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Randomized, double blind placebo-controlled trial: effects of Myo-inositol on ovarian function and metabolic factors in women with PCOS

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Abstract. – Oligomenorrhea and polycystic ovaries in women are one of the most important causes of the high incidence of ovulation failure. This is linked, perhaps, to insulin resistance and related metabolic features. A small number of reports show that myo-inositol improves ovarian function, but in these trials the quality of evidence supporting ovulation is suboptimal. Furthermore, few of them have been placebo-controlled. The aim of our study was to use a double-blind, placebo-controlled approach with detailed assessment of ovarian activity (two blood samples per week) to assess the validity of this therapeutic approach in this group of women. Of the 92 patients randomized, 47 received 400 mcg folic acid as placebo, and 45 received myo-inositol plus folic acid (4 g myo-inositol plus 400 mcg folic acid). The ovulation frequency assessed by the ratio of luteal phase weeks to observa-tion weeks was significantly ($P < 0.01$) higher in the treated group (25%) compared with the placebo (15%), and the time to first ovulation was significantly ($P < 0.05$) shorter [24.5 d; 95% confidence interval (CI), 18, 31; compared with 40.5 d; 95% CI, 27, 54]. The number of patients failing to ovulate during the placebo-treatment period was higher ($P < 0.05$) in the placebo group, and the majority of ovulations were characterized by normal progesterone concentrations in both groups. The effect of myo-inositol on follicular maturation was rapid, because the E2 circulating concentration increased over the first week of treatment only in the myo-inositol group. A significant increase in circulating high-density lipoprotein was observed only in the myo-inositol-treated group. Metabolic risk factor benefits of myo-inositol treatment were not observed in the morbidly obese subgroup of patients (body mass index > 37). After 14-wk myo-inositol or placebo therapy, no change in fasting glucose concentrations, fasting insulin, or insulin responses to glucose challenge was recorded. There was an inverse relationship between body mass and treatment efficacy. In fact a significant weight loss (and leptin reduction) ($P < 0.01$) was recorded in the myo-inositol group, whereas the placebo group actually increased weight ($P < 0.05$).

These data support a beneficial effect of myo-inositol in women with oligomenorrhea and polycystic ovaries in improving ovarian function.

Key Words:

Myo-inositol, PCOS, Ovarian function.

Introduction

Polycystic ovary syndrome (PCOS) is shared by many women like a common premenopausal disorder, characterized by hyperandrogenism and chronic anovulation[1,2]. Its etiology remains unsolved in spite of the fact that there have been no specific population-based studies, but probably only a 5-10% prevalence of this kind of disorder in women of reproductive age is a reasonable moderate value. This early is based to get the upper hand of any studies prevalency on polycystic ovaries which detected that a 20% of self-selected normal women had polycystic ovary morphology on ovarian ultrasound[3]. The most of them had a slight endocrine abnormality[3]. The lower amount is based on the reported 3% prevalence rate of secondary amenorrhea for 3 or more months[4]: an available datum shows that the 75% of women with secondary amenorrhea will fulfill diagnostic criteria for PCOS[5]. PCOS women can

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also have less profound disturbances in menstrual function[1,3,6]. Burghen et al.7 in 1980 affirmed that PCOS was in association with hyperinsulinemia, and then become clear that the syndrome has major metabolic as well as reproductive morbidities. The recognition of this association stired up the relationship between insulin and gonadal function[1,8]. Therefore, women with PCOS were undergoing a treatment with insulin sensitizing agents such as troglitazone[7], metformin[8] and myo-inositol[9-11]. A number of small randomized and non randomized study groups have shown that women with PCOS respond to this therapy increasing ovarian activity and menstrual frequency. The relationships between treatment outcome, anthropometric changes, glycemic, metabolic, and lipid profile adjustments, at any rate, are less comprehensively studied and is able to be argued about. Perhaps some differences in published results, may be in patient selection. In fact patient profiles can differ between infertility and endocrinology clinics and probably also in racial and socioeconomic training. Furthermore, some published studies employing myo-inositol are not double blind, placebo-controlled in design and the greater number having approximately 20 patients. A direct assessment of follicular development, ovulation or progesterone elevations is going too far away to be comprehensive. The latter point is relevant because a number of the ovulations in women with PCOS show subnormal progesterone concentrations[15], which may be a sign for a suboptimal follicular maturation and ovulation. The aim of this study was to search into the effects of myo-inositol on detailed ovarian function in women with oligomenorrhea and polycystic ovaries (PCOs) who were treated using a randomized, double blind placebo-controlled trial of 16-wk treatment duration.

Patients and Methods

Patients

Ninety-two women with oligomenorrhea (cycle length 41d; 8 cycles for year) or amenorrhea and PCOS, aged less than 35 years old, were recruited from gynecology, endocrine, and infertility outpatient clinics. There's not considered any patients with significant hyperprolactinemia, abnormal thyroid function tests, and congenital adrenal hyperplasia. By using transvaginal ultrasound, effected by a single observer (Z.E.H.), were undertaken to estimate ovarian appearance, and ovaries were described as polycystic (PCOs) about the criteria of Adams et al.[16]. None of the patients was taking medications likely to influence hormonal profiles. This diagnosis was used on the understanding that the great part of patients defined on this basis would show elevated androgen activity, symptoms of hyperandrogenism or both[17].

Protocol

Ovarian activity was established throughout the study, using two blood samples per week for assessment of reproductive hormone concentrations. Before randomization, all patients underwent a 4-wk period of investigation to confirm abnormal ovarian function. The same assessment schedule was maintained through a subsequent 16-wk treatment period after randomization to Inofolic® (LO.LI. Pharma, Rome, Italy) or matching folic acid as placebo. Anthropometric, endocrine, and ovarian ultrasound assessments were effected before and after 14-wk treatment (between 12-16 wk).

The last time window was used to take the measurements outside a luteal phase. The tests were performed only after confirmation that the circulating progesterone concentration was less than 6 nmol/liter.

Randomization and Study Power

Randomization was effected in a double blind fashion; patients received either Myo-inositol combined with folic acid (Inofolic®) or only folic acid as placebo, according to the code provided by computer-generated randomization. The study power was based upon predicted changes in the ovulation rate and circulating lipoprotein concentrations, using data derived from the literature[18]. The calculation was adapted to account for the fact that 70-80% of the cases would have classical PCOS, a significant dropout rate (15%), and a failure to attain normal menstrual frequency in another 15% of cases. It was estimated that 13 patients in each arm would detect changes in high-density lipoprotein (HDL) cholesterol with more than 90% power with a type 1 error (a) 0.05. It was predicted that the study required 35 cases in each arm to achieve the stated aim. Before randomization and during the ovarian function assessment, all patients were evaluated for endocrine factors while outside the luteal phase (progesterone concentration, 6 nmol/liter) when they attend the hospital after an overnight fast. Blood samples were taken for assays of E2, T, androstenedione, LH, FSH, triglycerides, choles-

terol, low-density lipoprotein (LDL) cholesterol, and HDL cholesterol. Then, a standardized 75-g oral glucose tolerance test (GTT) was undertaken with blood samples collected at 0, 60, and 120 min for determination of serum glucose and insulin concentrations. This process was repeated at the 14-wk assessment point.

Ovarian Activity Ovulation and the Luteal Ratio

Ovarian activity was monitored using serum E2 rapid (same day) measurements; where follicular activity was diagnosed (E2 > 300 pmol/liter), progesterone and LH concentrations were determined to diagnose ovulation and the luteal phase. Ovulation frequency was calculated using the ratio of luteal phase weeks to observation weeks (the luteal ratio), such that an individual with normal menstrual rhythm would show two luteal weeks in four observation weeks, yielding a ratio of 0.5, expressed as a luteal ratio of 50%. One patient conceived within a week of the end of her treatment schedule, and her data were included in the completed trial analyses, because all samples and tests had been undertaken for the treatment period.

Anthropometric and Lifestyle Parameters

Anthropometric data were collected (weight, height, waist and hip measurements) before and at the 14th week of treatment or placebo by a single trained observer (Z.E.H.) using standardized techniques[19]. The body mass index (BMI) was calculated using the standard formula. Each volunteer completed a questionnaire of medical and social history (desiring pregnancy, smoking habits), from which subjective information about menstrual patterns, skin oiliness, acne, and hirsutism were recorded. Ovarian ultrasound assessments were also effected before treatment and at 14 wk by the same observer.

Assay Methods

The reproductive hormones, E2 and progesterone, were assayed routinely using the semi automated Immulite technology (Diagnostic Products, Los Angeles, CA). The analytes T, LH, FSH, and human chorionic gonadotrophin were assayed retrospectively in batches using the same system. Inhibin-B was measured using the specific two-site immuno-assay (Serotec Ltd., Oxford, UK). Plasma total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol measurements were performed by a modification of the standard Lipid Research Clinics protocol[20]. Serum leptin concentrations were measured by a validated in-house RIA[21]. Plasma glucose was measured using the glucose oxidase method (Glucose Reagent Kit, Bayer, Newbury, UK), whereas insulin was measured using a competitive RIA (Coat-A-Count I, Diagnostic Products).

The intra- and inter-assay coefficients of variation were less than 7 and 10%, respectively, over the sample concentration range. The detection limit of the assay was 0.5 ng/ml.

Data Analyses and Statistics

Fasting and postglucose insulin [area under curve (AUC)], SHBG, waist to hip ratio (WHR), triglyceride, and the ovulatory function were compared between treatment and placebo groups. Hormone and comparative data were introduced with confidence limits at 95%. Statistical information was prepared using the SPSS for Windows software (SPSS, Inc., Chicago, IL). Hormone data were compared using *t* test after log transformation if distributions were normalized.

Ethical Approval

Ethical committee approval was obtained before the study, and written informed consent was given by each patient.

Results

Recruitment, Randomization, and Pretreatment Assessments

A total of 92 patients proceeded to randomization having either Myo-inositol combined with folic acid (Inofolic®) 2 g twice a day was administrated continuously and controls received folic acid only as placebo.

Infertility was an ailment in only about half of the patients in each group. There was no difference in the proportions of infertile women within the groups (Table I). Although patient selection was based on the more wide-ranging definition often used in Europe *(i.e.* ultrasound-diagnosed PCOS and oligomenorrhea), 90% had biochemical or clinical evidence of hyperandrogenism. Table 1 also shows that the Inofolic® and placebo groups were matched for menstrual frequency in the preceding year, age, BMI, T, SHBG, fasting glucose, hemoglobin A1c, and circulating lipid fractions before treatment. The proportions of

Table I. Characteristics of the patients randomized to receive myo-inositol or placebo treatment.

	Placebo		Inofolic®	
	Mean	*CIs*	*Mean*	*CIs*
Age (yr)	29.7	28.5-30.9	29.0	27.1-30.9
Menses per year	4.1	3.2-4.9	4.7	3.6-5.7
BMI (kg/m^2)	34.8	32.4-37.1	34.0	31.5-36.5
WHR	0.90	0.87-0.92	0.89	0.87-0.91
LH (IU/liter)	10.1	8.4-11.7	8.3	6.9-9.7
T (nmol/liter)	4.0	3.8-4.2	2.8	2.4-3.2
SHBG (nmol/liter)	27.8	23.1-32.5	29.3	24.8-33.8
Free androgen index	13.6	11.3-15.9	10.6	9.3-11.8
Fasting insulin (μU/ml)	18.4	15.0-21.8	16.3	13.2-19.3
Insulin AUC (GTT)	229	180-278	191	160-222
Fasting glucose (nmol/liter)	4.86	4.78-4.93	4.99	4.77-5.21
Leptin (ng/ml)	39.3	32.9-45.6	40.1	33.0-47.2
Inhibin-B (pg/ml)	80	65-95	99	89-109

No. of patients: placebo-treated, 47 (infertile, 19; hirsutism, 22); myo-inositol-treated, 45 (infertile, 23; hirsutism, 13). P values are NS. CIs, Confidence intervals (95%).

patients seeking fertility treatment were also similar in each group.

All women showed a classical picture of PCOS on vaginal ultrasound scan.

Conception During Treatment

There were eight conceptions in eight patients during the study, and one miscarried in the first trimester. However, only 42 of the patients declared before the study that they wished to conceive. Of these, the distribution of pregnancies was: placebo, 1 of 19 patients; and myo-inositol 4 of 23 patients.

The results are not significantly different *(P* = 0.23).

Ovarian Function: Ovulation

An intention to treat analysis revealed that 8 of 45 myo-inositol-treated patients failed to ovulate during treatment, compared with 17 of 47 placebo-treated. This difference was statistically significant (Fisher's exact test; *P* = 0.04; Odd's Ratio, 0.38).

Table II shows the data from all cases in which ovulation data (over any length of time) were available. The myo-inositol-treated group had a significantly increased frequency of ovulation compared with the placebo group, defined by the luteal ratio. The distributions show that the placebo group was dominant at low ovulation rate (zero and one ovulations), whereas the myo-inositol group was dominant in the high ovulation rate (two to four ovulations).

Table II also shows the frequency of ovulations with deficient luteal phases assessed by the maximum progesterone concentration less than 7 ng/ml.

Table II. Details of ovulations during placebo and myo-inositol treatment.

	Placebo	Inofolic®	*P*
Observation weeks	497	352	
Luteal weeks [luteal ratio (%)]	74 (15)	88 (25)	< 0.001
Luteal phases with P_{max} 7 ng/ml (%)	6 (14)	2 (9)	NS
Days to first ovulation, mean	40.5	24.5	0.02
(CIs, 95%)	(27, 54)	(18, 31)	

P_{max}, Maximum progesterone concentration.

According to these data, the concentrations of progesterone recorded during monitoring of ovarian function indicated that most of the ovulations showed normal endocrine profiles during both myo-inositol and placebo treatment. All patients started treatment outside the luteal phase, and the delay to the first ovulation after starting the program (Table II) was significantly shorter in the myo-inositol-treated group.

Initial Responses to Treatment: Follicular Development

Inhibin-B is a marker of early follicular granulosa cell activity, and circulating E2 represents follicular maturation. Table III shows the E2, inhibin-B, and T concentrations on the first and eighth days of treatment, showing that the Myo-inositol-treated group had a significant ($P = 0.03$, paired data) increase in mean E2, whereas the control group showed no change. There was no change in the circulating inhibin-B or T concentrations. These profiles suggest that although improved follicular maturation was detected, there appeared to be no change in the remainder of the ovarian metabolism (total immature granulosa cell activity and stromal androgen biosynthesis).

Metabolic and Anthropometric Assessments

Table IV shows that after 14-wk treatment, the BMI decreased significantly in the myo-inositol group, whereas it increased in the placebo group. There was no change seen in the WHR in either group. The circulating leptin concentration declined in the myo-inositol-treated group, in contrast to the control group, but there was no change recorded in the fasting glucose, fasting insulin, or insulin AUC in response to the glucose challenge in either group. Circulating very LDL (VLDL) showed little change during the treatment period, but the LDL showed a trend toward reduction, and HDL increased significantly in the myo-inositol-group. It is possible that the reduction in HDL was related to the weight loss achieved in the myo-inositol-treated patients, although the ANOVA ($r > 0.34$; $P > 0.07$) did not reach conventional levels of significance.

Subgroup Analyses Characteristics of the Group That Responded to Myo-Inositol With Normal Ovulation Frequency

A total of 12 patients who responded to myo-inositol by establishing normal ovulation frequency (n = 6) and/or pregnancy (n = 6) were compared with those patients who did not respond with establishment of normal ovarian function (less than three ovulations in 16 wk; n = 9). The two groups showed similar BMI, WHR, and circulating E2 and inhibin-B concentrations. However, responders to myo-inositol treatment showed significantly lower T (2.3 nmol/liter *vs.* 3.4 nmol/liter; 95% CI = 0.07 and 2.1, respectively; $P > 0.04$), higher SHBG (35.9 nmol/liter *vs.* 25.8 nmol/liter; 95% CI, 20.6 and 0.13; $P < 0.05$), and thus lower free androgen index (6.9 *vs.* 11.6; 95% CI, 1.2 and 8.1; $P = 0.01$). Fasting insulin or glucose concentrations or responses to the GTT were not significantly different.

Table III. The reproductive hormone changes over the first week of myo-inositol treatment.

	Day 1		Day 8		*P*
	Mean	*CIs*	*Mean*	*CIs*	
Placebo					
E2 (pmol/liter)	159	108-209	177	119-235	NS
Inhibin-B (pg/ml)	82	69-95	88	72-103	NS
T (nmol/liter)	4.2	3.6-4.7	4.1	3.4-4.8	NS
Myo-inositol					
E2 (pmol/liter)	141	122-159	224	147-300	< 0.03
Inhibin-B (pg/ml)	99	89-109	96	87-105	NS
T (nmol/liter)	2.9	2.3-3.5	3.3	2.5-4.0	NS

Table IV. Changes in metabolic parameters during placebo or myo-inositol treatment.

	Placebo			Inofolic®		
	Pretreatment	*14 wk*	*P*	*Pretreatment*	*14 wk*	*P*
BMI (SD)	35.2	35.5	0.04	35.0	34.4	0.03
WHR	0.90	0.90	NS	0.89	0.89	NS
Leptin (ng/ml) (SD)	40.5	39.0	NS	41.3	37.5	0.05
Fasting insulin (µU/ml)	18.1	17.3	NS	16.6	16.8	NS
GTT insulin AUC	218	220	NS	190	202	NS
Fasting glucose (nmol/liter)	4.9	5.0	NS	5.0	5.1	NS
Total cholesterol (nmol/liter)	4.85	4.92	NS	4.53	4.42	NS
Triglycerides (mmol/liter)	1.39	1.43	NS	1.59	1.60	NS
VLDL cholesterol (mmol/liter)	0.40	0.52	NS	0.50	0.55	NS
LDL cholesterol (mmol/liter)	3.25	3.32	NS	3.05	2.89	0.09
HDL cholesterol (mmol/liter)	1.15	1.15	NS	1.10	1.16	0.03

Statistical probability by t test for paired data.

Metabolic Responses and Obesity

It was observed that morbidly obese women (BMI > 37; n = 10) showed a similar number of ovulations (mean, 1.5) during 16-wk myo-inositol treatment to the leaner women (mean, 2.2), but they showed no indication of changes in either BMI (pretreatment, 42.6 kg/m^2; week 14, 42.4 kg/m^2) or HDL cholesterol (pretreatment, 0.94 mmol/liter; week 14, 0.94 mmol/liter). The leaner women (BMI < 37 kg/m^2) showed distinct changes during treatment as follows: BMI, pretreatment, 29.2 kg/m^2; week 14, 28.3 kg/m^2 *(P* = 0.01); or HDL cholesterol, pretreatment, 1.19 mmol/liter; week 14, 1.30 mmol/liter *(P* = 0.02).

Discussion

This study is the first to give a comprehensive, detailed endocrinological assessment of ovarian function in the context of a large randomized placebo-controlled trial of myo-inositol in women with abnormal ovarian function. Our data show clear beneficial effect of myo-inositol treatment upon ovarian function, anthropometric measures, and lipid profiles in women with oligomenorrhea and PCOS. We observed that more than 70% of the patients established normal ovarian rhythm (three or more ovulations) through the 16-wk treatment period. This contrasted with 13% for the placebo group. The luteal phases had normal progesterone concentration profiles in a high frequency of the cycles, showing that these were fertile cycles. The mean time until the first ovulation was significantly shorter in the myo-inositol-treated group (25 d) than in the placebo-treated group (41 d).

This suggests a relatively rapid effect of treatment upon ovarian function, which is further supported by the significant increase in E2 concentrations during the first week of treatment.

At week 14 assessment, the myo-inositol patients showed significant reductions in weight, in contrast to patients in the placebo group who actually increased their BMI. Associated with the weight loss were significant reductions in circulating leptin and increased HDL cholesterol concentrations in the myo-inositol-treated group. LDL cholesterol showed a trend toward reduction, and overall the LDL cholesterol to HDL cholesterol ratio improved significantly in the myo-inositol group.

For all increased ovulation frequency, there were no changes in circulating androgen concentrations, glycemic indices, basal or provoked insulin levels, or circulating VLDL cholesterol concentrations. Our data on HDL cholesterol are important, because no previous study has addressed this important issue.

Subgroup analyses comparing those patients who showed a high ovulation rate during myo-in-ositol treatment with those who were resistant to it, indicated that the least androgenic patients were more likely to respond with establishment of normal menstrual rhythm. Furthermore, the morbidly obese patients (BMI > 37) showed no cardiovascular risk factor (BMI and HDL cholesterol) benefit.

Taken together, these data suggest that either higher doses of myo-inositol may prove to be more beneficial in the morbidly obese patient or such patients may be resistant to this form of therapy. These assertions remain to be tested in future studies. A number of reports have indicated that insulin sensitizing agents improve ovulation rates in women with PCOS, and they have shown conflicting results with respect to changes in ovulation rate and also changes in endocrinology during myo-inositol treatment

On the other hand, a number of studies have shown decreases in hyperandrogenism and markers of insulin resistance with myo-inositol in PCOS[9-14]. A recent comprehensive multicenter, multidose study using the peroxisome proliferator-activated receptor (PPAR) agonist troglitazone[7] showed improvements in hyperan drogenism, mediated through circulating free androgens rather than total androgen concentrations, and also in glycemic indices. These changes were dose-related, as were improvements in ovulation rates. It is possible that patient selection criteria may have an impact on the potential for beneficial effects of myo-inositol on surrogate markers of insulin resistance and hyperandrogenism.

The principal inclusion criteria in our study was disturbances of ovarian function, whereas in other studies the emphasis may have been on more profound metabolic derangements, including clinical manifestations of hyperandrogenism. It is considerable that the higher doses of troglitazone treatment (300 and 600 mg) were associated with weight increase in women who were generally overweight at the time of starting[7]. Weight loss achieved in the myo-inositol-treated patients would be considered a beneficial effect of treatment. The increase in ovulation rate seen in the myo-inositol-treated patients appeared to take place rapidly, as evidenced by significant increases in circulating E2 concentrations, representing follicular maturation, within the first 8 d of treatment and also the shorter mean time to first ovulation. This effect is likely to have taken place before significant weight loss or changes in the lipid profiles, and also in the absence of changes in glycemic indices. This leads to the possibility of direct gonadal effects of myo-inositol as has been demonstrated for the PPAR agonist troglitazone[29,30].

These should be dose-determining and aimed to define patient characteristics that best predict beneficial response to myo-inositol treatment.

Furthermore, we also suggest that the problems of maternal obesity be carefully considered with such treatment, and that weight loss may be the better approach[31] in many circumstances.

Finally, the high dropout rate in the myo-inositol arm (more than 30%) is notable. Clinically, this observation is important and indicates that significant side effects on the dosage regime we used are common. Most of the discontinuation cases occurred at the early part of treatment, suggesting that women prescribed myo-inositol should be adequately counseled and perhaps actively supported through this stage.

In conclusion, using a comprehensive, detailed endocrinological assessment of ovarian function, we have shown that myo-inositol treatment increases ovulation rates by a significant degree in women with oligomenorrhea and PCOS. Continued treatment also resulted in significant weight loss (and leptin reduction) and an associated change in HDL cholesterol even if many different factors may contribute to the metabolic syndrome in PCOS patients. These beneficial effects of myo-inositol support a future therapeutic role in women with PCOS.

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