DR VALENTINA IMMEDIATA (Orcid ID : 0000-0001-8241-3949)

Received Date : 02-Nov-2016

Revised Date : 18-Dec-2016

Accepted Date : 10-Jan-2017

Article type : 2 Original Article - Europe, excluding UK

Title: Metformin versus myoinositol: which is better in obese PCOS patients? A randomized controlled crossover study.

Short title: comparison between metformin and myoinositol in PCOS

Valeria Tagliaferri MD, Daniela Romualdi MD, PhD, Valentina Immediata MD*, Simona De Cicco MD, Christian Di Florio MD, Antonio Lanzone MD, Maurizio Guido MD, PhD.

1 Department of Obstetrics and Gynaecology, Università Cattolica del Sacro Cuore, Largo "A. Gemelli" 8, 00168 Roma, Italy

2 Department of Obstetrics and Gynaecology, Ospedale Generale Regionale Ente Ecclesiastico F.Miulli, Strada Provinciale Acquaviva - Santeramo, Km 4, 70021 Acquaviva delle Fonti (BA), Italy

V.T and D.R contributed equally to this work

***Corresponding author:**

Valentina Immediata, MD, Department of Obstetrics and Gynecology, Università Cattolica del Sacro Cuore, L.go Agostino Gemelli,8, 00168 Roma, Italia. Tel/Fax +39063057794

vale.immediata@gmail.com

Acknowledgement: All authors have no conflicts of interest to declare.

Keywords: myoinositol, metformin, insulin, metabolism, polycystic ovary syndrome

Summary

Context: due to the central role of metabolic abnormalities in the pathophysiology of PCOS, insulin sensitising agents have been proposed as a feasible treatment option.

Objective: to investigate which is the more effective between metformin and myoinositol on hormonal, clinical and metabolic parameters in obese patients with polycystic ovary syndrome (PCOS).

Study design: crossover randomized-controlled study.

Patients: Thirty-four PCOS obese women (age: 25.62 ± 4.7 years; BMI: 32.55 ± 5.67 kg/m^2) were randomized to receive metformin (850 mg twice a day) or myoinositol (1000 mg twice a day) for six months. After a three month washout, the same subjects received the other compound for the following six months.

Measurements: Ultrasonographic pelvic examinations, hirsutism score, anthropometric and menstrual pattern evaluation, hormonal profile assays, oral glucose tolerance test (OGTT) and lipid profile at baseline and after 6 months of treatment were performed.

Results: Both metformin and myoinositol significantly reduced the insulin response to OGTT and improved insulin sensitivity. Metformin significantly decreased body weight, and improved menstrual pattern and Ferriman-Gallwey score. Metformin treatment was also associated with a significant decrease in LH and estradiol levels, androgens and AMH levels. None of these clinical and hormonal changes were observed during myoinositol administration.

Conclusions: Both treatments improved the glyco-insulinaemic features of obese PCOS patients,but only metformin seems to exert a beneficial effect on the endocrine and clinical features of the syndrome.

Trial registration number: NCT01791647

Introduction

During the past decades, increasing evidence has supported the central role of metabolic disturbances such as insulin resistance and/or compensatory hyperinsulinaemia in the pathogenesis of polycystic ovary syndrome (PCOS) (1). This endocrine disorder affects approximately 5-10% of women of reproductive age and is characterised by the heterogeneous combination of menstrual irregularities, chronic anovulation, hyperandrogenism and metabolic abnormalities (2).

Hyperinsulinaemia seems to directly stimulate both ovarian and adrenal androgen secretion and to suppress liver sex hormone binding globulin (SHBG) synthesis causing an increase in free, biologically active androgens. The excess in androgen production, worsened by hyperinsulinaemia, causes premature follicular atresia and anovulation. Moreover, at the central level, insulin seems to be involved in the dysregulation of LH secretion (3).

Based on this rationale, insulin-sensitising agents have been proposed as a useful primary or adjunctive treatment to improve clinical and biochemical parameters in patients with PCOS by lowering insulin secretion. In this group of compounds, the biguanide metformin has been the most extensively investigated: in randomized trials, it was reported to improve insulin sensitivity, body mass index (BMI), menstrual irregularities and hyperandrogenism in most PCOS women (4).

Despite the clinical efficacy, the metformin administration is burdened by several side-effects, such as nausea, vomiting and gastrointestinal discomfort (5-6) in about 30% of users, accounting for the poor compliance associated with this treatment.

Several reports in literature have also analysed the role of thiazolidinediones in PCOS treatment (7-8). These drugs are able to restore normal menstrual cyclicity and improve clinical signs of

hyperandrogenism: nonetheless, the widespread use of these substances is restricted by their documented hepatotoxicity (4).

Recently, attention has been given to the role of inositolphosphoglycan (IPG) mediators in insulin action. Molecular and animal studies showed that D chiro-inositol deficiency and an imbalance with its precursor myo-inositol (MYO) are directly related to the insulin resistance (9). In particular, the stereoisomer MYO is an important constituent of the follicular microenvironment, where it seems to play an important role in both nuclear and cytoplasmic oocyte development. It was demonstrated that in human follicular fluids higher concentrations of MYO are related to a better quality of oocytes (10).

Some authors have experimented with MYO supplementation in both obese and lean PCOS patients, reporting varying degrees of improvement in clinical, hormonal and metabolic features of the syndrome. However, previous reports in PCOS subjects exclusively evaluated the clinical effects of formulations containing MYO plus folic acid (11). In addition, no direct comparisons with other insulin sensitising compounds, regarding the effects on clinical and biochemical characteristics of the syndrome, are available in literature. The only published study comparing MYO and metformin focused on the ovulation and pregnancy rates in a population of infertile PCOS women (12).

Based on these evidences, in the present crossover study, we aimed at comparing for the first time the two insulin sensitising agents metformin and MYO in terms of efficacy in the treatment of overweight, obese PCOS patients.

MATERIALS AND METHODS

Participants

Thirty-four overweight/obese women with PCOS (mean age: 25.62 ± 4.7 years; mean BMI: 32.55 ± 5.67 kg/m^2) attending our divisional outpatient services were randomized for this study. PCOS was diagnosed according with the Rotterdam Consensus Conference criteria (13). The presence of a late-onset adrenal enzyme defect was excluded by an ACTH test (250 μg iv Synacthen; Ciba-Geigy, Basel, Switzerland) (14). Significant liver or renal impairment, pregnancy and nursing, neoplasm, cardiovascular disease and other hormonal dysfunctions were considered as exclusion criteria. Investigations were conducted during the early follicular phase of spontaneous or induced [medroxyprogesterone acetate (MPA) 10 mg/day for 7 days] menstrual cycles (day 3±7). All patients were evaluated for FSH, LH, estradiol, progesterone, prolactin, testosterone, androstenedione, 17-hydroxyprogesterone, SHBG, dehydroepiandrosterone sulfate, and anti-müllerian hormone (AMH). The ratio of testosterone x 100/ SHBG was used to calculate the free androgen index (FAI). The body mass index (BMI) was calculated as the ratio of weight (kilograms) to height^2 (square metres). A BMI > 25 kg/m^2 was considered suggestive for over-weight; a BMI >30 kg/m^2 was considered suggestive for obesity. Because of the impact of body fat distribution on androgen levels and glucose metabolism, waist to hip ratio (WHR) was measured. Waist circumference was determined as the minimum value between the iliac crest and the lateral costal margin, whereas hip circumference was determined as the maximum value over the buttocks. The cut-off point for high WHR for women was set at 0.80 (15). All patients also underwent transvaginal ultrasonography and the grade of hirsutism was assessed using the Ferriman-Gallwey (F-G) score performed at each visit by the same two members of our medical staff and the mean between the two determinations was considered for the analysis of the data (16).

An oral glucose tolerance test (OGTT) for insulin and glucose determinations was performed sampling 15 minutes before and 30,60,90,120,180 after the oral ingestion of 75g of glucose. Insulin

and glucose responses to the stimuli are expressed as the area under the curve (AUC). A normal insulinaemic response to OGTT was defined by a threshold AUC value of 12,000 IU/ml/180min, as previously described (17). On the following day, an euglycemic-hyperinsulinaemic clamp was performed to estimate peripheral insulin sensitivity. Peripheral glucose utilization, an expression of insulin sensitivity, was measured as M (mg/kg/min), with a threshold value for insulin resistance set at 4.5 mg/kg/min (18).

A transvaginal pelvic ultrasound was performed on each patient using a 6.5 MHz endovaginal probe (Esaote, AUC5). Ovarian volume was calculated for each ovary using the formula for a prolate ellipsoid: $\pi/6$ (D1xD2xD3), where D represents the maximum diameter in transverse, anteroposterior, and longitudinal axes.

Study design

This was a crossover, active-treatment-controlled, randomized, open-label, single centre study. Informed consent was obtained from all participants before enrollment. After the baseline evaluation, enrolled patients were divided into two equal groups by simple randomization with computer-generated random allocation sequence. One group was treated with metformin 850 mg twice a day and the other group with MYO 500 mg two oral pills twice a day for six months. At the end of this period the basal clinical and laboratory work-up was repeated in both groups. After a wash-out interval of three months, the patients who had been treated with metformin in the first stage were given MYO for six months, and the patients who had received MYO started the treatment with metformin for the same period of time. At the end of the six months the clinical and biochemical evaluation was performed again (fig. 1). During the study the subjects were advised to continue their usual diet and lifestyle.

Assays

All hormonal assays were performed with ECLIA (Electrochemiluminescence immunoassay) kits (Roche Diagnostics, Mannheim, Germany). Serum 17(OH)P concentrations were determined by radio-immunoassay; serum AMH concentrations were determined by enzyme immunoassay (EIA) (Immunotech–Beckman). The intra- and interassay coefficients of variation for all hormones were less than 8% and less than 15%, respectively. For each determination, all samples from the same patient were assayed simultaneously.

Plasma glucose concentrations were determined by the glucose oxidase technique with a glucose analyser (Beckman Coulter, Fullerton, CA). Total cholesterol and triglyceride concentrations were determined by an enzymatic assay (Bristol, Paris, France). HDL concentrations were determined after precipitation of chylomicrons, VLDL, and LDL (Roche, Mannheim, Germany), and VLDL was separated (as the supernatant) from LDL and HDL by lipoprotein ultracentrifugation. A magnesium chloride/phosphotungstic acid technique was used to precipitate LDL from the bottom fraction after ultracentrifugation. All lipid assay were performed according to standard laboratory procedures, as previously reported (19).

Statistical analysis

All results are presented as median and interquartile range (IQR).

Statistical analysis was performed by GraphPad Prism5 software. The distribution of data was tested by the Kolmorov-Smirnov test; we found that the variables were not normally distributed. The data from the study groups were compared by using Mann-Whitney *U* test. The significance of

differences between the same tests performed before and after treatment was assessed using the nonparametric Wilcoxon rank-sum test. $P < 0.05$ was considered statistically significant.

Ethical approval

This study was approved by our local ethics committees (Institutional Review Board of Department of Obstetrics and Gynaecology, Catholic University of Sacred Heart).

RESULTS

A total of 34 PCOS patients were enrolled between January 2013 and May 2015 and 26 completed the study without protocol violations; 7 dropped out due to mild gastrointestinal side effects during metformin treatment. One patient became pregnant during the administration of myoinositol.

The patients were categorized into two groups (each containing thirteen cases), via computer randomization. At baseline, the two groups were well matched in terms of clinical, endocrine and metabolic characteristics.

Table I shows the clinical, anthropometric and hormonal characteristics of participants before and after 6 months of metformin or MYO treatment. At the beginning of the study, all subjects were oligo-amenorrhoeic and had mild to moderate hirsutism. All the studied patients were overweight or obese (BMI > 25), with a predominantly central body fat distribution, as shown by the WHR values. Mean values of ovarian and adrenal androgens were at the upper limit of or above the normal range in both groups; mean SHBG levels, conversely, were at the lower limit of the normal range.

After six months of metformin treatment, independently of the sequence of drug administration, a significant decrease in body weight ($p<0.01$) and, consequently, BMI ($p<0.01$) was observed. Patients reported an improvement in menstrual cycle frequency ($p<0.01$) and F-G score ($p< 0.05$). In this group of patients, a significant reduction of androstenedione levels ($p<0.01$) and FAI (p

<0.05) was detected. Serum AMH levels, which were above the normal range at baseline, decreased significantly after six months of treatment ($p<0.01$). Both LH and estradiol levels dropped significantly with metformin treatment ($p< 0.05$). The other hormonal parameters under evaluation did not change significantly.

During MYO administration, none of the patients experienced any significant improvement in clinical, anthropometric or hormonal parameters.

Ovarian volume, as estimated by ultrasound scan, was not significantly affected by either of the treatments.

The effects of the two different treatments on the metabolic profile are shown in Table II. None of the studied subjects exhibited impaired glucose tolerance at baseline and the mean insulinaemic response to the oral glucose tolerance test was above the normal range. Evaluation of peripheral glucose utilization obtained by the euglycaemic hyperinsulinaemic clamp documented mild insulin resistance. Both metformin and MYO significantly reduced the insulinaemic response to OGTT ($p< 0.05$, $p < 0.01$ respectively); during treatments with the two different drugs peripheral insulin sensitivity even showed a trend towards an increase, although not statistically significant, as documented by the M value. Neither compound affected lipid profile (data not shown).

DISCUSSION

Some authors have suggested that hyperinsulinaemia and insulin resistance may represent the common determinant in the interplay of anovulation, hyperandrogenism and AMH overproduction that characterize PCOS (20-21). The high circulating levels of insulin act at various levels of the hypothalamic-pituitary-ovarian axis, as well as on the hepatic production of SHBG, thus contributing to the endocrine-reproductive abnormalities (22). Since the insulin sensitizing agents came into use in the management of PCOS, metformin has shown an overall positive effect on the

clinical, endocrine and metabolic features of PCOS. Recently, a new group of substances, nutrients, dietary supplements and herbal products has gained popularity in the management of the syndrome. In particular, the inositol isomers have been proposed for the long-term treatment of PCOS. Many authors suggest that a defect in tissue availability or altered metabolism of inositol or IPGs mediators may contribute to insulin resistance in PCOS subjects (23). Several studies have reported beneficial effects of MYO and D-chiroinositol in women affected by PCOS: most have documented an amelioration in the endocrine and clinical features of these subjects as well as in insulin metabolism (9,11).

Based on these data, the aim of our study was to evaluate for the first time the effect of the administration of myoinositol (2g daily) in comparison with the best known insulin sensitising drug metformin on hormonal, clinical and metabolic parameters in the same group of overweight/obese PCOS patients.

During metformin administration we observed a return to menstrual regularity in all treated subjects, confirming the ability of this drug to reduce plasma androgen concentrations in PCOS women. (24-25). Interestingly, a marked decrease of LH plasma levels was obtained in metformin treated patients, reaching statistical significance. Several lines of evidence suggest that insulin-lowering drugs are associated with a decrease of LH levels: metformin long-term administration was able to obtain this effect in obese PCOS subjects (26). In addition, AMH levels significantly decrease during metformin treatment, as previously demonstrated in a study from our group in obese PCOS patients (27).

These results are in line with the huge number of studies in the literature that document the positive benefits of metformin in the treatment of PCOS.

Notwithstanding the considerable number of published trials on myoinositol in PCOS patients, treatment schedules are not standardized. In line with previous studies (28-29), we chose to

administer a dose of 2g daily and to evaluate the efficacy of this compound in comparison with metformin in women affected by PCOS. Despite a significant decrease in insulin response to glucose load, we failed to find significant modifications in the clinical and biochemical parameters analysed. By contrast, several previous reports documented an improvement in the hormonal milieu and restoration of menstrual cyclicity after MYO treatment in PCOS patients (28,30). In a previous study, Artini *et al* observed a significant amelioration of both menstrual regularity and gluco-insulinaemic metabolism, expressed as glucose-to-insulin ratio and HOMA index, whereas no changes were reported in circulating androgen levels (31). Several hypotheses can be formulated in order to explain such inconsistencies. First, it could be speculated that the lack of changes in hormonal and clinical characteristics in our study may be due to the use of pure MYO. In the majority of the above mentioned studies, indeed, patients were treated with formulations containing a combination of MYO and folic acid and/or other substances (11). This hypothesis is further supported by the fact that, in vivo, folic acid may improve glycaemic control and insulin resistance in patients with type 2 diabetes (32-33). Through a synergic effect, the association of MYO and folic acid may account for the clinical improvements of PCOS abnormalities observed in the previous reports.

On the other hand, we cannot rule out the possibility that the low MYO dose we used might have been insufficient to disclose major clinical and hormonal changes (34).

Interestingly, the effect of the two different treatments on insulin metabolism on our PCOS group seems to be superimposable. These data further support the role of inositol as a modulator of insulin-mediated metabolic pathways and fit with previous evidence emphasising the association between insulin sensitivity and inositol–phosphoglican (IPG) intracellular balance (23). Myo-inositol administration positively affects hyperinsulinaemia and hormonal parameters in overweight patients.

In conclusion, this study confirms the beneficial effect of long term treatment with metformin on the clinical, biochemical and metabolic characteristics of PCOS as several lines of evidence have already suggested. Against this positive result in terms of efficacy, the 21% incidence of adverse effects associated with metformin treatment (7/33 subjects) should be considered. Myoinositol therapy was not able to ameliorate the endocrine or clinical features of the syndrome in our patients. Conversely, our analysis showed that a dose of 2g of MYO seems to result in a significant improvement in the metabolic parameters of PCOS patients.

This result may suggest the possible usefulness of a combination of lower doses of metformin and MYO in treating the metabolic derangements of PCOS. It is well known that the the magnitude of adverse effects associated with metformin is dose related and that the minimal effective dosage should be preferred in order to reduce patients' symptoms (35).

Finally, our study seems to suggest a clear advantage of metformin over myoinositol in overweight/obese hyperinsulinaemic PCOS patients.The major weakness of this study, however, is the small sample size and further randomised clinical trials are needed to compare the two molecules in a larger and more heterogeneous PCOS population.

Declaration of interest: All authors have no conflicts of interest to declare.

Sources of funding: none

Authors role:

Valeria Tagliaferri and Daniela Romualdi: analysis and interpretation of data, draft of the article

Valentina Immediata: interpretation of data and revision of the article

Simona De Cicco and Christian Di Florio : acquisition and analysis of data

Antonio Lanzone and Maurizio Guido: critical discussion, final approval of the version to be submitted

References

1. Morin-Papunen LC, Vauhkonen I, Koivunen RM *et al*. (2000) Insulin sensitivity, insulin secretion, and metabolic and hormonal parameters in healthy women and women with polycystic ovarian syndrome. *Human Reproduction*; **15**:1266–74.
2. McCartney CR & Marshall JC (2016) Polycystic ovary syndrome. *NEJM*; **375**:54-64
3. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. *Endocr Rev* 1997; **18**: 774-800.
4. Tang T, Lord JM, Norman RJ *et al.* (2012) Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. *Cochrane Database Syst Rev*; **16**; CD003053.
5. Palomba S, Falbo A, Zullo F *et al.* (2009) Evidence-based and potential benefits of metformin in the polycystic ovary syndrome: a comprehensive review. *Endocr Rev*; **30**:1-50.
6. Fulghesu AM, Romualdi D, Di Florio C, *et al.* (2012) Is there a dose-response relationship of metformin treatment in patients with polycystic ovary syndrome? Results from a multicentric study. *Hum Reprod.*; **27**:3057-66.
7. Azziz, R, Ehrmann D, Legro RS, *et al*. (2001) Troglitazone improves ovulation and hirsutism in the polycystic ovary syndrome: a multicenter, double blind, placebo-controlled trial. *J. Clin. Endocrinol. Metab.*; **86**, 1626-1632.

8. Romualdi D, Guido M, Ciampelli M, *et al*. (2003) Selective effects of pioglitazone on insulin and androgen abnormalities in normo- and hyperinsulinaemic obese patients with polycystic ovary syndrome. *Hum Reprod*; **18**:1210-8.

9. Larner J. (2002) D-chiro-inositol--its functional role in insulin action and its deficit in insulin resistance. *Int J Exp Diabetes Res*; **3**:47-60.

10. Chiu TT, Rogers MS, Law EL, *et al.* (2002) Follicular fluid and serum concentrations of myo-inositol in patients undergoing IVF: relationship with oocyte quality. *Hum Reprod*; **17**:1591-6.

11. Unfer V, Carlomagno G, Dante G, *et al.* (2012) Effects of myo-inositol in women with PCOS: a systematic review of randomized controlled trials. *Gynecol Endocrinol*; **28**:509-15.

12. Raffone E, Rizzo P & Benedetto V. (2010) Insulin sensitiser agents alone and in co-treatment with r-FSH for ovulation induction in PCOS women. *Gynecol Endocrinol*; **26**:275-80.

13. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long term health risks related to polycystic ovary syndrome (PCOS) (2003). *Human Reproduction*; **19**: 41-7.

14. New MI, Lorenzen F, Lerner AJ, *et al.* (1983) Genotyping steroid 21-hydroxylase deficiency: hormonal reference data. *Journal of Clinical Endocrinology and Metabolism*; **57**:320-6.

15. Taylor RW, Keil D, Gold EJ, *et al*. (1998) Body mass index, waist girth, and waist to hip ratio as indexes of total and regional adiposity in women: evaluation using receiver operating characteristic curves. *The American Journal of Clinical Nutrition*; **67**:44–49.

16. Ferriman D & Gallwey JD. (1961) Clinical assessment of body hair growth in women. *Journal of Clinical Endocrinology and Metabolism*; **21**: 1440-7.

17. Ciampelli M, Leoni F, Cucinelli F, *et al.* (2005) Assessment of insulin sensitivity from measurements in the fasting state and during an oral glucose tolerance test in polycystic ovary syndrome and menopausal patients. *Journal of Clinical Endocrinology and Metabolism*; **90**: 398-1406.

18. DeFronzo JP, Tobin JD & Andrei R. (1979) Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am. J. Physiol*; **237**, E214-E223.

19. Ciampelli M, Fulghesu AM, Cucinelli F, *et al.* (1999) Impact of insulin and body mass index on metabolic and endocrine variables in polycystic ovary syndrome. *Metabolism*; **48**: 167-72.

20. Jonard S & Dewailly D. (2004) The follicular excess in polycystic ovaries, due to intra-ovarian hyperandrogenism, may be the main culprit for the follicular arrest. *Hum Reprod Update*; **10**:107-17.

21. Chen MJ, Yang WS, Chen CL, *et al.* (2008) The relationship between anti-Mullerian hormone, androgen and insulin resistance on the number of antral follicles in women with polycystic ovary syndrome. *Hum Reprod*; **23**:952-7.

22. Teede HJ, Deeks A & Moran L. (2010) Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. *BMC*; **30**;8:41.

23. Baillargeon JP, Nestler JE, Ostlund RE, *et al* (2008). Greek hyperinsulinemic women, with or without polycystic ovary syndrome, display altered inositols metabolism. *Hum Reprod*; **23**:1439-46

24. Vrbíková J, Hill M, Stárka L, *et al.* (2002) Prediction of the effect of metformin treatment in patients with polycystic ovary syndrome. *Gynecol Obstet Invest*; **53**:100-4.

25. Ben-Haroush A, Yogev Y & Fisch B. (2004) Insulin resistance and metformin in polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol*; **10**;115:125-33.

26. Kowalska I, Kinalski M, Straczkowski M, *et al.* (2001) Insulin, leptin, IGF-I and insulin-dependent protein concentrations after insulin-sensitizing therapy in obese women with polycystic ovary syndrome. *Eur J Endocrinol*; **144**:509-15.

27. Romualdi D, De Cicco S, Tagliaferri V, *et al*. (2011) The metabolic status modulates the effect of metformin on the antimullerian hormone-androgens-insulin interplay in obese women with polycystic ovary syndrome. *J Clin Endocrinol Metab*; **96**:E821-4

28. Genazzani AD, Lanzoni C, Ricchieri F, *et al.* (2008) Myo-inositol administration positively affects hyperinsulinemia and hormonal parameters in overweight patients with polycystic ovary syndrome. *Gynecol Endocrinol*; **24**:139-44.

29. Genazzani AD, Prati A, Santagni S, *et al.* (2012) Differential insulin response to myo-inositol administration in obese polycystic ovary syndrome patients. *Gynecol Endocrinol*; **28**:969-73.

30. Checa MA, Requena A, Salvador C, *et al.* (2005) Reproductive Endocrinology Interest Group of the Spanish Society of Fertility. Insulin-sensitizing agents: use in pregnancy and as therapy in polycystic ovary syndrome. *Hum Reprod Update*; **1**:375-90.

31. Artini PG, Di Berardino OM, Papini F, *et al.* (2013) Endocrine and clinical effects of myo-inositol administration in polycystic ovary syndrome. A randomized study. *Gynecol Endocrinol*; **29**:375-9.

32. Gargari BP, Aghamohammadi V & Aliasgharzadeh A. (2011) Effect of folic acid supplementation on biochemical indices in overweight and obese men with type 2 diabetes. *Diabetes Res Clin Pract*; **94**:33-8.

33. Villa P, Perri C, Suriano R, *et al.* (2005) L-folic acid supplementation in healthy postmenopausal women: effect on homocysteine and glycolipid metabolism. *J Clin Endocrinol Metab*; **90**:4622-9.

34. Facchinetti F, Bizzarri M, Benvenga S, *et al.* (2015) Results from the International Consensus Conference on Myo-inositol and d-chiro-inositol in Obstetrics and Gynecology: the link between metabolic syndrome and PCOS. *Eur J Obstet Gynecol Reprod Biol*; **195**:72-6.

35. Bosi E. (2009) Metformin--the gold standard in type 2 diabetes: what does the evidence tell us? *Diabetes Obes Metab*; **11** Suppl 2:3-8

Table I: Clinical and hormonal features at baseline and after 6 months of myoinositol and metformin treatment

	Myoinositol		Metformin	
	Baseline	**After 6 months**	**Baseline**	**After 6 months**
BMI (kg/m^2)	31.5 (IQR 5.3)	31.2 (IQR 7.8)	29.7 (IQR 7.8)	32.5 (IQR7.9)*
WHR	0.84 (IQR 0.04)	0.85 (IQR 0.08)	0.83 (IQR 0.05)	0.81 (IQR 0.12)
FG score	13.5 (IQR 6.75)	11 (IQR 5.75)	12 (IQR 8)	9.5 (IQR 5)*
Cycles in 6 months	3 (IQR 2)	4 (IQR 2.75)	3.5 (IQR 1)	6 (IQR 0)*
FSH (IU/l)L)	5.1 (IQR 0.68)	5.25 (IQR 0.83)	5.5 (IQR 0.65)	5.5 (IQR 0.9)
LH (IU/Ll)	6.5 (IQR 1.43)	7 (IQR 2.85)	7 (IQR 8.85)	6.2 (IQR 0.9)
E2 (pmol/lpg/ml)	51.5109 (IQR 10.2538)	49.5182 (IQR 267)	45 165 (IQR 6618)	38 139 (IQR 226)*
Prolactin (ng/mlmU/l)	15.7314 (IQR 1366.8)	11.35227 (IQR 7.58152)	11 220 (IQR 1.530)	11.5230 (IQR 1.8537)
A (ng/mlnmol/l)	2.8910.1 (IQR 0.762.7)	2.368.2 (IQR 1.093.8)	3.1811.1 (IQR 1.154.0)	2.58.7 (IQR 1.475.1)*
T (ng/mlnmol/l)	0.41.4 (IQR 0.170.6)	0.411.4 (IQR 0.190.7)	0.561.9 (IQR 0.321.1)	0.551.9 (IQR 0.230.8)
FAI	1.55.5 (IQR 1.144.0)	1.545.3 (IQR 0.873.0)	2.167.5 (IQR 1.796.2)	1.55.2 (IQR 0.441.5)*
17(OH)P (ng/mlnmol/l)	0.82.4 (IQR 0.30.9)	0.75 2.3(IQR 0.351.1)	0.92.7 (IQR 0.451.4)	0.72.1 (IQR 0.20.6)
DHEAS (ng/mlµmol/l)	2066 5.6 (IQR 10594.3)	2454 6.7 (IQR 6761.8)	2460 6.7 (IQR 4501.2)	2500 6.8 (IQR 9702.6)
SHBG (nmol/lL)	25.6 (IQR 3.3)	25.1 (IQR 7.6)	25.9 (IQR 7.45)	31.2 (IQR 4)
AMH (µng/ml)	6.2 (IQR 4.65)	9.3 (IQR 5.13)	7.5 (IQR 1.85)	5.1 (IQR 2.1)*
Ovarian Volume (cm3)	12.05 (IQR 5.39)	10.52 (IQR 37.78)	11.20 (IQR 5.53)	10.02 (IQR 5.36)

Data are expressed as medians with their respective interquartile range (IQR)

Significances: * p<0.05

Abbreviations: BMI= body mass index; WHR= waist to hip ratio; FAI= free androgen index; FG= Ferriman-Gallwey SHBG: sex hormone binding globuline; PRL:prolactin; A: androstenedione; 17 OHP: 17 hydroxyprogesterone; AMH: anti mullerian hormone

Table II: Glyco-insulinaemic features at baseline and after 6 months of myoinositol and metformin treatment

	Myoinositol		Metformin	
	Baseline	**After 6 months**	**Baseline**	**After 6 months**
AUC-Insulin (µIU/ML/180 min)	13.059.28 (IQR 15077.63)	12063 (IQR 6238.38)*	11500 (IQR 6884.75)	7690.5 (IQR 6048.15)§
AUC-Glucose (µIU/ML/180 min)	19245 (IQR 4050)	18495 (IQR 2760.54)	18465 (IQR 3843.07)	19350 (IQR 2567.82)
M (mg x kg x min^{-1})	2.79 (IQR 2.43)	3.2 (IQR 1.43)	3.7 (IQR 1.62)	3.86 (IQR 1.52)

Data are expressed as medians with their respective interquartile range (IQR)

AUC: area under the curve, M: Peripheral insulin sensitivity.

*p<0.05 §p<0.01