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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 [illegible] was to use a double-blind, placebo-con[illegible]ed approach with detailed assessment of ov[illegible]n activity (two blood samples per week) to [illegible]sess the validity of this therapeutic approach [illegible] this group of women. Of the [illegible]ents ra[illegible]domized, 47 received 400 [illegible]acid as placebo, and 45 receive[illegible]yo-in[illegible]ol plus folic acid (4 g myo-inos[illegible]plus 40[illegible] folic acid). The ovulation fr[illegible]qu[illegible]as[illegible] ratio of luteal pha[illegible]vee[illegible]bserva-tion weeks was signifi[illegible]ly ($P < 0.$[illegible]igher in the treated group (2[illegible]mpared wi[illegible] placebo (15%), and th[illegible]e[illegible]t ovulatio[illegible]as signif-icantly (P [illegible]5) sh[illegible][24.5 d; 95% confidence int[illegible]l (CI), 18, [illegible]mpared with 40.5 d; 95% [illegible]27, 54]. The nu[illegible]of patients failing t[illegible]ulate d[illegible]ing the pla[illegible]bo-treatment period[illegible]s high[illegible] < 0.05) in the placebo group, and [illegible]y of o[illegible]lations were characterized b[illegible]al pro[illegible]rone concentrations in gro[illegible] The[illegible]ct of myo-inositol on follic[illegible] mat[illegible]was rapid, because the E2 cir[illegible]ating c[illegible]ntration increased over the fi[illegible]k of treatment only in the myo-inositol [illegible]nificant increase in circulating [illegible]gh-density lipoprotein was observed only in [illegible] myo-inositol-treated group. Metabolic risk [illegible] benefits of myo-inositol treatment were n[illegible]bserved 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. [illegible]here wa[illegible]inverse relationship between [illegible] mass and[illegible]ment efficacy. In fact a s[illegible]t weight los[illegible]d leptin reduction) ([illegible].01) was recorded in the myo-inositol [illegible]p, whereas [illegible]e placebo group actually in-[illegible]sed weight ([illegible]0.05).
[illegible]se data [illegible]port a beneficial effect of my[illegible]sitol i[illegible]omen with oligomenorrhea and p[illegible]ovaries in improving ovarian function.

[illegible]s:
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 i sign and the greater number having approx ly 20 patients. A direct assessment of foll r development, ovulation or progesterone el tions is going too far away to be comprehensi The latter point is relevant be umber the ovulations in women wi COs ow sub-normal progesterone conc tions[15] ich may be a sign for a suboptim licul and ovulation. The o udy was to search into the effe of myo-in l on detailed ovarian functio men with o enorrhea and polycysti ari COs) who re treated using a ra nized, de blind placebo-controlled t 16-wk treati duration.

Patients and Methods

Patients

ety-two en with oligomenorrhea (cycle gth 41d; cycles for year) or amenorrhea ed less than 35 years old, were recruited from gynecology, endocrine, and infertility outpatient clinics. There's not considered any ts 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 pa tients defined on this basis would sh evate androgen activity, symptoms of erandrogenism or both[17].

Protocol

Ovarian activity was e shed throughe study, using two blood ples week for sessment of reproducti m concent tions. Before randomiza all s unde a 4-wk period of i tigation fir normal ovarian func e same ass t schedule was maint d th a subsequ 16-wk treatment period after r mization to Inofolic® (LO rma, Rome,) or matching folic ac placebo. Anthropo etric, endocrine, and an ultrasound assessments were effected before and after 14- reatment (between 12-16 wk). last time ndow was used to take the mea ents side a luteal phase. The tests were p only after confirmation that the circulating progesterone concentration was less mol/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 under[illegible] for the treatment period.

Anthropometric and Lifestyle Parame[illegible]

Anthropometric data were collected (weig[illegible] height, waist and hip measure[illegible]efore an[illegible] at the 14th week of treatment[illegible]lace[illegible]y a single trained observer (Z.E[illegible] using s[illegible]ardized techniques[19]. The body [illegible]inde[illegible] calculated using the [illegible]dard[illegible]. Each [illegible]olunteer completed [illegible]estionnai[illegible] medical and social history [illegible]ng pregna[illegible]smoking habits), from[illegible]ch [illegible]tive inform[illegible]ion about menstrual [illegible]tterns, ski[illegible]ness, acne, and hirsutism [illegible] recorded. O[illegible] ultrasound assessm[illegible] were also effecte[illegible]before treatment and [illegible]4 wk h[illegible]e same observer.

Assay [illegible]ods

[illegible]e rep[illegible]cti[illegible]ormones, E2 and progesteron[illegible] were [illegible]d routinely using the semi automated Immuni[illegible] technology (Diagnostic Prod[illegible]geles, CA). The analytes T, LH, [illegible]SH, and human chorionic gonadotrophin were [illegible]ayed retrospectively in batches using the same [illegible]n. 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, Newbu[illegible] whereas insulin was measured using [illegible]mpet[illegible]tive RIA (Coat-A-Count I, Diagnos[illegible]roducts).

The intra- and inter-assay coeffi[illegible] of variation were less than 7 and 10% respe[illegible] over the sample concentration[illegible]ge. The [illegible]ion limit of the assay was 0.5[illegible]ml.

Data Analyses and [illegible]isti[illegible]

Fasting and p[illegible]luce[illegible]ulin [a[illegible]under curve (AUC)[illegible]SHBG, [illegible]t t[illegible]p ratio (WHR), tri[illegible]le, and the[illegible]tory function wer[illegible]mp[illegible] between [illegible]eatment and placebo groups. Ho[illegible]e and comparative data were[illegible]uced with [illegible]lence limits at 95%. St[illegible]ical information w[illegible] prepared using the S[illegible]S for Wind[illegible]ws software (SPSS, Inc., [illegible]ago, IL). H[illegible]one data were compared using [illegible]test after [illegible] transformation if distribution[illegible]n no[illegible]ized.

Ethical Approval

[illegible]al committee approval was obtained be[illegible] 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	0.9
Menses per year	4.1	3.2-4.9	4.7	6-5.7
BMI (kg/m^2)	34.8	32.4-37.1	34.0	6.5
WHR	0.90	0.87-0.92	0.89	0.
LH (IU/liter)	10.1	8.4-11.7	8.3	6.9-
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	4.8-33.8
Free androgen index	13.6	11.3-15.9	10.6	9.3-1
Fasting insulin (μU/ml)	18.4	15.0-21.8	3	13.2
Insulin AUC (GTT)	229	180-278	1	2
Fasting glucose (nmol/liter)	4.86	4.78-4.93	4.99	-5.21
Leptin (ng/ml)	39.3	32.9-45.6	1	3.0-47.2
Inhibin-B (pg/ml)	80	65-95		89-109

No. of patients: placebo-treated, 47 (infertile, 19; hirsutism, 22); myo-i ated, 45 (infert 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 pict PCOS on vaginal ultrasound scan.

Conception During Treatment

There were eight conceptio t patien during the study, and one m rried the first trimester. However, only 42 ne patie declared before the study that they d to Of these, the distribution reg as: placebo, 1 of 19 patients; an o-inositol 23 patients.

The results a ignificantly rent (P = 0.23).

Ovaria nction: Ov n

An ition t treat analys revealed that 8 of 45 inosit eated patients failed to ovulate dur atme ompared with 17 of 47 placebo-trea difference was statistically significant (Fisher's exact test; P = 0.04; Odd's Ra- 8)

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.

Ta I. Detai ulations during placebo and myo-inositol treatment.

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

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 the 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. Circulating HDL 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. 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 ANOVA ($P > 0.34$; $P > 0.07$) did not reach conventional levels of significance.

Subgroup Analyses Characteristics of the Group That Responded to Myo-inositol With Normal Ovulatory 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, 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 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*	
BMI (SD)	35.2	35.5	0.04	35.0	34	0.03
WHR	0.90	0.90	NS	0.89		NS
Leptin (ng/ml) (SD)	40.5	39.0	NS	41.3	37	0.05
Fasting insulin (µU/ml)	18.1	17.3	NS	16.6	16.8	NS
GTT insulin AUC	218	220	NS	190	202	
Fasting glucose (nmol/liter)	4.9	5.0	NS	5.0	5	
Total cholesterol (nmol/liter)	4.85	4.92	NS	4.5		N
Triglycerides (mmol/liter)	1.39	1.43	NS	1.5	0	NS
VLDL cholesterol (mmol/liter)	0.40	0.52	NS	0	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		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) but they showed no indication of change ther BMI (pretreatment, 42.6 kg/m^2; wee 4, 42.4 kg/m^2) or HDL cholesterol (pretreatm 0.94 mmol/liter; week 14, 0.94 mmol/liter). T leaner women (BMI < 37 kg/m^2 d distin changes during treatment as ows MI, pre- treatment, 29.2 kg/m^2; we 4, 28.3 m^2 (P = 0.01); or HDL cholester retre mmol/liter; week 14 m (P = 0.02).

Discussion

This dy is the first to giv a comprehensive, det endo logical assessment of ovarian func in conte of a large randomized placeb trolle al of myo-inositol in n wi ovarian function. Our data sho lear be al effect of myo-inositol treatm upon ovarian function, anthropometric d lipid profiles in women with gomenorrhea and PCOS. We observed that re than 70% of the patients established normal an rhythm (three or more ovulations) thr gh 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, s ing that these were fertile cycles. The mean t until the f ovulation was significantly sh in the m nositol-treated group (25 d) tha pla treated group (41 d).

This a relatively rapid effect of treatent upon ovarian function, which is further ted by the significant increase in E2 one ations 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-inosit surrogate markers of insulin resistance a perandrogenism.

The principal inclusion criteria in our s was disturbances of ovarian function, whereas other studies the emphasis m been c more profound metabolic de geme includ- ing clinical manifestation hyperan genism. It is considerable that the r d tazone treatment (30 d 6 were asso- ated with weight ease in w n who were generally over at the tim tarting[7]. Weight loss a ved he myo-ino ol-treated patients w ld be cons d a beneficial effect of treatm The increase ulation rate seen in the o-inos ol-treated p ents appeared to tak ce rapi as evidenced by significant increa ating concentrations, repre- senting lar m tion, within the first 8 d atme d the shorter mean time to fir ulatio effect is likely to have taken pla efore sig ficant weight loss or changes in t les, and also in the absence of anges in glycemic indices. This leads to the sibility of direct gonadal effects of myo-inosi- 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 m tol arm (more than 30%) is notable ically this observation is important and cates that significant side effects on the dos gime we used are common. Most of the disc uation cases occurred at the early of treatm ug- gesting that women pr ibed myo-in should be adequately iseled perhaps tively supported throu st

In conclusion, g a hensiv tailed endocrinologic ssessment vari unction, we have sh t myo-inos atment increases o ion by a signi ant degree in women with oligom hea and PCOS. Continued t also res in significant weight lo and leptin reductio and an associated c ge in HDL cholesterol even if many differ- e factors may tribute to the metabolic syn- d in PCOS ents. These beneficial effects of i osit pport a future therapeutic role in wom PCOS.

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