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ORIGINAL ARTICLE

Effects of myo-inositol, gymnemic acid, and L-methylfolate in polycystic ovary syndrome patients

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine and metabolic disorder, characterized by chronic anovulation/oligomenorrhea, hyperandrogenism, and insulin-resistance. Moreover, some studies propose a possible association between insulin resistance and hyperhomocysteinemia, which is a significant long-term risk for factor for atherogenesis and chronic vascular damage, especially in situations where insulin levels are increased. Insulin-sensitizing agents are used in the treatment of PCOS: in fact, inositols were shown to have insulin-mimetic properties. Synergic action to myo-inositol is that of gymnemic acids that have antidiabetic, anti-sweetener, and anti-inflammatory activities. Gymnemic acid formulations have also been found useful against obesity due to their ability to delay the glucose absorption in the blood. L-methyl-folate increases peripheral sensitivity to insulin, maintaining folatemia stable, and thus restoring normal homocysteine levels. Unlike folic acid, L-methyl folate has a higher bioavailability, no drug/food interferences, high absorption, and it is stable to UV-A exposure. The aim of our study is to compare the clinical, endocrine, and metabolic parameters in 100 PCOS women treated with myo-inositol, gymnemic acid, and l-methylfolate (Group A) or myo inositol and folic acid only (Group B), continuously for 6 months. From a clinical point of view, it was noticed a more significant improvement of the menstrual cycle regularity and a more significant reduction of BMI in Group A. Moreover, a more significant decrease of total testosterone and increase of SHBG serum levels were noticed in Group A. The metabolic assessment found a more significant decrease of total cholesterol and homocysteine levels; OGTT glycemia and insulinemia values were significantly more improved after treatment with myo-inositol + gymnemic acid. In conclusion, we can state that a good option for the treatment of PCOS is the combined administration of myo-inositol + gymnemic acid + l-methyl-folate, especially for overweight/obese patients with marked insulin resistance and with associated hyperhomocysteinemia.

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PCOS; insulin resistance; inositol; gymnemic acid; methylfolate

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine and metabolic disorder, characterized by chronic anovulation/oligomenorrhea, hyperandrogenism, and insulin resistance [1].

The syndrome is present in approximately 5–10% of reproductive-age women and it is considered the most frequent endocrine abnormality in females [2,3]. Approximately, 85–90% of women with oligomenorrhea have PCOS, while 30–40% of women with amenorrhea suffer from PCOS [4].

Insulin resistance (IR) and the associated metabolic abnormalities (visceral obesity and hypertension) are frequent findings in women with PCOS [5].

IR, and consequent compensatory hyperinsulinemia, appears to be the central pathophysiologic mechanism that links PCOS to its metabolic disorders; in fact, few studies reported that PCOS women are more insulin resistant than controls who are matched for age and BMI [6].

Disturbance in the insulin's ability to bind to its receptor or in the transport mechanism across the cell membrane may lead to a state of a reduced sensitivity to insulin, or IR. Furthermore, pancreatic β-cell secretory dysfunction has also been reported [7,8], and a reduction in hepatic insulin extraction contributes to the high insulin levels as well [9,10].

Compensatory hyperinsulinemia is important in the development of metabolic abnormalities and also contributes to the high androgen levels, peculiar of PCOS women [1].

There is a post-binding defect in insulin signaling in PCOS women, resulting in marked insulin sensitivity decrease. The defect is due to serine phosphorylation of the insulin receptor and IRS-1 secondary to intracellular serine kinases. This causes a decreased activation of PI3K mediated by insulin and resistance to the metabolic actions of insulin too [11].

Some studies have shown that insulin action on steroidogenesis in granulosa and theca cells is mediated via insulin receptor, both in normal and PCOS women [12,13]. Moreover, in PCOS granulosa cells, increased insulin levels might cause premature LH receptor expression in small follicles, leading to premature granulosa terminal differentiation and the arrest of follicular growth, which is the basis for anovulation.

Furthermore, human studies have demonstrated that insulin can increase circulating androgen levels in PCOS women.

Physiologically, insulin acts as a 'co-gonadotropin' to increase androgen synthesis induced by LH in theca cells [14–16] and to boost FSH-mediated estrogen production and LH-induced luteinization in granulosa cells [17].

Moreover, insulin can also act indirectly to raise free testosterone serum concentration by inhibiting the hepatic production of SHBG [1].

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Although obesity is a major factor for the development of IR in PCOS, it is now well known that a component of IR is independent of body weight [18].

For all these reasons, insulin-sensitizing agents are used in the treatment of PCOS, particularly metformin and inositol.

Myoinositol (MI) and D-chiro-inositol (DCI) were shown to have insulin-mimetic properties and to be efficient in the treatment of PCOS.

MI is naturally present in animal and plant cells, as free form, as inositol-containing phospholipid (phosphoinositides), or as phytic acid (IP6) [19]. The greatest amounts of MI in common foods are found in fresh fruits and vegetables and in peas, beans, grains, and nuts [20]. Originally, MI was considered one of the B-complex vitamins, but now it is no more reputed an essential nutrient because it was shown that it is produced in sufficient amount in the human body from D-glucose. *In vivo*, conversion of MI to DCI can occur in tissue expressing the specific epimerase.

The exact mechanisms of action of MI and DCI with insulin-mimetic activities are still unclear [1].

One of the most interesting models is the one elaborated by Larner et al. [21]. According to this model, insulin binding to its receptor (IR) causes the autoactivation of the receptor, and the activated IR can transduce the signal through two parallel signaling pathways, which act together to mediate insulin action in a complementary and synergistic manner [22]: the first one implies the recruitment and activation of substrate of insulin receptor (IRS) by the activated IR. Subsequent protein activations (PI3K and PDK-1) finally lead to PKB-Akt recruitment and activation at the plasma membrane. Activated PKB-Akt induces GLUT-4 translocation to the plasma membrane, improving glucose entry into the cell.

When insulin binds to its receptor, the epimerase converts MI molecules to DCI. In the second pathway, the IR is fixed to a G protein itself attached to a phospholipase that catalyzes the hydrolysis of a GPI [23]. The insulin-induced hydrolysis of the GPI releases an inositol phosphoglycan containing DCI (DCI-IPG), which acts as a probable second messenger of insulin (INS-2) mediating insulin effects on glucose oxidative and non-oxidative clearance.

Moreover, it is known that part of MI supplementation effect on insulin sensitivity may come from its partial *in vivo* intracellular epimerization to DCI [22].

Recently, it was highlighted how epimerase activity/expression seems to be abnormal in diabetic patients and abnormal/reduced in those PCOS patients that have familial diabetes [24].

MI intracellular concentration is regulated through processes, such as extracellular MI uptake, *de novo* biosynthesis, regeneration, efflux, and degradation. IR is associated with: abnormally low levels of DCI in urine, plasma, and insulin target tissues (liver, muscle, and fat), excessive MI urinary excretion and intracellular MI deficiency in insulin-sensitive tissues.

Synergic action to myo-inositol is that of gymnemic acids that have antidiabetic, anti-sweetener, and anti-inflammatory activities [25]. Gymnemic acid (GA) formulations have also been found useful against obesity [26] due to their ability to delay the glucose absorption in the blood [25].

GA is a saponin extracted from *Gymnema sylvestre*, which is a slow growing herb, found ideally in tropical and subtropical humid climate and common in hills of evergreen forests [27].

It is shown that GA increases the activities of enzymes responsible for utilization of glucose by insulin-dependent pathways, increasing phosphorylase activity and decreasing gluconeogenic enzymes, and sorbitol dehydrogenase; it also causes inhibition of glucose absorption from small intestine [25]. GAs curb the binding of carbohydrates to the receptors in the intestine and hence, the 'empty calories' are taken care of so that the body does not go into obese stage [25].

Hypoglycemic effects of GAs include a cascade of events starting from modulation of incretin activity, which triggers insulin secretion and release; it also increases regeneration of pancreatic islet cells to enhanced enzyme-mediated uptake of glucose [27]. This process decreased glucose and fatty acid assimilation in the small intestine and interferes with the ability of receptors in mouth and intestine to sensation of sweetness. It has been previously reported in literature that the action of GA is similar to that of incretin-mimetic mechanism of action [28]. Toxicity studies of *G. sylvestre* extract have shown its safety when taken in recommended doses; high doses may lead to side effects including hypoglycemia, weakness, shakiness, excessive sweating, and muscular dystrophy [27].

Moreover, some studies propose a possible association between IR and hyperhomocysteinemia (HHcy) [29] due to a documented increased incidence of the latter in PCOS women [30]. HHcy is a significant long-term risk for factor for atherogenesis and chronic vascular damage, especially in situation s where insulin levels are increased [31,32].

Classic homocysteinemia has been characterized as the accumulation of Hcy due to defects in enzymatic pathways [29]. Insulin levels have also been implicated as a modulating factor of Hcy, in that insulin inhibits hepatic cystathione B synthase activity [33,34].

Apart from the thrombogenic effect of elevated Hcy on pregnant PCOS women (resulting in microthrombus formation causing placental dysfunction), recent findings have implicated the adverse effect of HHcy on the defect in folliculogenesis [35], embryo quality [36], oocyte number, and maturation [37].

L-methyl-folate increases peripheral sensitivity to insulin, maintaining folateemia stable, and thus restoring normal homocysteine levels. Unlike folic acid, L-methyl folate has a higher bioavailability, no drug/food interferences, high absorption, and it is stable to UV-A exposure [38].

The aim of our study is to compare the clinical, endocrine, and metabolic parameters in 100 PCOS women treated with myo-inositol, GA, and l-methylfolate, or myo inositol and folic acid only, continuously for 6 months.

Materials and methods

All the patients enrolled in this study attended the 'Gynecological Endocrinology Outpatient Clinic' at the Institute of Obstetric and Gynecological Pathology ('Santo Bambino' Hospital, Catania) from January to December 2016.

In the 12 months enrollment phase, a total of 100 PCOS women were selected. Each patient received and signed an informed consent.

Inclusion criteria were:

- Patient's age >20, but <35 years old;
- BMI >25, but <34.99;
- Family history of type 2 diabetes mellitus;
- Patients not on any medications for menstrual disorders (oral contraceptives and metformin) or other pathologies (hypertension, dyslipidemia, or any other medical or psychiatric illness) for at least 3 months before study enrollment;
- Diagnosis of PCOS, according to Rotterdam criteria [20];

- Exclusion of other endocrine diseases (hyperprolactinemia, thyroid dysfunctions, Cushing's syndrome, congenital adrenal hyperplasia, or androgen-secreting tumors).

The study was double-blind designed and, according to a randomization table, patients were divided into two groups: patients of Group A intook 2 g of myo-inositol +400 µg of methyl-folate +75 mg of GA, while Group B intook 2 g of myo-inositol +400 µg of folic acid only. Both groups took the treatment continuously for 6 months per oral.

All the patients followed the same diet and performed a similar physical activity.

Six months after the treatment was carried out a clinical, endocrine, and metabolic assessment of patients enrolled.

Clinical evaluation: BMI, W/H ratio, acne score (according to Cremoncini Score), hirsutism score (according to Ferriman and Gallwey's score), relief of the number of the spontaneous menstrual cycle, and side effects.

Endocrine parameters: serum assay of LH, FSH, estradiol, prolactin, total testosterone, DHEA, and SHBG (blood sample taken within the 8th day of spontaneous or progesterone-induced cycle).

Table 1. Results of the study.

	Group A		Group B		
	Pre	Post	Pre	Post	*p* Value
BMI (kg/m^2)	27 ± 0.8	22 ± 0.6	27 ± 0.9	25 ± 0.8	**.04**
W/H ratio	0.86 ± 0.4	0.83 ± 0.2	0.85 ± 0.3	0.84 ± 0.4	**.19**
Acne score	3.4 ± 0.6	2.9 ± 0.5	3.3 ± 0.8	3 ± 0.4	**.09**
Hirsutism score	16 ± 1	15 ± 0.8	16 ± 0.6	14 ± 1	**.21**
Cycle (no. of pts)	13	33	10	19	**.01**
LH (mIU/ml)	12 ± 2	11 ± 1.7	12 ± 2.6	10 ± 1.5	**.06**
FSH (mIU/ml)	8 ± 1.8	7 ± 1	9 ± 1.4	8 ± 0.9	**.06**
Estradiol (pg/ml)	58 ± 4.5	55 ± 3	62 ± 6	57 ± 4.4	**.06**
Prolactin (ng/ml)	16 ± 1.8	16 ± 0.9	15 ± 0.8	14 ± 0.7	**.1**
Tot testost (ng/ml)	0.61 ± 0.5	0.55 ± 0.7	0.63 ± 0.8	0.60 ± 0.6	**.002**
DHEA (ng/ml)	11 ± 2.3	9 ± 1.7	10 ± 0.8	10 ± 0.8	**.08**
SHBG (nmol/ml)	23 ± 4	38 ± 3	25 ± 3.4	32 ± 2.8	**.00007**
Tot Chol (mg/dl)	172 ± 18	164 ± 13	170 ± 23	167 ± 17	**.0002**
HDL (mg/dl)	43 ± 6	48 ± 5.8	44 ± 7.2	46 ± 6.3	**.04**
Triglyc (mg/dl)	50 ± 3	49 ± 4	55 ± 2.7	54 ± 3.2	**.07**
Homocysteine (umol/l)	16 ± 2.4	11 ± 3	15 ± 3.2	13 ± 2.8	**.004**
Basal glycemia (mg/dl)	92 ± 2	87 ± 1	90 ± 3	88 ± 2	**.03**
Glycemia 60′ (mg/dl)	168 ± 4	160 ± 5	172 ± 4	168 ± 2	**.02**
Glycemia 120′ (mg/dl)	98 ± 3	91 ± 2	95 ± 3	93 ± 1	**.04**
Basal insulin (mcU/ml)	19 ± 4.5	11 ± 1.6	17 ± 3	14 ± 2.9	**.002**
Ins 60′ (mcU/ml)	131 ± 5	91 ± 4.1	128 ± 3	111 ± 4.8	**.0004**
Ins 120′ (mcU/ml)	87 ± 3	63 ± 2.1	90 ± 2.5	82 ± 1.7	**.00001**

Metabolic evaluation included oral glucose tolerance test (OGTT), total and fractionated cholesterol, triglycerides, and homocysteinemia.

The comparison between Group A and Group B was performed using:

- Student *t* test for quantitative data normally distributed (age, BMI, W/H ratio, OGTT results, hormones, cholesterol, triglycerides, and homocysteine serum levels);
- U test for quantitative data not normally distributed (number of patients with regular menstrual, hirsutism, and acne score).

Results

The mean age of Group A was 27 y ± 3 m, whereas the mean age of Group B was 28 y ± 4 m, without statistically significant difference between the two groups.

After 6 months of therapy, from a clinical point of view, our study showed a more significant improvement of the menstrual cycle regularity in Group A compared to Group B (66 vs. 38%; $p = .01$) (Table 1; Figure 1).

Similarly, a more significant reduction of BMI was detected in Group A than in Group B ($p = .04$) (Table 1; Figure 2).

No significant adverse effects were reported in both groups.

Despite being highlighted a reduction of W/H ratio, acne, and hirsutism score in both groups, Group A does not show a more statistically significant improvement compared to Group B (Table 1).

Concerning the hormonal evaluation, no statistical changes between the two groups were found for LH, FSH, estradiol, prolactin, and DHEA serum levels, whereas a more significant decrease of total testosterone ($p = .002$) and increase of SHBG ($p = .00007$) serum levels, were noticed in Group A (Table 1; Figure 3).

The metabolic assessment found a more significant decrease of total cholesterol ($p = .0002$) and homocysteine levels ($p = .004$) (Figure 4) in Group A than in Group B.

OGTT glycemia and insulinemia values were significantly more improved after treatment with myo-inositol + GA (Table 1; Figure 5).

Discussion

A supplementation with MI or DCI was found to be safe and effective in improving metabolic and hormonal parameters in

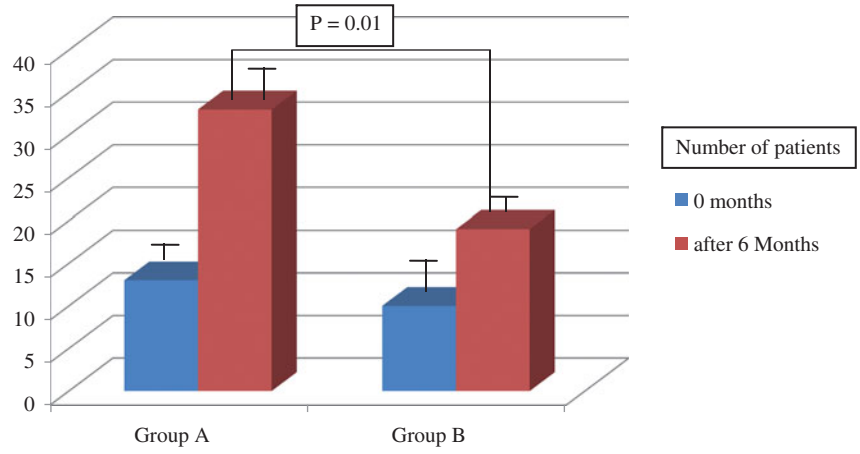


Figure 1. Retrieval of menstrual-cycle regularity before and after treatment with myo-inositol + gymnemic acid + L-methyl folate (Group A) and myo-inositol + folic acid (Group B).

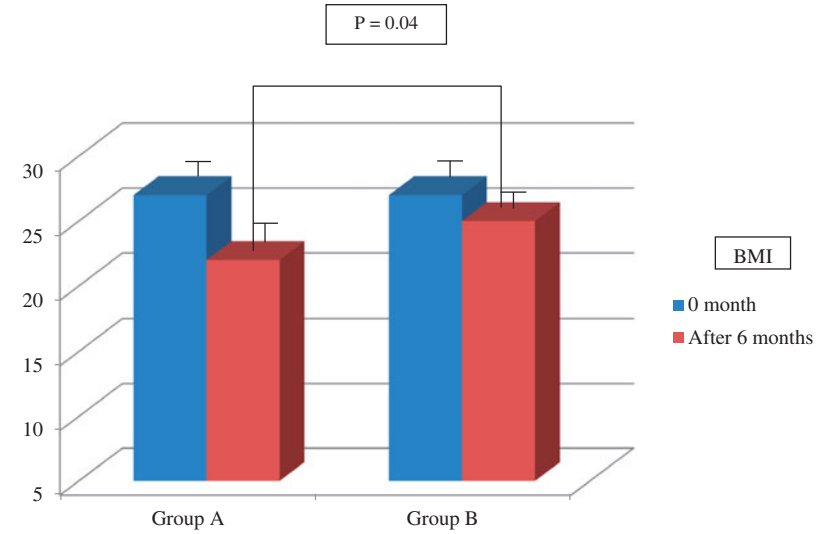


Figure 2. Patients' BMI before and after treatment with myo-Inositol + gymnemic acid + L-methyl folate (Group A) and myo-inositol + folic acid (Group B).

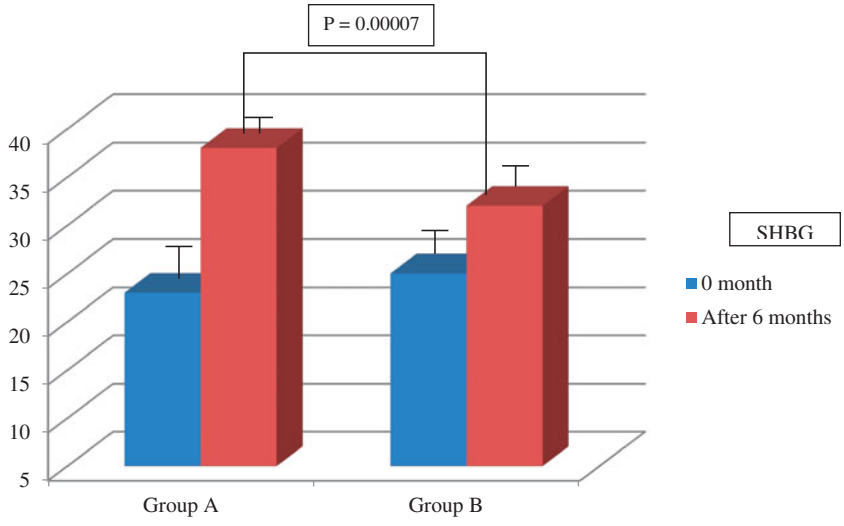


Figure 3. SHBG value before and after treatment with myo-Inositol + gymnemic acid + L-methyl folate (Group A) and myo-inositol + folic acid (Group B).

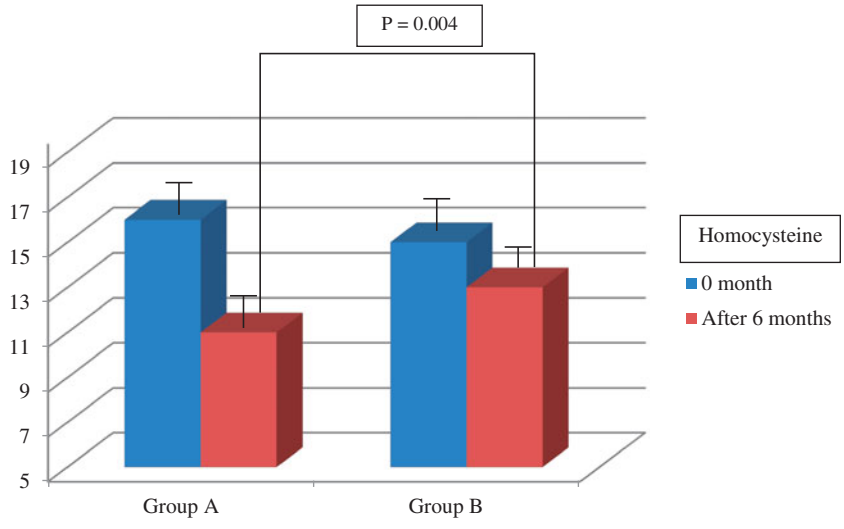
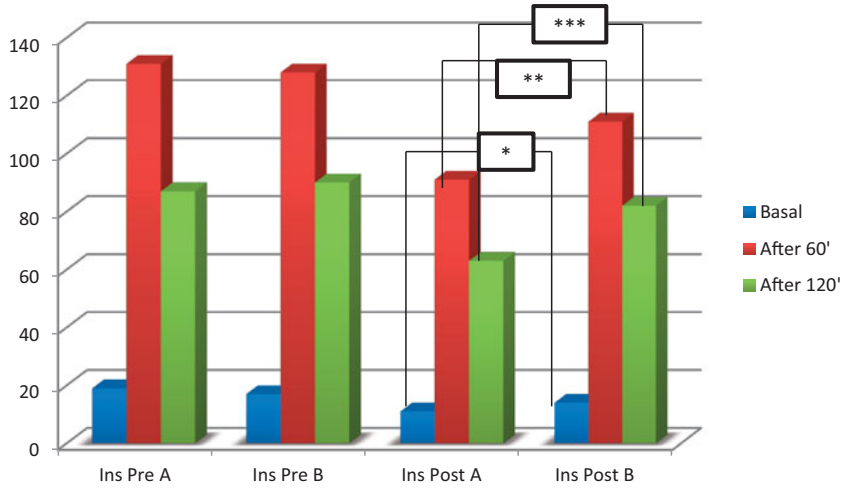


Figure 4. Homocysteine levels before and after treatment with myo-Inositol + gymnemic acid + L-methyl folate (Group A) and myo-inositol + folic acid (Group B).



* P = 0.002

** P = 0.0004

*** P = 0.00001

Figure 5. OGTT insulinemia values before and after treatment with myo-Inositol + gymnemic acid + L-methyl folate (Group A) and myo-inositol + folic acid (Group B). $^{*}p = .002$, $^{**}p = .0004$, and $^{***}p = .00001$.

PCOS patients: the main mechanism of action is based on improving insulin sensitivity of target tissues, resulting in the reduction of insulinemia, which has a positive effect on the reproductive axis and metabolism.

It was demonstrated in various studies that MI is able to:

- Reduce LH levels, LH/FSH ratio, and testosterone levels [39–43].
- Restore spontaneous ovulation and menstrual cycles [39,40,44–46].
- Improve cutaneous disorders of hyperandrogenism, reducing hirsutism, and acne score [41].
- Decrease HOMA index [40,42,47].
- Reduce systolic arterial blood pressure [42,43].
- Reduce leptin, LDL cholesterol levels, and triglycerides [46].
- Increase HDL cholesterol level [44,46].

In addition to these findings, our study particularly demonstrated that myo-inositol associated to GA and L-methylfolate is more effective in improving some clinical (BMI, retrieval of menstrual cycle regularity), hormonal (SHBG, total testosterone), and metabolic (total cholesterol, HDL, homocysteine, OGTT glycemia, and insulinemia) parameters of PCOS patients compared to the treatment with only myo-inositol and folic acid.

Despite predisposition to diabetes probably affects epimerase expression/synthesis, reducing endogenous MYO conversion to DCI [24], the improvement of the parameters tested is probably deputed to the synergic action of GA.

G. sylvestre 75 is a herbal preparation which contains 75% GA from leaf extract and provides nutritional support to pancreas and maintain healthy blood sugar balance when used as part of diet [27].

The mechanism of action of GA on glucose-insulin homeostasis is not well elucidated. Some studies have shown preservation of β cell function while others have shown improvement in insulin sensitivity [48,49].

It attaches to the receptor present in external layer of intestine, thereby preventing the absorption of sugar molecules by intestine, leading to reduction in blood sugar levels [50]. GA inhibits the sodium-glucose transporter (SGLT1), a membrane integral glucose conveyor located in the intestinal worm brush. This enzyme favors the passage of glucose from extracellular compartments within the cells. The molecular geometry of GA is similar to the glucose one and this molecule is capable of reversibly saturating the glucose receptor, thereby countering intestinal absorption. GA, lowering blood glucose levels, causes a reduced secretion of insulin by the pancreas, leading to increased exposure of the insulin receptors [25,51].

Moreover, GA determines a transient dysgeusia: it reduces the intake of sugars binding to sweet taste receptors [26,52,53].

A recent Indian study demonstrated that GA decreases the levels of fasting plasma glucose accompanied with decrease in fasting plasma insulin, reduction in systolic blood pressure and modest improvement in lipid profile. This was associated with decrease in HOMA-IR, suggesting that GA has beneficial effect on improvement insulin sensitivity [54], due to the increased cell permeability to insulin [55].

Our study confirmed the data in literature, even if we could not demonstrate that the clinical and metabolic improvements found in Group A were due to the intrinsic insulin-sensitizing action of GA or to the weight loss: anyhow, we can assert that at least GA allows better dietary adhesion.

Moreover, it is important to underline that the administration of GA does not cause any interference with the intestinal absorption of myo-inositol.

Indeed, Scalera et al. [56–58] demonstrated that SGLT1 is a high-affinity glucose carrier but is not involved in the carriage of myo-inositol, while SMIT is a low-affinity glucose carrier but responsible for the transport of myo-inositol both at the intestinal and peripheral levels. GA partially inhibits SGLT1, but not SMIT, thus it contrasts the absorption of glucose but not of myo-inositol, in contrast to other polyphenols' action, such as florizine that works on both transporters and, therefore, it selectively inhibits the absorption of inositol by the cell membranes.

Epidemiological and experimental studies also link HHcy to IR; elevated levels of homocysteine have been observed in a variety of subjects with type 2 diabetes [59], metabolic syndrome

[60], and obesity [61,62]. On the other hand, HHcy has induced IR itself. Prolonged folate treatment decrease homocysteine and insulin levels, thus improving IR in patients with metabolic syndrome [63]. The molecular mechanism by which homocysteine promotes IR remains unclear; a recent study has demonstrated that homocysteine upregulates the expression of resistin (a mediator of IR), and the oxidative stress produced by Hcy thiolactone interrupts the insulin signaling pathway by inhibiting the phosphorylation of insulin receptor tyrosine kinase, phosphatidylinositol 3 kinase, and glycogen synthase kinase 3β [64–66].

The accumulation of misfolded proteins within the endoplasmic reticulum triggers that activation of an unfolded protein response (UPR) [67]. It is possible that homocysteine induces IR by impairing glucose homeostasis through the activation of UPR pathway [64].

Our study also demonstrated a significant decrease of homocysteinemia, due to the reduction of insulin-resistance and to the action of L-methyl-folate that is now considered the 'gold standard' for the treatment of hyperhomocysteinemia (HHcy).

Methylfolate (6S-5-Methyl-THF) is a natural folate that constitutes 98% of the plasmatic folates. Bioavailability studies have shown that supplementation with methylfolate is as effective as treatment with folic acid in improving folate levels in the body [68].

Unlike folic acid, methylfolate does not need to be biotransformed by DHFR enzyme to become active. Therefore, the bioavailability of methylfolate is not limited by saturation of this enzyme and its intake does not involve the presence of non-metabolized forms in the blood. Moreover, methylfolate is not affected by functional polymorphisms in *MTHFR* and *DHFT* genes, which instead limit the efficacy of folic acid [69].

Compared to folic acid, methylfolates have a lower risk of interaction with DHFR inhibitors (e.g. Methotrexate) and are less likely to mask the hematologic symptoms due to vitamin B12 deficiency in patients with pernicious anemia [69,70].

In addition, methylfolate is stable to UV-A exposure that, on the contrary, degrades folic acid [71]. Therefore, during sunlight exposure, supplementation with methylfolate ensures stability of blood folate levels that cannot be guaranteed by folic acid.

In conclusion, we can state that a good option for the treatment of PCOS is the combined administration of myo-inositol + GA + l-methyl-folate, especially for overweight/obese patients with marked IR and with associated hyperhomocysteinemia.

Disclosure statement

The authors declare that there is no conflict of interest regarding the publication of this article.

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