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

PhD thesis

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Budapest
2025

1. INTRODUCTION

In many circumstances, insulin resistance and its most prevalent phenotypic expression, polycystic ovarian syndrome, which exhibits characteristic hormonal aberrations, can be linked to gynacological 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.

2. OBJECTIVES

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 disosders 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. 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 ;
- 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 reeducations 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. Data on menstrual cycle length was retrieved; a typical menstrual cycle was determined between 25 and 35 days. In line with clinical guidelines, where normal menstrual cycles range between 24 and 38 days, with inter-cycle variability not exceeding 7–9 days 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.

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. 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 . 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 glucuse levels.

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

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

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.

Table I. The average age of patients in the groups at the beginning of treatment. At baseline mean ages of the various groups were statistically indifferent.

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

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 . 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 II), 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 . 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

4.1.3. 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).

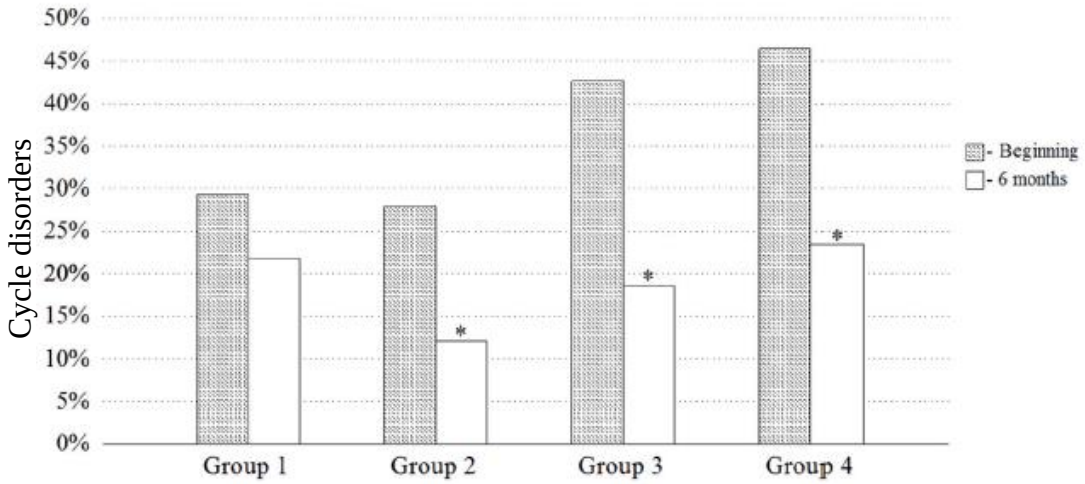

Figure I. Rate of irregular cycles at the start of treatment and six months later. Fisher's exact test, * $p<0.05$ indicates statistically significant results . Menstrual cycle disorders were substantially reduced in the therapy groups, at the same time the lifestyle group did not reach statistical significance, 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 III). Regarding age, there were no differences between the groups with normal and decreased insulin sensitivity (Table III).

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

	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**

Table IV. Insulin sensitivity characteristics and basic parameters
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).

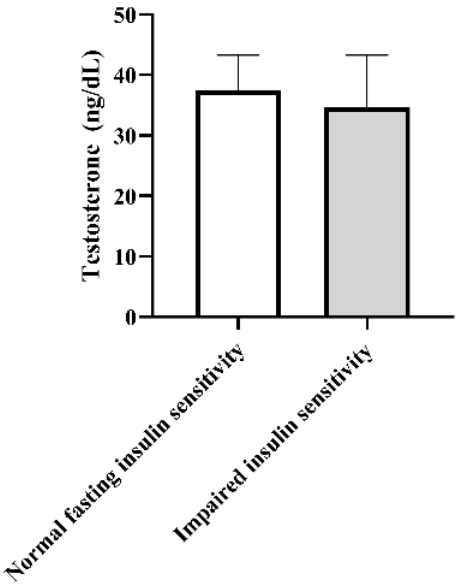

Figure II. Testosterone levels. Testosterone levels did not differ among the groups. Mann-Whitney test.

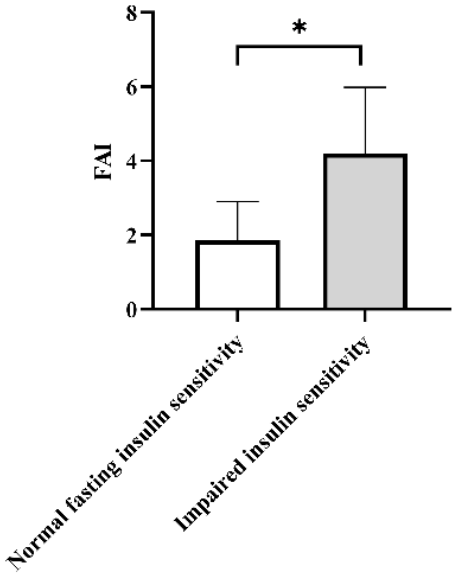

Figure III. Free androgen index. The FAI was significantly higher in the impaired insulin sensitivity group than in the normal fasting insulin sensitivity group. Mann-Whitney test, * $p < 0.05$.

5. CONCLUSIONS

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 connencted 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 testostesterone levels across the groups, the impaired insulin sensitivity group had a considerably higher FAI level. Measuring FAI during regular check-ups could possibly allow to 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.

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.

6. BIBLIOGRAPHY OF PUBLICATIONS

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