulin impede the biosynthesis of SHBG (32, 33). These correlations provide possible chance to describe insulin sensitivity or hyperinsulinemia using repeatedly determined serum SHBG levels (34).

1.1.2. Treatment of insulin resistance

In the treatment of IR and PCOS life-style changes are strongly recommended, showing outstanding results combined with complex tailored treatment (35).

To stay healthy and prevent non-communicable diseases, people of all ages need to establish healthy habits, in accordance with medical advice. Additionally, monitoring nutritious food intake is crucial when there are risk factors for cardiovascular disease (36). Furthermore, the most popular recommendations for enhancing insulin sensitivity include weight loss and lifestyle changes (e.g., eating a balanced diet and exercising) (37). Regular exercise and lifestyle changes that target weight loss and calorie reduction are part of the complex tailored treatment. It is believed that many dietary patterns, including the Mediterranean, can lower IR and help maintain a healthy body weight (6). Women displaying insulin resistance (IR) and associated higher insulin levels are frequently subscribed insulin sensitizers. This approach is utilized to treat IR-related conditions including PCOS, with the aim of reducing insulin release and restoring adequate endocrinological and clinical characteristics (38). Among the most researched and frequently prescribed for these patients is a biguanide, metformin (MET). MET promotes peripheral insulin sensitivity, inhibits gluconeogenesis, and slows absorption of glucose in the gastrointestinal tract (39).

According to several studies (40-44), PCOS may cause insulin resistance by impairing the inositol phosphoglycan (IPP) mediated second messenger pathway. Identified as a member of the vitamin B group, myo-inositol (MYO) is an insulin-sensitizing substance and an isomer of a C6 sugar alcohol (45). Recent research has shown that MYO acts as an essential component in the regulation of glucose metabolism by triggering enzymes (40, 42), and PCOS patients with IR have been reported to have lower MYO levels (46). MYO supplementation has been shown to improve ovulation and reduce levels of serum insulin and testosterone, thereby improving the metabolic and hormonal characteristics of PCOS women with IR (46, 47).

1.2. Polycistic Ovarian Syndrome (PCOS)

At present, the most prevalent factor causing fertility problems in women is PCOS (48, 49). The identification of related metabolic conditions has altered the

perspective of PCOS, which is now seen as a sign of subsequent chronic illnesses (50-52). In addition to preserving and improving the female cycle and fertility, its medical approach has come to focus on the early detection, treatment, and, if feasible, eradication of metabolic disorders (53, 54).

With a current prevalence of 5–10% (55–57) globally and about 7–10% in Europe, PCOS displays an alarming rate in the population. (55-57). This condition, which is influenced by both external and genetic aspects, is crucial for a number of diseases, including Polycystic Ovary Syndrome (PCOS), being currently one of the most prevalent endocrinopathies affecting women before menopause (58-60).

Over the past ten years, PCOS' manifestation has considerably changed. In addition to the typical obese phenotype, PCOS can also present as normal or low body weight, as has been found in recent decades (61, 62). Along with the typical obese characteristics, PCOS has been found in the more recent years to present also in case of healthy or low body weight (63, 64). A greater proportion of females with regular or even low body weights have been identified as an addition to the traditional profile of overweight individuals (65-68). Additionally, unlike healthy women, regular or lean patients with PCOS are at greater metabolic and cardiovascular risk (69, 70). Insulin response to glucose load improves when PCOS is treated with modern, individualized, efficient therapy (71, 72).

Nowadays, as PCOS being a highly prevalent reason behind fertility problems (48), its perception has evolved as a result of the identification of related metabolic anomalies, and it is currently regarded as an early indicator of subsequent chronic illnesses. (50). Besides restoring the female cycle and fertility, its therapeutic strategy increasingly focuses on early detection, healthcare, and, if feasible, eradication of metabolic disorders (53).

The appearance of PCOS is known to be significantly influenced by the effect of hyperinsulinaemia on the ovaries and enhanced androgen synthesis in theca cells in response to insulin (65, 73). A condition known as the "insulin resistance paradox" occurs in PCOS, when sensitivity to insulin is maintained in the female reproductive organs but reduced in the fat cells, liver, and muscles (74, 75). Key differences in how insulin secretion adapts to carbohydrate consumption during meals provides insight into how ovarian hormone secretion is altered parallel to beta cell function. Evidence

shows that the onset of insulin resistance at the early stages of metabolic disorders is marked by significant changes is insulin response (76). Therefore insulin action is marked by a delayed insulin peak (due to secondary beta cell dysfunction). Impairment of beta cells can also manifest mainly as a primary insulin response alteration characterized by an early onset of decreased insulin secretion (77, 78). As a result, the early, initial insulin peak is lost or diminished, which minimizes the inhibition of glucagon release and enables constant glucose release in the liver (79).

Due to the decreased early insulin and the delayed second phase insulin response, an extended insulin action induces the significant rise of blood glucose levels in the initial few minutes after meals, as well as a higher second (slow) phase insulin production (80). A few hours after meals, the occurring prolonged insulin response promotes hypoglycaemia. The reduced basal metabolic rate and recurrent hypoglycaemia are linked to weight gain and the development of characteristic insulin resistance with a high HOMA score (81, 82).

Between 85 and 90 percent of women referring to longer than 35 day cycle lengths and hereby experiencing oligomenorrhea, can also be diagnosed with PCOS (83). Numerous factors, including body size, physical activity, smoking, alcohol use, and pathologic conditions such polycystic ovarian syndrome, influence fundamental characteristics of the menstrual cycle (84-88). As science has evolved, so has the diagnosis of PCOS. There are now 3 different sets of criteria for PCOS (56, 89, 90) overlapping, without more thorough consistency. All three classifications of PCOS cover irregular menstrual cycles and lengths. Uneven and prolonged menstrual cycles have been associated with lower levels of sex hormone binding globulin (SHBG) and higher levels of androgen (91-93).

Numerous studies have examined the connection between serum SHBG levels and insulin sensitivity (measured by the HOMA index) (94, 95). Research has shown that SHBG and the Homeostasis Model Assessment (HOMA) index have an inverse linear association, determining SHBG as an efficient indicator for metabolic characterisation (96-98). However, because of its great variability, several studies have cautioned about using SHBG as a biomarker in PCO syndrome (99).

1.2.1. Diagnosis of PCOS

To maintain or restore fertility, mitigate symptoms, and avoid complications that may arise in women with PCOS from adolescence to the postmenopausal stage, it is critical to diagnose and treat the condition. Multiple PCOS phenotypes, substantial individual variation in clinical features, and conflicting diagnostic criteria from the National Institutes of Health (NIH), the Androgen Excess and PCOS Society (AE-PCOS), the European Society for Human Reproduction and Embryology and American Society for Reproductive Medicine (ESHRE/ASRM Rotterdam), and others show that PCOS is a complex and not always easily diagnosed multifactorial condition (100, 101).

No single diagnostic criterion (like hyperandrogenism or polycystic ovary) is sufficient for the clinical diagnosis because the classic presentation, as described by Stein and Leventhal, with features of obesity, amenorrhea, and hirsutism, is at one end of the spectrum that, at the other end, includes women with normal menstrual cyclicity but with polycystic ovarian appearance on ultrasound (102).

Oligo-ovulation, clinical or biochemical hyperandrogenism, and the exclusion of other recognized conditions including late-onset congenital adrenal hyperplasia and Cushing's syndrome were the main concluded requirements at the 1990 NIH-sponsored conference for definition (12). The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group revised the syndrome's diagnostic criteria in 2003. The criteria were sonographically confirmed PCOS, oligo/amenorrhea, together with clinical and biochemical signs of hyperandrogenism (56). After ruling out other causes such congenital adrenal hyperplasia, androgen-secreting tumors, or Cushing's disease, two of the three criteria are necessary for the diagnosis. The presence of 12 or more follicles, each measuring 2 to 9 mm in diameter, and/or a larger ovarian volume (10 ml) are sonographic characteristics of PCOS. This does not depend on the distribution of follicles or the echogenicity of the ovarian stroma. PCOS can be defined with just one ovary meeting these criteria (89, 103).

According to the 1990 NIH criteria, women who have previously been diagnosed with PCOS are included in the patient population defined by the 2003 Rotterdam criteria. The NIH 1990 standards have been expanded upon, not replaced, by the 2003 Rotterdam criteria. The recognition of two new PCOS phenotypes with polycystic ovaries, hirsutism, and/or hyperandrogenemia but normal ovulation, and

women with polycystic ovaries and irregular ovulation but no indication of androgen excess—has consequently expanded the range of patients with this condition (103-105). The Androgen Excess PCOS Society recommended in 2006 the diagnostic criteria's adjustment to exclude those who do not exhibit symptoms (such as PCO on ultrasonography and oligomenorrhea/amenorrhea but no hyperandrogenism) (89).

It should be mentioned that PCOS is a diagnosis of exclusion, meaning that less common illnesses (Cushing's syndrome, virilizing tumors, etc.) should be excluded clinically, while more frequent health issues as thyroid dysfunction and hyperprolactinemia should be excluded biochemically. Nevertheless, insulin resistance and cardiometabolic characteristics are not currently included in the diagnostic criteria for PCOS. This is partly because of techincal limits at measuring insulin resistance in everyday clinical practice (106).

A 2-hour oral glucose tolerance test should be used to screen for impaired glucose tolerance (IGT) in all women with PCOS, according to the AE-PCOS Society. Women who had normal baseline glucose tolerance should also be screened for IGT at least every two years, or sooner if they have other risk factors for type 2 diabetes, such as obesity, a family history of diabetes, or metabolic syndrome (107, 108).

1.2.2. The role of insulin resistance in PCOS pathogenesis

Between 50 and 90 percent of women with PCOS are thought to have insulin resistance (13). Hyperinsulinemia, which arises as a compensatory reaction to insulin resistance, acts together with luteinizing hormone (LH) as a co-gonadotrophin in the cells of the ovaries (109). Consequently, the production and release of androgens are enhanced by ovarian androgen biosynthesis (110). Insulin additionally triggers termination of pre-antral follicle growth in the ovaries (111). Other extra-ovarian pleiotropic effects of hyperinsulinemia include reduction of hepatic sex hormone binding globulin synthesis (112), stimulation of adrenal P450c17α activity, and amplification of LH pulses (75).

Reproductive, hyperandrogenic, and metabolic characteristics are improved when insulin sensitivity enhances in PCOS (either by medication or weight loss), which supports the idea that insulin resistance is involved in the pathophysiology of the disorder (113). On the contrary, women who are genetically susceptible to PCOS

frequently exhibit the clinical characteristics of the syndrome as a result of excess weight (along by developing insulin resistance). Both insulin resistance and hyperinsulinemia play a significant role in the development of PCOS (113, 114). Mitogen-activated protein kinase (MAP kinase) and phosphatidylinositol 3-kinase (PI3-kinase) are the two key mechanisms through which insulin exerts intracellular effects. The MAP kinase route mediates cell growth and steroidogenic effects, while the PI3-kinase pathway promotes metabolic effects, such as the disposal of glucose into skeletal muscle (115). It seems that the MAP kinase pathway functions normally in PCOS, but the PI3-kinase pathway is affected (113). As a result, cells react differently to insulin, exhibiting resistance to its metabolic effects and simultaneously increasing steroidogenesis, leading to reproductive issues, hyperandrogenism, and metabolic disorders (14). By exhibiting increased 5α reductase activity, the intact MAP kinase receptor pathway plays a significant role in insulin resistance that enhances the onset of hyperandrogenism in PCOS (116). The hyperandrogenism associated with PCOS is probably caused by the increased conversion of testosterone to the more powerful androgen, 5α-dihydrotestosterone. The breakdown of cortisol with less negative feedback on the pituitary is another consequence of increased 5α reductase activity. As a result, PCOS causes the hypothalamo-pituitary adrenal axis to turn hyperactive, that results in increased synthesis of adrenal androgen (116).

1.2.3. Treatment of PCOS targeting carbohydrate metabolism disorders

1.2.3.1. Lifestyle, physical exercise, diet

Medical care options should be customized according the medical condition. Reducing the detrimental effects of this chronic disease and reducing anxiety can be achieved by receiving education on the short-term and long-term consequences of PCOS from a trustworthy, objective source. To enable lifestyle change, which is unlikely to be successful without first addressing education and psychosocial difficulties, psychological elements must be realized, discussed, and counselled parallel to therapy (117).

According to an evidence-based approach, changing one's lifestyle is the first line of treatment for the majority of overweight PCOS women (118). Additionally, it is important to prevent excessive weight gain in all women with PCOS, regardless of their baseline weight. Reproductive aspects (menstrual cyclicity, ovulation, and fertility) (119, 120), metabolic features (impaired insulin sensitivity and/or hyperinsunilaemia are risk factors for cardiovascular disease and type 2 diabetes), and psychological outcomes (121) can all be improved with already 5% to 10% weight loss. Research proves that even when women continue to be in the overweight or obese weight range, changing their lifestyles with modest, attainable goals has a positive clinical impact (119, 122, 123).

1.2.3.2. Insulin sensitizing drugs

Type 2 diabetes mellitus is commonly treated with the antihyperglycemic biguanide metformin. Metformin reduces the effect of glucagon and inhibits hepatic gluconeogenesis, which lowers the level of insulin and glucose in the blood. It seems that inhibition of mitochondrial complexes is the background mechanism. Insulin-sensitizing medications (metformin, rosiglitazone, pioglitazone, inositol) have downstream effects on cyclic (adenosine monophosphate) AMP and protein kinase signaling pathways. Lipid production may potentially be modulated by the impact on protein kinase.

1.2.3.2.1. Metformin

A biguanide insulin sensitizer is metformin (124). It enhances insulin action but has no effect on insulin secretion (125). It lowers blood lipid levels, reduces the synthesis of glucose in the liver, encouraging insulin-mediated glucose absorption in the liver and skeletal muscles, and inhibiting the use of gluconeogenic substrates (126). In terms of insulin resistance and hyperinsulinemia, obese women with PCOS have metabolic consistencies with those who have type 2 diabetes (127).

Metformin has been used to treat polycystic ovarian syndrome since 1994 as an insulin sensitizer (128). It is well recognized that metformin affects a number of organs impacted by insulin resistance, such as the ovaries, liver, and adipose tissue (129).

Because of its beneficial effects on insulin resistance and menstrual cycle, metformin, a well-known insulin sensitizer (130, 131), is often administered in PCOS (132). In obese women with PCOS, body mass index decreases by an average of 1.25 kg/m2 during MET treatment (133). Weight loss is the primary mechanism for improved insulin sensitivity during MET administration (134). Reduced insulin resistance (131), which has a direct impact on ovarian insulin sensitivity (135), is linked to a better menstrual cycle following MET treatment. Women with PCOS did not experience any changes in their quality of life throughout MET (136, 137). Treatment interruption is often caused by adverse effects during MET, particularly gastrointestinal side effects (131, 138).

1.2.3.2.2. Thiazolidendiones

Rosiglitazone and pioglitazone are thiazolidinediones. The nuclear transcription factor perioxisomes proliferator activated receptor γ is selectively activated by thiazolidinediones. These are drugs used to treat non-insulin-dependent diabetes mellitus (NIDDM). While they reduce high blood sugar in diabetics, they have no effect on blood glucose levels in non-diabetics despite lowering insulin levels.

Both pioglitazone and rosiglitazone are frequently used in clinical trials on women with PCOS, although due to the possibility of fetal development limitation in animal tests, the Food and Drug Administration (FDA) has categorized them as pregnancy category C medicines (139). Their usage in obese women with PCOS is further limited by possible weight gain among users (39). Although rosiglitazone is

currently marketed in the United States, the European Medicines Agency has banned its usage in the European Union and withdrawn it from South Africa, New Zealand, and India. Because of a long-term use link to bladder cancer, pioglitazone has been banned in various countries (140, 141).

1.2.3.2.3. Incretins, Glucagon-like peptide 1 (GLP-1) receptor agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors

Glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), which are both gut-secreted hormones in response to meal consumption (incretins) that increase glucose-stimulated insulin production (142). By lowering hepatic glucagon release, delaying stomach emptying, and decreasing food cravings, incretin hormones also preserve glucose homeostasis, supporting weight control and enhancing glycaemic control (143). Researches with appropriate case numbers indicate mostly lower GLP-1 levels and activity in overweight/obese PCOS individuals (143, 144).

Following an OGTT women with PCOS have been found to have higher GIP and lower GLP-1 values (145). Compared to GIP, which has a half-life of five minutes, endogenous GLP-1 has a short half-life of one to two minutes and is rapidly deactivated by the pro-teolytic enzyme dipeptidyl peptidase-4 (DPP-4) (146). Oral DPP-4 inhibitors raise endogenous physiological levels of GLP-1 and GIP, which in turn improves glycaemic control (146). GLP-1 agonists function similarly to native GLP-1, withstanding DPP-4 degradation. GLP-1 receptor agonists and DPP-4 inhibitors are relevant treatment tools for PCOS management and improving metabolic parameters (147).

GLP-1 modulates nitric oxide and endocannabinoid pathways the postsynaptic gonadotropin hormone-releasing hormone (GnRH) neurons (148). It further influences the release of GnRH from the hypothalamic neurons. Additionally, the region that overlaps the hypothalamic arcuate nucleus and is home to proopiomelanocortin (POMC) neurons exhibits elevated expression of GLP-1 receptors. The effects of GLP-1 receptor agonists are also expressed in the ovaries (149). A significant decrease in androstenedione, free testosterone, and an increase in SHBG were observed in obese women with PCOS who received liraglutide treatment (150).

GLP-1 enhances insulin sensitivity in peripheral tissues in addition to its glycaemic impact. Insulin sensitivity and glucose absorption in muscle and adipose tissue can be improved by raising GLP-1 concentrations, using GLP-1 receptor agonists or DPP-4 inhibitors (151). By lowering overall glucagon secretion and altering the insulin/glucagon ratio, GLP-1 promotes glucose elimination in an insulin-independent manner (152). Treatment with GLP-1 analogues decreases the inflammatory response in obese patients by decreasing the release of inflammatory cytokines by macrophages, such as interleukin-1 β, interleukin-6, and tumour necrosis factor-β (153). GLP-1 promotes insulin sensitivity by lowering the inflammatory response (154).

Because of decreasing gastric smooth muscle activity, GLP-1 delays glucose absorption and reduces postprandial glucose rises, by reducing intestinal motility and gastric emptying (155). Furthermore, GLP-1 has a major role in reducing hunger and increasing the sense fullness, which lowers food consumption and aids weight loss (156). Pancreatic β-cells express GLP-1 receptors, which trigger the release of insulin into the circulation (143, 157). By stimulating β-cell growth, differentiation, and proliferation, GLP-1 increases the mass of β-cells in the pancreas (158, 159). By decreasing apoptosis brought on by different cytotoxic stressors, GLP-1 improves β-cell survival (143).

1.2.3.3. Inositol

Nine stereoisomers of the polyalcohol inositol exist. Myo-inositol and D-chiro-inositol (DCI) are two of them that have been demonstrated to mediate the postreceptor actions of insulin. Fruits, especially citrus fruits, beans, corn, and nuts have the highest alimentary inositol contents (160). MYO absorption is adversely affected by DCI in the gut.

The lack of MYO is linked to ovulatory failure in PCOS (161), and MYO is a second messenger in the follicle-stimulating hormone (FSH) signaling pathway. Women with PCOS who sought conception experienced an increase in ovulation frequency during MYO therapy (162, 163). Furthermore, investigations of women with PCOS have shown that MYO has favorable effects on insulin resistance, lipids, and testosterone (46, 164). These findings imply that MYO may be used in PCOS not only in fertility contexts.

MYO raises high-density lipoprotein (HDL) cholesterol, lowers body weight, and reduces leptin levels (165), but the metabolic risk factor advantages of inositol therapy did not apear in morbidly obese women. MYO enhances cell development and structure, as well as the synthesis of lipids involved in cell membranes, because of its antioxidant effect. A malfunction in MYO implementation may affect insulin and FSH signaling in PCOS. Every organ has a unique MYO/DCI ratio that is associated with its function (166). DCI causes the ovary to produce too much insulin-dependent testosterone, while MYO increases the action of FSH by producing anti-Müllerian hormone (AMH). MI has been detected in follicular fluid and seems to enhance the quality of oocytes and embryos (167).

The MI/DCI ratio is typically 100:1, but in PCOS, it is 0.2:1 (168). Epimerase activation is excessive when the concentration of MI in the follicular fluid is decreased (as it is in PCOS, when it is 500 times lower), which results in an excess of DCI, an increase in insulin resistance, and a rise in LH levels. Blastocyst quality was reduced when DCI concentrations in the follicular fluid exceeded the 70:1 MYO/DCI cut off ratio. A MYO/DCI ratio of 40:1 is ideal for supplementing, aiming to restore ovulation and menstrual cycle while lowering levels of LH, testosterone, and insulin and raising progesterone and SHBG levels (169). By lowering testosterone and androstenedione levels, adjusting the LH/FSH ratio, restoring regular menstrual cycles, and inducing ovulation, MYO promotes spontaneous conceptions through sufficient luteal phase progesterone production (41).

1.2.3.4. Vitamin D

Vitamin D is a steroid hormone, mostly produced by the skin when exposed to UV light, with less than 10% to 20% originating from food (170, 171). Vitamin D receptor (VDR), a member of the steroid/thyroid nuclear hormone receptor superfamily, mediates the physiological effects of vitamin D (48, 172). VDR has been found in many different reproductive organs, including the ovary (especially granulosa cells), uterus, placenta, testis, hypo-thalamus, and pituitary, in addition to calcium-regulating tissues such the bowels, bones, and parathyroid glands (173-175). This varied VDR expression relates to the function of vitamin D in the physiology of female reproduction (172).

Influencing AMH signaling, follicle-stimulating hormone sensitivity, and progesterone synthesis in human granulosa cells, vitamin D has a physiological function in reproduction, including ovarian follicular development and luteinization (176). It affects glucose homeostasis in many ways: through the vitamin D receptor in skeletal muscle and pancreatic β-cells, the expression of the enzyme 1-α-hydroxylase, which can catalyze the conversion of 25-hydroxy vitamin D [25(OH)D] to 1,25-dihydroxyvitamin D, and the presence of a vitamin D response element in the human insulin gene promoter (177).

About 67–85% of women with PCOS are deficient in vitamin D, with blood levels of 25(OH)D <20ng/ml (178). Insulin resistance, ovulation, irregular menstruation, infertility, hyperandrogenism, obesity, and an increased risk of cardiovascular issues are some of the symptoms of PCOS that can be associated with low 25(OH)D levels (179). In vitamin D-deficient women with PCOS, vitamin D administration can raise serum anti-inflammatory soluble receptor for advanced glycation end products and decrease excessively increased blood AMH levels (176). Specifically, women with PCOS experiencing menstrual cycle disorders and anovulation may benefit from vitamin D and calcium supplements in addition to metformin treatment. There is a substantial correlation between insulin resistance and low 25(OH)D levels (180). Expression of insulin receptors increases with sufficient vitamin D supplementation, at the same time improving insulin production and release (181, 182).

Vitamin D levels are correlated with the likelihood of ovulation in PCOS: 68% below 50 nmol/l, 77% between 50-75 nmol/l, and 78% over 75 nmol/l (183). An increased rate of miscarriages in PCOS may possibly be due to insulin resistance. In PCOS patients, vitamin D administration increases endometrial receptivity and decreases the frequency of recurrent miscarriages (184).

As part of the Hungarian consensus recommendation on the role of vitamin D in disease prevention and treatment (185) adequate vitamin D intake is an important part of women's health at all stages of life. Vitamin D levels should be measured in preconception care, especially in cases of infertility or recurrent miscarriage. In PCOS, normalisation of vitamin D levels is strongly recommended. Preventing deficiencies is the primary intent of vitamin D supplementation. There is no apparent advantage to

taking vitamin D supplements over the suggested normal range of 75–125 nmol/l. Adults should supplement 2000 IU per day during the UV-B radiation-free period to maintain the normal range (185).

1.3. Gonadal disfunction

IR with consequential hyperinsulinemia (186) is the key factor in the pathogenesis of anovulation (187) and hyperandrogenism (12), possibly resulting in a major metabolic disease (188) and gonadal dysfunction (12). Patients in reproductive age with IR are often affected by infertility, ovarian dysfunction and menstrual irregularity (189, 190).

The luteal phase, defined as the interval between ovulation and the onset of menstruation, exhibits significantly less inter-cycle variability compared to the follicular phase. This relative stability allows for the retrospective estimation of its length, even in the absence of direct ovulatory markers such as LH surge or ovulation detection.

Multiple studies have demonstrated that the luteal phase typically ranges between 12 and 14 days, with an average duration of approximately 13 days. Lenton et al. (1984), in a seminal study of 327 cycles, reported a mean luteal phase of 14.13 ± 1.41 days (191). More recent large-scale investigations, including app-based ovulatory cycle tracking and prospective hormonal monitoring studies, confirm this finding. Ecochard et al. (2015) found the luteal phase averaged 12.4 ± 2.4 days in over 100,000 cycles (192), while Baird et al. and the BioCycle study similarly report average durations of 13.9 ± 2.4 days (193, 194).

Due to this relative consistency, retrospective evaluation of the luteal phase can be reasonably approximated by subtracting 13 days from the expected start of menses. Accordingly, measuring serum progesterone approximately 7 days before the anticipated menstruation—rather than on a fixed cycle day (e.g., day 21)—is more physiologically sound, especially in cycles that deviate from the standard 28-day length. This approach is endorsed by clinical guidelines, including the American College of Obstetricians and Gynecologists (ACOG), which recommend mid-luteal progesterone testing at 7 days before the expected next period, regardless of total cycle length (195).

In retrospective or observational studies using historical cycle data, estimating the ovulation day as cycle length minus 13 days provides a valid basis for interpreting luteal phase hormonal data. This methodology is especially pertinent in research involving menstrual cycle irregularities, where ovulation can vary widely, but luteal phase duration remains relatively constrained.

Between 50 - 90% of women with PCOS have shown insulin resistance (13). Due to different cellular reactions to insulin, such as resistance to its metabolic effects and simultaneous increased steroidogenesis, which may present as hyperandrogenism, metabolic malfunction, and reproductive issues (14).

1.3.1. Hyperandrogenism and ovarial function, irregular cycles

Chronic oligo-/anovulation frequently results in oligomenorrhea/amenorrhea, which is a common indicator of ovarian dysfunction (149). 70% to 80% of women with PCOS suffer from either amenorrhea or oligomenorrhea, indicating that ovarian dysfunction is prevalent in the majority of PCOS patients. 80% to 90% of people with oligomenorrhea will receive a PCOS diagnosis (196). The start of oligomenorrhea is typically linked to weight gain and typically occurs in adolescence. Oral contraceptives frequently disguise irregular menstruation. Oestrogen dominance and endometrial hyperplasia can lead to menorrhagia, which is worsened by obesity-boosted oestrogen levels. PCOS can be found also in women who have regular menstrual cycles (197).

The most frequent cause of anovulatory infertility is PCOS. 90% to 95% of women with anovulation at infertility clinics have PCOS. Although the time to conceive is usually prolonged, 60% of women with PCOS are fertile. 90% of women with PCOS who are infertile are overweight. Obesity increases the incidence of miscarriage, affects fertility, and decreases the effectiveness of infertility treatments (196). Weight should ideally be optimized before becoming pregnant (197).

Increased synthesis and release of ovarian androgens lead the clinical and/or biochemical androgen excess in PCOS. Insulin and elevated luteinizing hormone together boost androgen production. Insulin resistance causes hyperinsulinemia, lowers SHBG, and increases free circulating testosterone levels. Hyperandrogenism and hyperinsulinemia impair the growth of ovarian follicles. Hyperandrogenism reduces in PCOS women when insulin sensitivity improves (198).

Impaired folliculogenesis is a fundamental effect of androgen excess in PCOS. Primordial follicle development and the growth of small antral follicles are promoted by increased androgens in the early gonadotropin-independent stage (199). Increased LH stimulation results in many arrested follicles in the ovaries' theca cells, primarily in the preantral and antral stages. This leads to the hyperplasia of theca cells and follicular fluid build-up, forming cyst-like structures along the ovary's periphery, presenting in a string of pearls (200). Male pattern baldness, acne, and hirsutism are the main signs of clinical hyperandrogenism (197, 201).

In most PCOS patients biochemical hyperandrogenism can be detected. Measuring free androgen index is revealing adequate information, free androgen index (FAI) is obtained in the laboratory from total testosterone and SHBG levels (202-204).

1.3.2. Effect of prolactin on ovarial function

Prolactin (PRL), beyond its lactogenic role, acts at physiological levels on granulosa and luteal cells to promote progesterone production, supporting follicle maturation and corpus luteum maintenance (205-207).

Pathologically elevated PRL suppresses hypothalamic GnRH and downstream LH/FSH secretion, resulting in hypoestrogenism, anovulation, luteal insufficiency, and oligo- or amenorrhea (208-211). At the ovarian level, high PRL directly inhibits granulosa cell aromatase, reduces estradiol/progesterone output, and induces oxidative stress and apoptosis (206).

Hyperprolactinaemia has a 9–17% prevalence in women with reproductive disorders (212). Elevated prolactin levels can be associated with impaired insulin sensitivity, particularly in women with reproductive disorders and in individuals with metabolic comorbidities (213, 214), by influencing glucose homeostasis through pancreatic β-cell stimulation, adipose tissue metabolism, and modulation of insulin receptor signaling (215). Prolactin may also act centrally by affecting dopamine pathways that indirectly regulate insulin action (216). Clinical studies have shown elevated fasting insulin levels and higher HOMA-IR in patients with hyperprolactinaemia (217).

Prolactin is physiologically upregulating 3β-hydroxysteroid dehydrogenase activity, supporting progesterone secretion by luteal cells (218, 219). Transient prolactin

drops during luteal phase lower progesterone output and lead to suboptimal luteal support (220, 221). Prolactin suppresses the hypothalamic-pituitary-gonadal axis by inhibiting GnRH pulsatility, which in turn reduces LH (luteinizing hormone) and FSH (follicle-stimulating hormone) secretion, causing hypogonadotropic hypogonadism in case of persisting hyperprolactinaemia (222, 223).

Normal range prolactin is essential to sustain corpus luteum steroidogenesis and regular menstrual cycle duration. In healthy ovulatory women the average level of prolactin was around 300 mU/l with ranges 60–800 mU/L, conception cycles showing slightly lower mean values (~180 mU/l) and ranges of 58–600 mU/l, suggesting optimal fertility within this margin (224). Mild elevations of prolactin levels, particularly near the upper normal limit (20–50 ng/mL; 400–1000 mU/L), can stabilize cycles by reinforcing luteal progesterone synthesis without inducing anovulation (225-228). Higher prolactin concentrations even within high-normal- have been associated with shorter luteal phase and luteal insufficiency. High prolactin levels (50–100 ng/ml; 1000–2000 mU/l) trigger hypogonadotropic hypogonadism, leading to oligomenorrhea or amenorrhea and significantly reduced fertility through suppression of GnRH, LH, and FSH secretion (205, 229, 230).

Lowering prolactin levels excessively via dopamine agonists such as bromocriptine can impair progesterone production and destabilize luteal phase, even if hyperprolactinemia is not present (218). Therapeutic normalization of prolactin often restores ovulatory cycles and fertility (ovulation or pregnancy in up to 90% of cases), whereas oversuppression may precipitate luteal defect and compromised fertility (231). Therefore maintaining prolactin within the optimal range (~100–600 mU/l; ~5–30 ng/ml) is essential (227, 232).

2. OBJECTIVES

In many circumstances, insulin resistance and its most prevalent phenotypic expression, polycystic ovarian syndrome, which exhibits characteristic hormonal aberrations, can be linked to gynaecological problems. However, in day-to-day clinical practice, the number of clinical manifestations beyond the classic profile is escalating.

Our research aimed to assess the fundamental clinical characteristics of women with IR and PCOS and track the effectiveness of the implemented tailored treatment. Besides the patient's phenotype, clinical test results, symptoms, and the severity of PCOS and IR, customized therapy may also vary with respect to the patient's personal preferences. Our goal was to assess the efficacy of the administered medicinal treatments, specifically the efficacy of myo-inositol and metformin.

We used the clinical database of our internal medicine and endocrine practice at EndoCare Institute, Endocrinology Center, Budapest, Hungary, to determine the answers to the following questions.

1) After receiving tailored treatment, did progesterone levels improve in IR women?
2) Did tailored treatment result in improvement of prolactin levels in IR women?
3) After receiving tailored treatment, did menstrual cycle disorders improve in IR women?
4) How effectively does metformin treatment improve IR?
5) To what extent does myo-inositol influence IR?
6) Is the combination of metformin and myo-inositol more effective than single-use treatment?
7) Does insulin sensitivity affect the levels of testosterone and FAI in any way?

3. METHODS

3.1. Study I

3.1.1. Study design

Using our records from October 2013 to February 2016 in EndoCare Institute, Endocrinology Center, Budapest, Hungary, a diverse group of women with insulin resistance was selected. All participants provided written informed consent, and the study structure was implemented in compliance with the Declaration of Helsinki. Using the following inclusion criteria, 237 of the 411 IR patients were included in this study:

- Identified as having IR, indicated by HOMA-IR>2 (233);
- Diagnosed menstrual irregularities;
- Women between the ages of 25 and 45;
- Routine examinations spaced no more than six months apart. A small percentage of PCOS women were within this diverse group of patients.

Criteria for exclusion were:

- Amenorrhea patients;
- Individuals suffering from premature ovarian failure;
- Females with insulin dependent diabetes mellitus (IDDM), non insulin dependent diabetes mellitus (NIDDM), or any pre-diabetic condition: impaired glucose tolerance (IGT) or impaired fasting glucose (IFG).

The tailored treatment included diet, exercise depending on body composition and BMI (assessed with InBody R20), medication with metformin (750–2550 mg/day), myo-inositol (4 g myo-inositol plus 400 µg folic acid/day), or both. Changes in lifestyle were heavily encouraged and thoroughly monitored, with restrictions and re-educations provided as necessary. An average of six months of treatment for each patient was used for obtaining data. Four groups of patients were created based on the customized treatments that were used:

- Group 1: patients receiving only lifestyle treatment (41 patients)
- Group 2: therapy with myo-inositol and lifestyle (62 patients)
- Group 3: therapy with metformin and lifestyle (81 patients)

- Group 4: treatment with metformin, myo-inositol, and lifestyle (53 patients).

This study was retrospective in design, therefore the selection of treatment modalities was not standardized or randomized but was instead influenced by a range of clinical and contextual factors. Selection of treatment regimens was guided by individual drug tolerability, patient preferences, and the clinical judgment of the treating physician.

Progesterone, prolactin levels were gathered during the luteal phase, between 8-6 days before the expected date of menstruation, evaluating the average menstrual cycle lengths of 1 year prior to baseline measurements and 3 months prior to '6 months' data collection. Fasting insulin and glucose levels were obtained independent of the menstrual cycle. The formula (Basal glucose) x (Basal insulin)/22.5 was used to calculate HOMA-IR index (20). Data on menstrual cycle length was retrieved; a typical menstrual cycle was determined between 25 and 35 days (234). In line with clinical guidelines, where normal menstrual cycles range between 24 and 38 days, with inter-cycle variability not exceeding 7–9 days (235, 236) we caracterised cycles shorter than 25 days and longer than 35 days, or with substantial irregularity (cycle-to-cycle variation >9 days) as menstrual cycle disorders (237).

Additionally, during the analyzed time, we were looking for data on pregnancies that occurred and issues with conception, characterized by failure to achieve a clinical pregnancy after ≥ 12 months of regular, unprotected intercourse in women under 35 years of age; or six months in women aged 35 and older, or immediately in women over 40 years or with known risk factors (238, 239).

3.1.2. Statistical Analysis

Statistics and data analysis were carried out using the R 3.2.3 software. 95% confidence bounds were applied to the data (a p-value of < 0.05 was deemed statistically significant).

Pair wise comparisons of proportions were used to ascertain the distribution of pregnancy rates among the groups (p-value correction method: Bonferroni). Fisher's exact test was used to examine menstrual cycle problems, and the McNemar test was used to assess any group differences. Normality was assessed using the Shapiro-Wilk test. The Mann-Whitney test (for non-normal distributions) was applied for serum

fasting insulin and HOMA-IR values, while the paired t-test (for normal distributions) was chosen for assessing changes in progesterone, prolactin, and fasting glucose levels.

3.2. Study II

3.2.1. Study Design

Accessing a Hungarian data banks' (EndoCare Institute, Endocrinology Center, Budapest, Hungary) follow-up data on 136 PCOS (Caucasian population) women aged 18 to 45, this retrospective cohort analysis was performed as part of the Hungarian Polycystic Ovarian Syndrome (HCPOS) study. All participants provided written informed consent, and the study structure was implemented in compliance with the Declaration of Helsinki and authorized by the ethics committee (identification number: ETT TUKEB 49591-1/2019/EKU). Rotterdam criteria (two of the following three: oligo-amenorrhea, biochemical or clinical hyperandogenism, and polycystic ovaries on transvaginal ultrasonography) were applied to diagnose PCO syndrome (240). Based on their fasting insulin levels, these women with PCO syndrome were divided into two groups:

- Group A, which had normal insulin sensitivity (fasting insulin level < 8 mU/l; n = 88)
- Group B, which had impaired insulin sensitivity (fasting insulin level > 8 mU/l; n = 46).

Criteria for exclusion were:

- Treatment of oral contraceptives
- antiandrogenic medications
- hyper- or hypothyreosis
- type 1 or type 2 diabetes
- menopause

The following information was gathered: age, height, weight, and laboratory results: FAI, SHBG, testosterone, fasting plasma insulin, and fasting plasma glucose levels.

The HOMA-IR index was determined using the following equation (20):

$$\text{HOMA-IR index} = \frac{FPI * FPG}{22.5}$$

where FPG is the fasting plasma glucose expressed in mmol/L and FPI is the fasting plasma insulin expressed in mU/L.

3.2.2. Statistical Analysis

The Mann-Whitney test (for non-normal distributions) or the 2-tailed unpaired Student's test were used. Values that were lacking were regarded as missing. A value of $P < 0.05$ was deemed statistically significant. Statistics are displayed as median with 95% confidence intervals (CIs) (if the distribution was not normal) or mean ± Standard Error of Mean (SEM). GraphPad Prism 6.0 programme was applied for data interpretation.

4. RESULTS

4.1. Study I

4.1.1. Age of patients

The average age of patients in each group was evaluated at the beginning of treatment showing no statistical differences in between the groups.

Group	Mean Age ± SD (years)	Min (years)	Max (years)
Group 1	35.15 ± 3.36	29.31	45.00
Group 2	33.96 ± 3.84	25.10	42.56
Group 3	35.84 ± 3.11	29.55	43.63
Group 4	37.58 ± 2.94	30.93	42.45

Table I. The average age of patients in the groups at the beginning of treatment. One-way analysis of variance (ANOVA) was performed. $p = 0.059$. $p<0.05$ indicates statistically non significant results. At baseline mean ages of the various groups were statistically indifferent.

4.1.2. Progesterone levels

After six months of treatment, progesterone levels during the luteal phase of the menstrual cycle in each group increased substantially; however, there were no differences between the four groups (Table II).

Table II. Serum progesterone levels at baseline (T0) and six months (T6) after treatment were evaluated. Paired T-test, * $p<0.05$ indicates statistically significant results (241). Each group's progesterone levels during the luteal phase of the menstrual cycle significantly increased after six months of treatment; however, the four groups did not differ from one another.

Progesterone levels (nmol/l)	**T0**	**T6**	**p**
Group 1	33.6 ± 7.64	51.7 ± 5.09 *	0.033
Group 2	35.3 ± 5.66	47.3 ± 47.3 *	0.039
Group 3	32.1 ± 8.91	56.4 ± 8.49 *	0.034
Group 4	28.6 ± 7.35	41.8 ± 41.8 *	0.048

4.1.3. Prolactin levels

Prolactin showed similar results (Table III), declining significantly across all groups, with the combination group showing the most notable decline. The groups did not differ significantly from one another.

Table III. Serum prolactin levels at baseline (T0) and six months (T6) of treatment are compared. Paired T-test, * $p<0.05$ indicates statistically significant results (241). Prolactin levels showed significant decline in all groups, with the combination group showing the most notably decreasing prolactin levels. The four groups did not show relevant differences.

Prolactin levels (mIU/l)	**T0**	**T6**	**p**
Group 1	410.28 ± 67.81	325.96 ± 26.91 *	0.036
Group 2	381.15 ± 68.52	254.80 ± 28.85 *	0.023
Group 3	494.73 ± 85.98	382.3 ± 39.46 *	0.048
Group 4	479.3 ± 101.12	271.1 ± 48.22 *	0.017

4.1.4. Menstrual cycle

The treatment groups showed a significant improvement in menstrual cycle disorders, however the lifestyle group (Group 1) showed a statistically non significant improvement: Group 1 (p=0.094), Group 2 (p=0.027), Group 3 (p=0.039), and Group 4 (p=0.025) (Figure I).

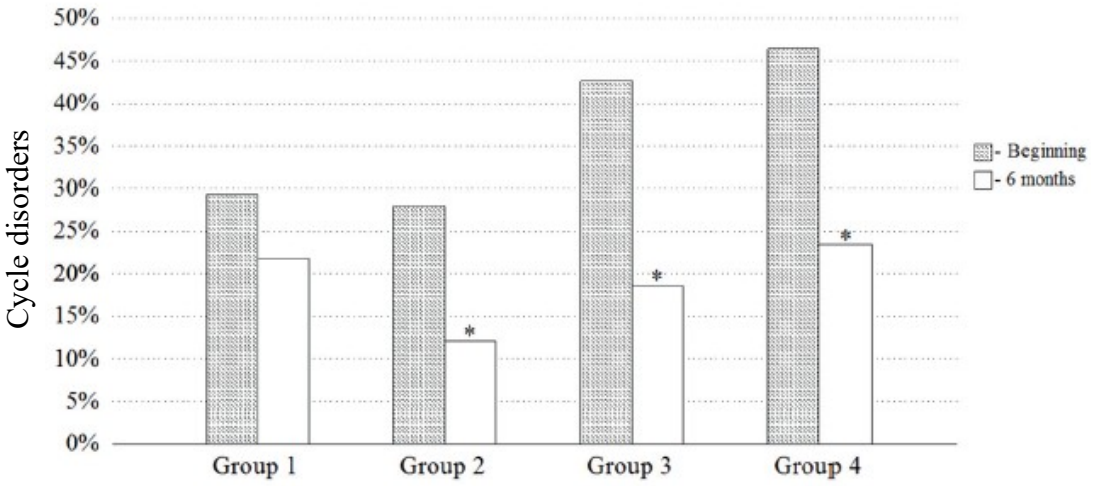

Figure I. Rate of irregular cycles at the start of treatment and six months later. Fisher's exact test, * $p<0.05$ indicates statistically significant results (241). Menstrual cycle disorders were substantially reduced in the therapy groups, at the same time the lifestyle group did not reach statistical significance, but improved.

168 (71%) of the 237 patients experienced problems conceiving. The percentages of infertile women in each group did not differ significantly. 71 (42.26%) of the 168 patients who wanted to conceive, got pregnant during the observation period. Likewise, throughout the whole course of treatment, no variations were found between the groups in relation to this measure or blood fasting glucose levels. Serum fasting insulin levels were initially higher in the MET (Group 3) and MET+MYO groups (Group 4), and after six months of complex tailored treatment, there were noticeable but insignificant improvements.

At the onset of treatment, both Group 1 and Group 2 had HOMA-IR index values below 2.5. The groups treated with MET (Group 3; value at the start of treatment: 2.95; value after 6 months of treatment: 2.41) and MET+MYO (Group 4; value at the start of treatment: 3.4; value after 6 months of treatment: 3.05) exhibited declining tendencies in the HOMA-IR index values, but there was no statistical significance within or between the groups at the 6-month point.

Although there was no discernible difference in the groups' average BMIs, Groups 3 and 4 had somewhat higher BMIs. Group 1's average BMI was 22.94, Group 2's was 22.1, Group 3's was 24.61, and Group 4's was 25.3.

4.2. Study II

4.2.1. Insulin sensitivity characteristics and basic parameters

The group in our study with decreased insulin sensitivity weighed substantially more (Table IV). Regarding age, there were no differences between the groups with normal and decreased insulin sensitivity (Table IV).

HOMA-IR values appeared significantly higher in the group with impaired insulin sensitivity (Table IV).

Table IV. Insulin sensitivity characteristics and basic parameters (242)

	Normal fasting insulin sensitivity group	**Impaired insulin sensitivity group**	**Statistical level**
Age (years)	35±6	36+6	p=0.73
Body weight (kg)	62 (59 - 65)	80 (74 - 86)	**p<0.0001**
HOMA-IR	0.96 (0.83 – 1.15)	2.38 (2.26 – 2.89)	**p<0.0001**

The 2-tailed unpaired Student's test or the Mann-Whitney test (in cases of non-normal distribution) was performed. Data are presented as mean ± SEM or median with 95% confidence intervals (in cases of non-normal distribution). The group with reduced insulin sensitivity had a significantly higher weight. The groups with normal and reduced insulin sensitivity did not differ in terms of age.
The group with reduced insulin sensitivity had noticeably higher HOMA-IR findings.

4.2.2. Testosterone, free androgen index

There was no difference in testosterone levels between the groups (Figure II). The group with decreased sensitivity had a significantly higher free androgen index (FAI) (Figure III).

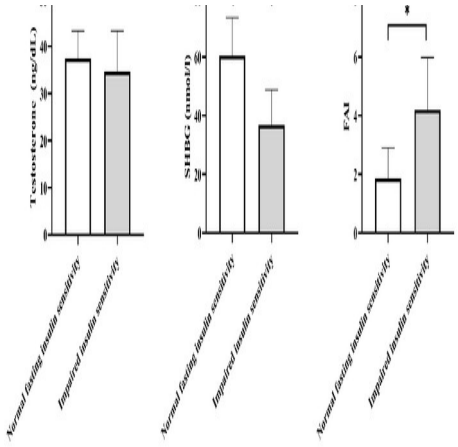

Figure II. Testosterone levels. Testosterone levels did not differ among the groups. Mann-Whitney test (242).

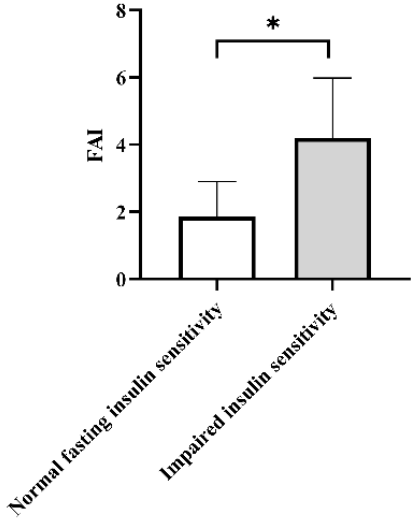

Figure III. Free androgen index. The FAI was significantly higher in the impaired insulin sensitivity group than in the normal fasting insulin sensitivity group. Mann-Whitney test, * $p < 0.05$ (242).

5. DISCUSSION

Our study focused on the effectivity of the complex tailored treatment applied at our clinic, where patients with IR and/or PCOS are treated by a multidisciplinary team of highly qualified specialists and dieticians. Each patient enters a wide range condition assessment at the time of diagnosis and is followed up on a regular basis, including check-ups every 3-4 months that allows adapting treatments as soon as possible to changes in condition. The basis of our therapeutic approach is lifestyle treatment, including dietary and physical activity features. Medical treatment is mainly determined by current health and physical status, laboratory tests and imaging test results.

Because MET is so effective at lowering insulin levels, it is nowadays regarded as one of the most widely used treatments for type 2 diabetes mellitus. Additionally, MET is frequently prescribed in conditions like IR and PCOS that are characterized by altered insulin sensitivity (55). The idea that insulin resistance is independent of weight is strongly supported by the description of severe insulin sensitivity problems with a compensatory hyperinsulinemic state in both lean women and obese PCOS patients.

However effective, metformin's side effects are widely documented in the literature, which is why using metformin is not always advised (96, 243).

Relevant data about MYO as a substitute insulin-sensitizing therapy for PCOS is confirmed by research (244, 245). Specifically, MYO has shown promising outcomes in a number of studies, improving metabolic and hormonal changes, hyperandrogenism, and menstrual abnormalities (246, 247). Considering these results, we aimed to find out if MYO would be a viable substitute for traditional methods in treating individuals with IR and associated menstrual abnormalities.

According to research data, MYO is a viable insulin-sensitizing substitute for PCOS. Several studies have shown that MYO can ameliorate menstrual cycle deviations, hyperandrogenism, as well as hormonal and metabolic changes (42). These findings are particularly intriguing. Given these results, we aimed to find out if MYO would offer an alternate strategy for treating individuals with IR and associated irregular

menstrual cycles. As a result of our complex tailored treatment significant changes in our patients' medical conditions were provable. Each group's progesterone levels in the luteal phase of the monthly period significantly increased after six months of treatment; however, the four groups did not differ from one another. Similar outcomes were observed for prolactin, which decreased noticeably in each group but especially in the combination treatment group. There were no major differences between the groups. Menstrual cycle disorders significantly improved in the therapy groups, without statistical significance in the lifestyle group.

Furthermore, we investigated whether there is a difference in testosterone and free androgen indices between groups in PCOS with adequate or impaired insulin sensitivity. Patients with reduced insulin sensitivity presented significantly higher HOMA-IR values and higher free androgen index (FAI). This confirms previous observations that hyperandrogenic symptoms are more common in overweight people. In everyday practice measuring FAI during the regular follow-ups could back up clinical improvement, allowing the number of OGTT tests to be reduced.

5.1. Progesterone levels

PCOS is known to cause a drop in progesterone levels (248). A major cause of infertility or spontaneous miscarriage in luteal phase defect conditions, such as IR or PCOS is the lack of luteal phase progesterone production and/or activity (249).

In previous studies progesterone, LH, and insulin levels did not differ statistically significantly between PCOS women on a natural cycle treated with clomiphene citrate and those treated with clomiphene citrate plus metformin (250). However, when compared to PCOS women on a natural cycle and PCOS women treated with just clomiphene citrate, there was a notable rise in luteal progesterone concentration in PCOS women receiving metformin. The group receiving metformin showed a rise in progesterone concentration during the mid- and late-luteal phases. During the luteal phase PCOS patients also showed elevated insulin levels, which were negatively correlated with progesterone levels (250). In another study using a classical dose of 2x2000mg myo-inositol and 200ug folic acid per day significant increase of progesterone levels were confirmed at an average treatment period of 20 weeks (251).

Our findings show significant improvement in progesterone levels in case of all treatment groups, without any differences in serum progesterone levels at the beginning point. In accordance with other findings lifestyle treatment (Group1), MYO supplementation (Group 2), metformin supplementation (Group 3) and MYO+MET administration (Group 4) all proved to be efficient strategies for improving progesterone levels. Similar to our results, a 2022 randomised controlled trial found that both metformin and myo-inositol increased progesterone levels and that there was no difference in progesterone levels between metformin alone and myo-inositol alone (252). Our conclusion is that no significant differences were detectable between the various groups within 6 months of treatment, indicating that both long-term used drugs like MET, and novel supplements like MYO can be used efficiently as part of complex tailored treatment for IR and /or PCOS.

5.2. Prolactin levels

It was known from the literature that metformin has a prolactin-lowering effect. Krysiak et al investigated whether metformin reduces prolactin levels in drug-induced (antipsychotic-induced) hyperprolactinemia. While hyperprolactinemic and normoprolactinemic patients with type 2 diabetes were treated with high-dose metformin (2.55–3 g daily), hyperprolactinemic patients with prediabetes were given moderate dosages of the medication (1.7 g daily) for six months. Only when administered in large dosages, did metformin lower raised prolactin levels (253). Furthermore, it was also known from the literature that myo-inositol can also reduce prolactin levels. Myo-inositol decreased the plasma levels prolactin when given in conjunction with folic acid (254) Even though baseline prolactin levels were within the established range, its prolactin-lowering action was noted (255). A 12 week administration of 2g MYO and 200mg folic acid significantly lowered serum prolactin levels, whereas patients treated with folic acid below did not experience any changes (254).

Our intention was to evaluate the effectiveness of combined metformin and inositol treatment on prolactin levels. All groups in our study showed a considerable drop in prolactin levels, while the combination group's decline was the most pronounced. There were no differences between the four groups comparing the six

month results. Our results were later confirmed by further research: Karadag et al compared the effects of 4 g myo-inositol (+ 400ug folic acid) and an average of 1700 mg metformin daily, used separately and in combination, on women with PCOS. Compared to either medication alone, the combination of myo-inositol and metformin did not provide any extra advantages on prolactin levels (256). Further prospective studies with higher case numbers might prove significant differences in between the sixth month results. Comparison of combined MYO and MET treatment with dopamin receptor agonists, such as bromocriptin, quinagolid or cabergoline could result in more extensive scientific evidence. The fact that life-style changes alone resulted in significant decrease of prolactin levels indicates that factors other than drugs of supplements might be of importance.

Clinical relevance of lowering prolactin within the normal range lies in optimizing reproductive function without inducing hormonal imbalance. Therapeutic normalization of prolactin has been shown to restore ovulatory cycles and fertility (218). However, excessive suppression of prolactin below the optimal physiological range can lead to luteal phase defects and impaired fertility outcomes (231). Therefore, maintaining prolactin within optimal range is a critical aspect for supporting ovulation, corpus luteum function, and overall reproductive success (227, 232).

5.3. Irregular cycles

In terms of clinical presentation, PCOS can range from a modest menstrual issue to a serious disruption of metabolic and reproductive processes (100). Irregular periods and longer menstrual cycles are considered to be hallmarks of PCOS and IR caused by hormonal alterations in these conditions (86, 87), and are typically characterized by a cycle lengths longer than 35 days (83). Improvement of hyperinsulinemia reduces the symptoms of PCOS, especially the ovulation and conception rate (257).

A widely recognized insulin sensitizer is metformin (130, 131). Because MET improves insulin resistance and cycle irregularities, it is recommended for PCOS (132). Reduced insulin resistance (131), which has a direct impact on ovarian insulin sensitivity (135), is associated with a more stable menstrual cycle following MET treatment. Treatment interruption is often due to adverse effects during MET, particularly gastrointestinal side effects (131, 138).

Myo-inositol insufficiency has been demonstrated in women with insulin-resistant PCOS. The underlying insulin resistance in PCOS causes hyperinsulinemia, which in turn causes the change in ovarian inositol metabolism. To restore normal ovulatory activity in women with PCOS, the vitamin B complex cofactor and insulin sensitizer MYO enhances insulin signaling, lowers blood insulin, and lowers serum testosterone (258).

Our results show that although the lifestyle group (Group 1) had a statistically non-significant improvement in menstrual cycle abnormalities, all drug groups significantly improved menstrual cycles. However, when used separately or together, there was no difference between the metformin and inositol groups. Improving monthly period abnormalities without ovulation induction and other hormon supplementation is a remarkable result. Irregular cycles were considerably improved by both MYO and MET therapies, which were linked to lifestyle modifications. The outcomes appear to be closely linked to how medical treatment raises progesterone levels while, conversely, lowering prolactin levels. A meta-analysis of randomised controlled trials published in 2023 also confirmed these results: inositol was as effective as metformin in improving menstrual cycle regularity (259).

5.4. Pregnancy rate

The most frequent cause of anovulatory infertility is PCOS, linked to amenorrhea or oligomenorrhea, that can result in infertility (260); approximately 90–95% of anovulatory women who seek infertility therapy are suffering from this disease (261). Women may discover they have PCOS and or IR only after undergoing treatment for infertility.

Even when all other variables are optimized and ovulation induction is effective, the pregnancy rate among PCOS women is only 40–50% (262). In PCOS patients, the progesterone level is associated with clinical pregnancy rate while progesterone/estradiol ratio is not (263).

Although MET treatment improved insulin sensitivity and reduced testosterone levels, its usage is limited by gastrointestinal side effects. In these PCOS cases the relevance of MYO was assessed. Research by Minozzi et al. (43) and Zacché et al. (264) proves that MYO promotes the reduction of insulin resistance, and improves LH,

and testosterone levels. By lowering androgen levels (androstendione and testosterone), adjusting the LH/FSH ratio, restoring regular menstrual cycles, and inducing ovulation, MYO promotes spontaneous conceptions through sufficient luteal phase progesterone production (41).

While we were measuring hormone levels and following the regularity of menstrual cycles we wanted to evaluate, whether the efficacy of MYO might be comparable with the effect of MET, especially in those cases, where MET was poorly tolerated. Of the observed 237 patients in our study, 168 (71%) had infertility issues. There was no obvious difference in the percentage of infertile women in each group. During the monitoring period, 71 (42.26%) of the 168 patients who desired to become pregnant conceived. This is a remarkable result, as they were achieved by treatments targeting PCOS, IR and hyperinsulinaemia, without using hormones, clomiphene citrate or letrozole for ovulation induction.

In a study of 116 women with infertility PCOS, the effect of 1500 mg metformin + 4g myo-inositol vs. 4g myo-inositol alone on fertility was investigated. After 6 months of treatment, there was no difference in clinical pregnancy rate (42% vs 45%) between the groups (265). In addition, Greff et al in a meta-analysis based on the 2023 RCT also examined pregnancy rates for metformin and inositol in women with PCOS. Compared with metformin, inositol showed similar pregnancy rates with and without additional therapy (letrozole, ovulation induction) (259).

5.5. Insulin sensitivity characteristics and basic parameters

Insulin sensitivity, the body's capacity to regulate glucose and muscular strength progressively deteriorate with age (266). Furthermore, the risk of developing type 2 diabetes rises in parallel with the aging process. According to studies, the prevalence of diabetes, impaired fasting glucose, or impaired glucose tolerance was 20.9% among Americans aged 20 to 39, 46.9% among those aged 40 to 59, 67.4% among those aged 40 to 59, and 75.6% among those aged 75 and older (267). IR and PCOS are linked with higher risk of prediabetes and diabetes mellitus (268-270). Because of these reasons it is important, that statistical analysis of PCOS and IR women compare age-matched

groups. Regarding age, there were no essential differences between the groups with normal and decreased insulin sensitivity in our study.

A bolus intake of 75-g glucose after an overnight fast is used in an oral glucose tolerance test to evaluate a person's capacity to eliminate circulating glucose and insulin sensitivity (271). However, OGTT has several drawbacks (high cost, non-physiologic, unsettling, low repeatability, and time-consuming) (272). That is why in addition to the traditional OGTT assessment, a straightforward metric that closely matches daily variations in serum insulin levels would be helpful to track changes in the metabolism of carbohydrates. It is commonly recognized that fasting insulin and BMI have a positive relationship, and that increasing body weight is linked to higher levels of HOMA index and fasting serum insulin (273). Prior research comparing serum insulin and HOMA index values of lean and obese PCOS patients revealed notable variations in the two groups' fasting insulin and HOMA index levels (273). The HOMA index is based on fasting insulin and glucose levels (HOMA = (fasting insulin uIU/ml × fasting glucose mmol/l)/22.5), that is typically normal in women with normal/lean PCOS (274), making it an inappropriate metric to describe insulin sensitivity in women with normal/lean PCOS. This can be explained by the absence of enhanced hepatic glucose production and fasting hyperinsulinemia in women with normal/lean PCOS (274, 275). However, it is important to remember that these women may have periodic hyperinsulinemia even when their HOMA index is normal. This can negatively impact their metabolic, cardiovascular, and reproductive health (274). OGTT can reliably detect post-load hyperinsulinemia, late insulin peak, and delayed insulin response in PCOS patients with normal weight or lean body mass (276).

Insulin promotes adipose accumulation in a variety of ways. The transformation of preadipocytes into adipocytes is stimulated by insulin. Insulin stimulates the uptake of glucose and fatty acids generated from lipoproteins, which in turn promotes lipogenesis in adipocytes. This controls genes that support the production of fatty acids and lipogenesis in both hepatocytes and adipocytes (277). The group in our study with decreased insulin sensitivity weighed substantially more. As a consequence, HOMA-IR values appeared significantly higher in the group with impaired insulin sensitivity. This outcome is consistent with current scientific research results (278) and backs up the connection between IR, PCOS and obesity (279). Moreover, mean plasma insulin

concentration was proven to be significantly greater in obese individuals than in thin patients, despite the fact that the mean baseline plasma glucose concentrations did not differ substantially between the two groups (280, 281). It is commonly recognized that fasting insulin and BMI have a linear relationship, and that increasing body weight is linked to higher levels of HOMA index and fasting serum insulin (273). Obese people appear to have a higher incidence of endothelium hyperplasia and hypertension, which puts them at a higher risk of developing health issues at an earlier age than lean people. This suggests that overweight women with PCOS may require closer health management (282).

5.6. Testosterone levels and free androgen index (FAI)

One of the main characteristics of PCOS is insulin resistance with compensatory hyperinsulinemia, increasing intra-ovarian androgen production with negative effects on follicular development and ovulation (283).

In PCOS women, associated hyperinsulinemia may cause an excess of androgen production in two ways: by directly stimulating the ovaries to create androgens and by lowering serum levels of SHBG (284), both resulting in higher free androgen index.

By limiting the liver in synthesizing SHBG, insulin lowers the concentration of SHBG in the blood (285), which raises the total of physiologically active free testosterone. This exacerbates IR and lowers the insulin clearance rate even more, starting a vicious cycle. The ovaries and adrenal glands of women with PCOS may also be directly stimulated to create excessive amounts of androgens by hyperinsulinemia brought on by obesity (286). A useful supplement to androgen status in assessing bioavailability of androgens leading to hyperandrogenic manifestations is the calculation of FAI using testosterone and SHBG values. When tests and results were evaluated, the FAI assessment performed better than the more intricate measurement of women's free testosterone levels for hypoandrogenism (287, 288). The development of gestational diabetes mellitus, which raises maternal and newborn morbidity, has also been linked to lower levels of SHBG in the blood. To improve results, it's critical to identify and treat these women early in pregnancy (289). According to scientific data, FAI appeared to be an intriguing variable since some PCO syndrome patients showed a linear association among FAI and HOMA index (51, 97, 290), whereas others did not

recommend it as an indicator in PCO syndrome because of its high variability (291). Our research shows that the normal insulin sensitivity group and the decreased insulin sensitivity group's testosterone levels did not differ from one another. The free androgen index was considerably higher in women with impaired insulin sensitivity.

5.7. Limitations

A longer observation time and increasing and balancing the numbers of cases in the groups may result in more distinct variations between the groups, potentially reaching significance, particularly when it comes to lowering serum insulin levels, which affect the outcome of pregnancy.

As both performed analyses were using follow-up data, there is a possibility that medical practitioners tend to start complex tailored treatment with MET or MET combined with MYO in the more severe cases and choose MYO or lifestyle changes alone at patients showing milder symptoms or at patients' adverse reactions or explicit request. It is necessary to conduct additional research to determine whether combining MET and MYO can improve the therapeutic efficacy of medical and lifestyle treatment.

Our second study's a limitation is that we did not separate all phenotypic categories, such as ovulatory and anovulatory subgroups, as doing so would have drastically decreased the number of cases in the groups. Additionally, primary beta cell dysfunction can occasionally result in weight gain; in these situations, changes in SHBG levels occur as predicted by elevated basal insulin levels, but there are also changes in insulin secretion and action (insulin resistance).

5.8. Relevance

In all situations when metformin is not an option, we discovered that MYO may be a viable substitute for treating IR patients without diabetes or pre-diabetes. In fact, in this study MYO and MET shown equal effectiveness in treating menstrual cycle problems associated with IR in this clinical investigation. In any case, no adverse effects of MYO have been documented to date, despite the fact that MET side effects are extensively documented in the literature. This may provide an effective treatment option for those who cannot tolerate MET.

Our data analysis showed that not all PCO syndrome phenotypes exhibit a direct proportion connection of FAI with HOMA index and serum insulin; as a result, this metric has limited benefit for identifying changes in blood insulin levels and carbohydrate metabolism. By examining standard clinical data, such as OGTT and FAI evaluations, FAI is safe to evaluate the metabolic status of obese and insulin-sensitive

PCOS patients. Another advantage of our research is that it enables us to identify the PCOS subtypes and where FAI is ineffective as a metabolic status monitoring parameter.

6. CONCLUSIONS

Based on our research, the following statements can be made:

1. We found that MYO could represent a possible alternative in the treatment of IR patients without diabetes or pre diabetes in all those cases where metformin cannot be used.

2. MYO and MET treatments both resulted in significant improvement of menstrual cycle disorders related to IR.

3. PCOS women with impaired insulin sensitivity have significantly higher body weight than those with normal insulin sensitivity. There were no differences between groups in age. However, the testosterone level did not differ among the groups, the FAI level was significantly higher in the impaired insulin sensitivity group.

7. SUMMARY

A prevalent condition that can affect female fertility and manifests in phenotypic heterogeneity is IR. In order to restore adequate endocrinological and clinical characteristics, women with IR and the resulting hyperinsulinemia are frequently treated with lifestyle changes and insulin sensitizers. Although myo-inositol has shown encouraging outcomes in the treatment of metabolic disorder, metformin is still regarded as one of the initial therapies to IR and PCOS.

Two retrospective studies were performed using our clinical database. In one study a diverse group of 237 IR reproductive age females' data was evaluated to compare the therapeutic effectiveness of 4 g myo-inositol with 400 mcg folic acid/day and metformin (median 1225 mg/day). The patients had received treatment for six months (MYO, MET, or both insulin sensitizers). In spite of not having diabetes or prediabetes, the individuals displayed signs of IR. As a result, every group had relevant improvement ($p<0.05$) in clinical parameters. We conclude that individuals without severe malfunctions in carbohydrate metabolism, treated with MYO and MET and connected lifestyle changes, had significant positive impact on their serum progesterone and prolactin levels, monthly period problems, and pregnancy rates. However, there were no major differences between the MYO and MET groups. While fasting serum insulin levels were somewhat improved, the combination of both therapies produced significant changes in hormone levels and symptoms. Additionally, the MET and MET+MYO groups had a somewhat but not substantially higher BMI.

In the second study we compared women with PCOS who had normal fasting insulin sensitivity ($n = 88$) with females who had reduced insulin sensitivity ($n = 46$).

Body weight was considerably higher in the group with decreased insulin sensitivity. Regarding age, there were no differences between the groups with normal and decreased insulin sensitivity. HOMA-IR values were considerably higher in the group with reduced insulin sensitivity. While there was no substantive deviation in the testosterone levels across the groups, the impaired insulin sensitivity group had a considerably higher FAI level. Measuring FAI during regular check-ups could possibly allow reduce the number of OGTT-s performed, if the clinical condition shows improvement and is consistent with hormonal, fasting and long term carbohydrate metabolism parameters.

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9. BIBLIOGRAPHY OF PUBLICATIONS

Publications related to the thesis:

1. Nas K, Tűű L. A comparative study between myo-inositol and metformin in the treatment of insulin-resistant women. Eur Rev Med Pharmacol Sci. 2017; 21: 77-82.

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2. Tűű L., Nas K., Török M. and Várbíró Sz. SHBG Levels Do Not Correlate with Insulin Levels in PCOS with Appropriate Fasting Insulin Sensitivity. J Clin Med. 2024¸1;13(3):838-

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Publications not related to the thesis:

1. Várbíró S, Takács I, Tűű L, Nas K, Sziva RE, Hetthéssy JR, Török M. (2022) Effects of Vitamin D on Fertility, Pregnancy and Polycystic Ovary Syndrome-A Review. Nutrients, 14.

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3. Dr. Nas Katalin, Dr. Tűű László (2015) Nőgyógyászati és Szülészeti Továbbképző Szemle; Szénhidrát anyagcserezavar kezelésének eredményessége meddőségben

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