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The 40:1 myo-inositol/D-chiro-inositol plasma ratio is able to restore ovulation in PCOS patients: comparison with other ratios

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Abstract. – OBJECTIVE: The aim of this clinical trial was to evaluate the efficacy of seven different ratios between two inositols stereoisomers, myo-inositol (MI) and D-chiro-inositol (DCI), in the therapy of polycystic ovary syndrome (PCOS).

PATIENTS AND METHODS: fifty-six PCOS patients (8 for each group) were treated by oral route using the following formulations: DCI alone, and 1:3.5; 2.5:1; 5:1; 20:1; 40:1, 80:1 MI/DCI ratio. They received 2 g of inositols twice a day for 3 months. The primary outcome was ovulation, the secondary outcome included the improvement of FSH, LH, Sex Hormone Binding Globulin (SHBG), 17-beta-Estradiol (E2), free testosterone, basal and postprandial insulin levels, as well as HOMA index, BMI and menses.

RESULTS: We found that the 40:1 MI/DCI ratio is the best for PCOS therapy aimed at restoring ovulation and normalizing important parameters in these patients. The other formulations were less effective. In particular, a decreased activity was observed when the 40:1 ratio was modified in favour of DCI.

CONCLUSIONS: Our data demonstrated that DCI activity is beneficial mainly at a specific ratio with MI, whereas the increase of DCI causes the loss of the beneficial effects at reproductive level. These results in humans validate a previous preclinical study with different MI/DCI ratios carried out in an experimental model of PCOS mice.

Key Words:
Myo-inositol, D-chiro-inositol, Different ratios, Polycystic ovary syndrome, Ovulation

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine pathology, affecting up to 10-15% of women during the reproductive age, and constitutes one of the leading causes of infertility in industrialized areas[1]. Irving F. Stein and Michael L. Leventhal[2] were the first in 1935 to identify this syndrome on the base of the simultaneous presence of polycystic ovary and anovulation. Amenorrhea, obesity, hirsutism and multiple follicular cysts were considered, as well as other symptoms, frequently involved in PCOS and useful for its diagnosis[1]. These signs, although characteristic, are quite restrictive and exclude some important disorders, such as hyperinsulinemia, usually present in the syndrome. In fact, PCOS is characterized also by several reproductive, metabolic and cardiovascular problems, that can exert a high number of health consequences during the life span[3,4]. Currently, the Rotterdam criteria 2004[5] focused their attention on three symptoms: chronic ovulatory disorder, hyperandrogenism and polycystic ovaries. According to these Criteria, it is mandatory for PCOS diagnosis that the patient shows at least two of the above-mentioned clinical and endocrine features. As far as the therapy is concerned, there is a general consensus that its profile requires a preliminary careful and overall analysis of the patient, taking into consideration her reproductive expectations, among other things. Therefore, tailored strategies should include weight loss and dietary habits modifications, since lifestyle changes significantly ameliorate ovarian function and can avoid later PCOS-related risks[6]. The hormonal approach mainly considers the use of oral contraceptives or metformin, even if the therapy should be tailored according to specific clinical situations and to patient's needs. Although combined oral contraceptives (estro-progestin compounds) yield satisfying results[7], they cannot be prescribed to patients requiring the restoration of ovulation to achieve pregnancy. For a long time, metformin

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and, at a lesser extent, thiazolidinediones were a choice in PCOS management in women wishing for motherhood. Albeit metformin obtained interesting improvements, mostly evident in obese insulin-resistant PCOS women, controversial outcomes were found for its efficacy in non-obese non-insulin-resistant patients[8,9]. However, metformin induces frequent gastrointestinal adverse events, such as diarrhea, nausea, vomiting and abdominal bloating, and metabolic complications. On the other hand, thiazolidinediones can give rise to fluid retention, body weight gain, coronary artery disease, myocardial infarction and bladder cancer.

Recently, myo-inositol (MI) therapy has gained momentum in the treatment of PCOS patients, due to its satisfying performance and the absence of side effects. Inositol is a hexahydroxycyclohexane, a 6-carbon ring compound with a hydroxyl group attached to each carbon of the ring. There are nine possible stereoisomeric forms of inositol, related to the epimerization of the six-hydroxyl groups. Among them, MI is the most represented isoform, however another therapeutically useful stereoisomer is D-chiro-inositol (DCI)[1]. Due to their well-known ability to reduce insulin resistance and to positively affect metabolism, MI and DCI are considered particularly beneficial to PCOS patients. They play the role of insulin's second messengers and are mediators of different insulin actions. MI is transformed into an inositolphosphoglycan (IPG) insulin second messenger (MI-IPG) that is involved in cellular glucose uptake. On the other hand, DCI is used to make an IPG insulin second messenger (DCI-IPG), which plays an important function in glycogen synthesis[10]. Moreover, MI-based second messenger increases glucose uptake in the ovary and is crucial for follicle stimulating hormone (FSH) signaling, whereas DCI-based second messenger is involved in the insulin-mediated androgen production. Both MI and DCI can reduce the levels of luteinizing hormone (LH) and testosterone, as well as LH/FSH ratio, counteracting the consequences of hyperandrogenism, with the final result of decreased hirsutism and acne[10-13]. The combination of these actions fully justifies the therapeutic use of the two stereoisomers in PCOS; however, it may be difficult to determine the optimal amount of each one to be used in therapy; therefore, some important considerations should be taken into account. DCI originates from MI through the action of a nicotinamide adenine dinucleotide-dependent epimerase, which operates under insulin stimulus and control, and the entity of such transformation is determined by the physiological needs of the single cell. According to this picture, it was seen that MI and DCI display a specific ratio between them in the different tissues: glycogen-storing tissues (muscle, fat, liver) have generally considerable DCI levels (although lesser than MI concentration); on the other hand, tissues with high glucose consumption (brain, heart) contain low DCI amounts. If we analyze a pathologic condition such as type 2 diabetes, we find a reduced insulin sensitivity in many tissues, which determines a reduced epimerase activity and, consequently, a lower DCI production. Unlike other tissues, the ovary never becomes insulin resistant and hyperinsulinemia overstimulates epimerase activity in PCOS women, as suggested by Unfer et al[14], causing a disproportionate DCI synthesis and an associated MI deficiency. Indeed, MI/DCI ratio decreased from 100:1 in healthy women to 0.2:1 in PCOS women. In this condition, the administration of excessively elevated DCI doses is incorrect. With the aim of defining the ideal clinical dosage, the ratio between MI and DCI in plasma was taken into consideration as the most appropriate indicator to understand the physiologic balance at systemic level between these stereoisomers in humans. Such plasma ratio was determined to be about 40:1 and, according to this evidence, a treatment based on the same ratio was used. This choice is supported by several studies[15-20] and by a recent animal research where an experimental animal model of PCOS was obtained exposing mice to continuous light for 10 weeks[21]. Interestingly, such research, demonstrated that the 40:1 ratio between MI and DCI is the most effective treatment, in comparison with other different ratios (5:1, 20:1 and 80:1).

In keeping with this animal research, the present clinical study was aimed at directly comparing, for the first time, the efficacy of different MI/DCI ratios in PCOS patients in order to select the best therapeutic formulation in humans.

Patients and Methods

Type of study: Randomized (using SAS® software), Interventional, Open-label.

The different MI/DCI ratios tested were labelled as follows: 0:1 (DCI alone), 1:3.5; 2.5:1;

5:1; 20:1; 40:1 and 80:1. These different MI/DCI blends were prepared by Pharcoterm S.P.A. (Cusano Milanino, MI, Italy).

Inclusion criteria: age 18-45 years; PCOS diagnosed according to the Rotterdam ESHRE-ASRM consensus workshop group[4] (PCOS diagnosed if 2 out of the 3 following conditions were met: a) oligo- or anovulation, b) clinical and/or biochemical signs of hyperandrogenism, and c) polycystic ovaries). Oligo-/anovulation or infertility > 1 year.

Exclusion criteria: presence of other pathologic or age-related conditions causing ovulatory dysfunction (such as hyperprolactinemia or hypothyroidism), androgen excess (such as adrenal hyperplasia or Cushing's syndrome) or poor ovarian reserve; the intake of other drugs that could potentially influence ovulation. Also, obese women (BMI > 29.9) were excluded, as well as women with partners with sperm abnormalities. In this way, we ruled out the concomitant factors that interfere with the possibility of becoming pregnant as potentially confounding variables. After the enrolment, the patients were invited to avoid any change of usual habits both for food, physical activity and lifestyle.

All the participants have signed an informed consent before entering the study. The present study complies with the current laws of the country in which it was performed and with the ethical principles of the Declaration of Helsinki. All data were anonymized and held securely in the outpatient care center.

Total number of groups: 7 (with 8 patients for each one).

Treatment dose: 2 g (total MI + DCI at different ratios) twice a day (morning and evening), at least 15 minutes before meals, by oral route for 3 months.

Outcomes

Primary outcome: ovulation (checked every month by means of progesterone assay performed in the medium luteal phase).

Secondary outcome: improvement of the following metabolic parameters: FSH, LH, Sex Hormone Binding Globulin (SHBG), 17-beta-Estradiol (E2), free testosterone, HOMA index, basal and postprandial insulin). BMI and menses were also included. These clinical tests were performed at baseline and after three months (end of the treatment).

Statistical Analysis

Comparisons between the different times were performed using the one-way analysis of variance (ANOVA) and the post hoc Bonferroni adjustment.

Comparisons in pairs between the different dosages were performed using the Chi-Square Test, while Student's *t*-test was used in order to compare quantitative variables between pairs of different dosages

Statistical analyses were implemented at two-sided with a 0.05 significance level, using Stata™ version 8.2.

Results

All the fifty-six patients completed the study, except for one of the groups treated with 2.5:1 MI/DCI ratio, that withdrew for reasons not related to the clinical study.

The averages of patients' age and BMI at baseline did not display any significant difference among all the groups. No changes were found in the BMI between the baseline and the third month (Table I).

As general observation, we can reveal in advance that a clear improvement of several parameters occurred in many cases, though with

Table I. Averages of patients' age at baseline and averages of patients' BMI at baseline and at the 3rd month.

MI/DCI	No.	Age	BMI 0	BMI +3rd month
0:1	8	30.5 ± 2.9	23.48 ± 3.0	23.48 ± 2.9
1:3.5	8	28.9 ± 3.4	24.52 ± 2.8	25.12 ± 3.3
2.5:1	7	29.3 ± 3.1	22.87 ± 2.9	23.35 ± 3.1
5:1	8	31.2 ± 2.7	24.12 ± 2.7	24.01 ± 2.9
20:1	8	30.6 ± 3.0	23.21 ± 3.1	23.61 ± 3.0
40:1	8	31.1 ± 3.2	24.08 ± 3.0	23.91 ± 2.9
80:1	8	29.7 ± 2.8	22.98 ± 2.9	23.11 ± 3.1

The values were similar, without significant differences. No changes were found in the BMI between the baseline and the third month.

considerable differences of effect depending on the treatment administered. All the improvements achieved the highest level using the 40:1 ratio, followed by the 20:1 and 80:1, instead the other combinations between MI and DCI gave less relevant outcomes. It is noteworthy that the difference between the effects due to the 40:1 treatment and those exerted by the other formulations was found often significant, especially when they were compared to the highest DCI dosages.

Progesterone

Progesterone assay was performed at the 1st, 2nd, and 3rd month (end of the therapy). The treatments, with 20:1, 40:1 and 80:1 ratio, were able to restore ovulation, with the best data using the 40:1 ratio, as shown by the rise of progesterone levels, which become significant already from the first month (Figure 1). Instead, the improvement was very low or no detectable when high DCI levels (0:1, 1:3.5, 2.5:1, 5:1) were given to patients. The effect reached by 40:1 and 80:1 ratios showed a significant difference in comparison with 0:1, 1:3.5, 2.5:1 and 5:1. On the other hand, 20:1 ratio gave halfway results (Figure 1).

The reappearance of menses agrees with progesterone increase (Table II).

Follicle Stimulating Hormone and Luteinizing Hormone

No changes were observed for FSH levels with any type of treatment, whereas LH decreased with all the different administrations; however, the reduction was significant only with the 40:1 and 80:1 ratios, in comparison with the baseline values (Figure 2).

Sex Hormone Binding Globulin and 17-beta-Estradiol

All the treatments increased SHBG and E2 levels, however statistically significant effects were reached using the 40:1 ratio and, to a lesser extent, the 20:1 and 80:1 ratio, whereas the other treatments gave no significant improvements (Figures 3 and 4). The comparisons between groups at the 3rd month mirrored this situation.

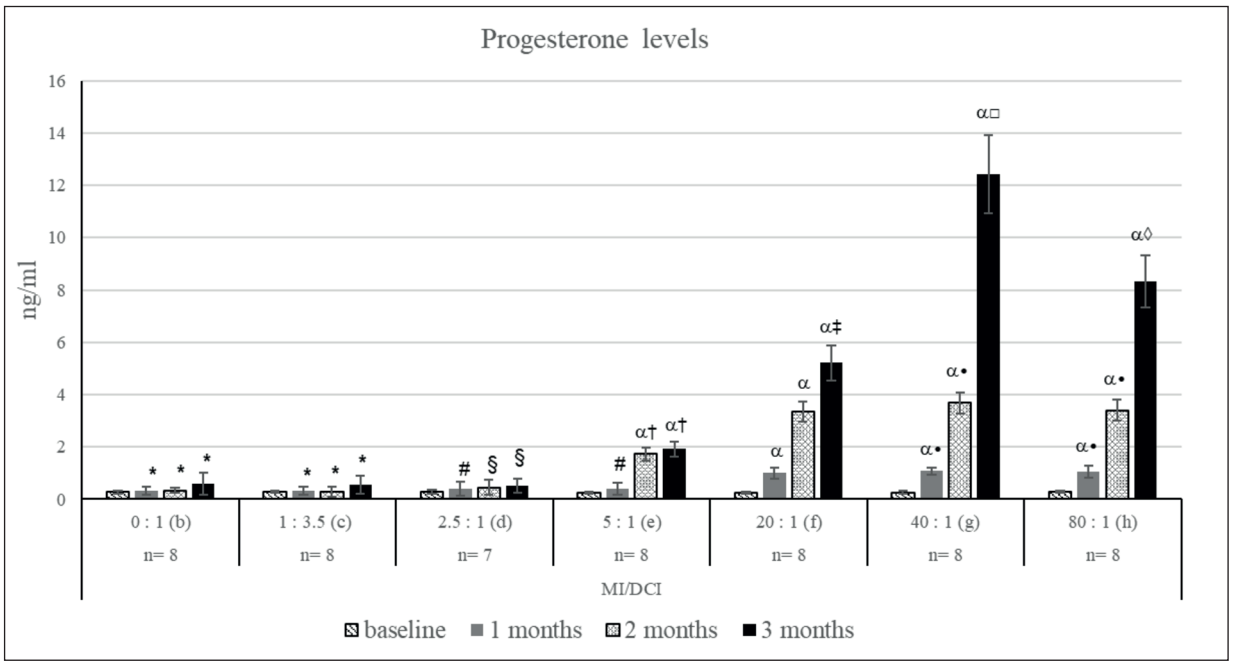

Figure 1. Progesterone levels at four different timepoints in patients administered with various MI/DCI ratio. Treatment dose: 2 g (total MI + DCI) twice a day. Values are expressed as mean ± SD. *p*: value at the 1st, 2nd, and 3rd month *vs.* baseline and between treatments at the same time. The level of statistical significance was set at $p \leq 0.05$. The significant difference between means was calculated *vs.*: α = *vs. baseline*, same formulation; * = *vs. d, e, f, g, h* same time; # = *vs. b, c, f, g, h* same time; § = *vs. b, c, e, f, g, h* same time; † = *vs. b, c, d, f, g, h* same time; ‡ = *vs. h, g* same time; • = *vs. b, c, d, e* same time; □ = *vs. b, c, d, e, f, h* same time; ◊ = *vs. b, c, d, e, f, g* same time.

Table II. Number of patients with menses for each MI/DCI ratio.

MI/DCI	0:1 (b)	1:3.5 (c)	2.5:1 (d)	5:1 (e)	20:1 (f)	40:1 (g)	80:1 (h)
N.	8	8	7	8	8	8	8
Menses	0/8*	0/8*	0/7*	1/8$^{\#}$	3/8	5/8$^{\alpha,\S}$	4/8$^{\alpha,\S}$

The level of statistical significance was set at $p \leq 0.05$. The significant difference between means was calculated vs.: α = *vs.* baseline, same formulation; * = *vs*. f, g, h same time; $^{\#}$ = *vs*. g same time; § = *vs*. *b*, *c*, *d*, *e* same time.

Free testosterone and HOMA Index

The MI/DCI ratios gave interesting results for free testosterone, however again the best ones were attained using the 40:1 ratio and, to a lesser extent, the others (Figure 5).

Post-treatment HOMA index was significantly reduced vs baseline by all the formulations administered; however, no significant differences were detected among the different treatments (Table III).

Basal and Postprandial Insulin

Finally, basal and postprandial insulin decreased after all the treatments, although with some differences. Concerning these parameters, the performance due to DCI alone was satisfying, nevertheless the top result was achieved by the 40:1 ratio. The most relevant difference for basal insulin compared to the other treatments was found only with such ratio and with 80:1. Instead, the data obtained for postprandial insulin did not show any significant difference among them, albeit they significantly improved vs baseline (Figure 6).

Discussion

This is the first clinical trial aimed at comparing the results obtained in PCOS patients after the administration of different MI and DCI ratios. Our evidence shows unquestionably that these two stereoisomers, even if they share almost the same chemical structure, in some cases exert very different clinical efficacy. This study demonstrated that overall the 40:1 ratio is the most effective, although some other ratios were able to display similar activities. Furthermore, it can be stated that the increase of DCI does not bring about any positive contribution to the

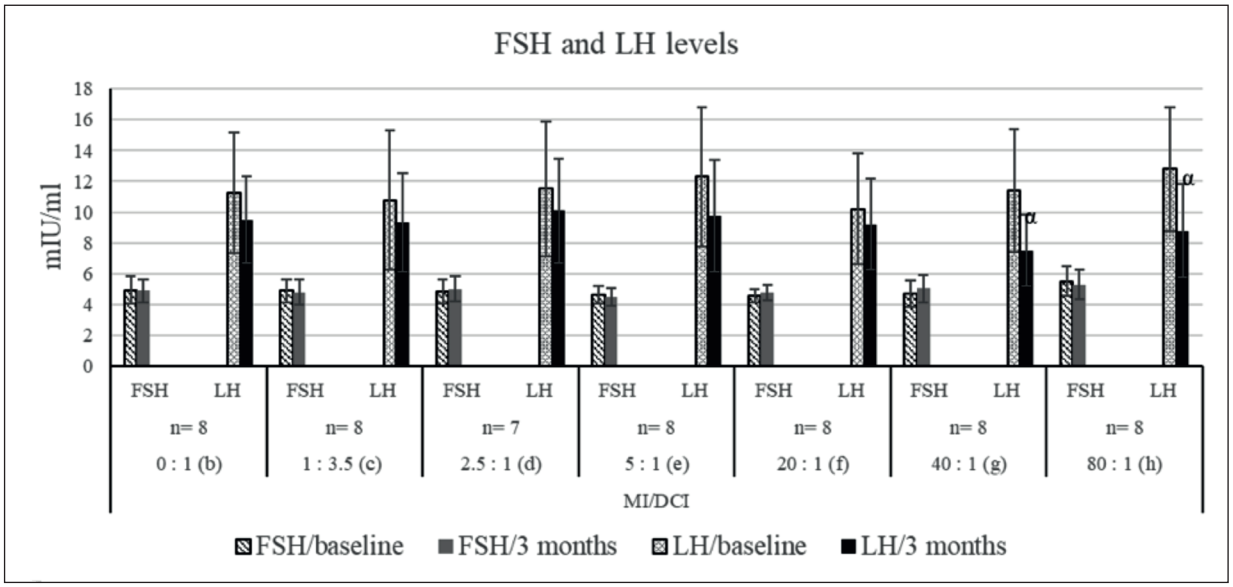

Figure 2. FSH and LH levels at two different time points in patients administered with various MI/DCI ratio. Treatment dose: 2 g (total MI + DCI) twice a day. Values are expressed as mean ± SD. *p*: value at the 3rd month *vs.* baseline and between treatments at the same time. The level of statistical significance was set at $p \leq 0.05$. The significant difference (α) between means was found only *vs.* baseline of the same formulation.

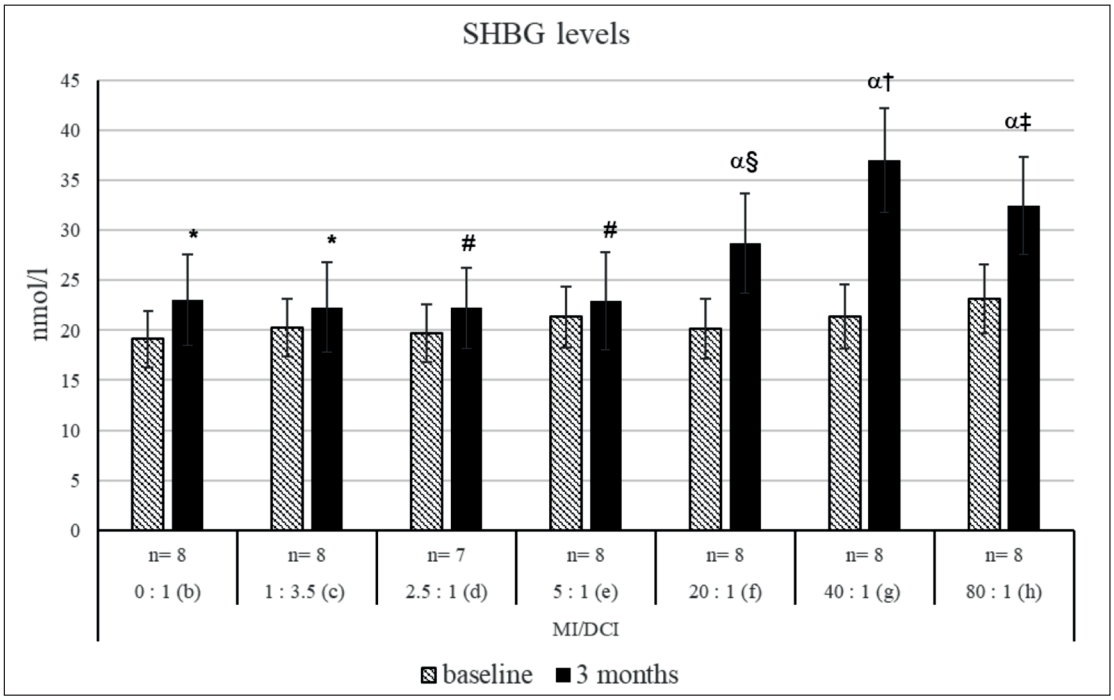

Figure 3. SHBG levels at two different time points in patients administered with various MI/DCI ratio. Treatment dose: 2 g (total MI + DCI) twice a day. Values are expressed as mean ± SD. *p*: value at the 3rd month *vs.* baseline and between treatments at the same time. The level of statistical significance was set at $p \leq 0.05$. The significant difference between means was calculated *vs.*: α = *vs. baseline*, same formulation; * = *vs. g, h* same time; # = *vs. f, g, h* same time; § = *vs. b, c, d, e, g, h* same time; † = *vs. b, c, d, e, f* same time; ‡ = *vs. b, c, d, e* same time.

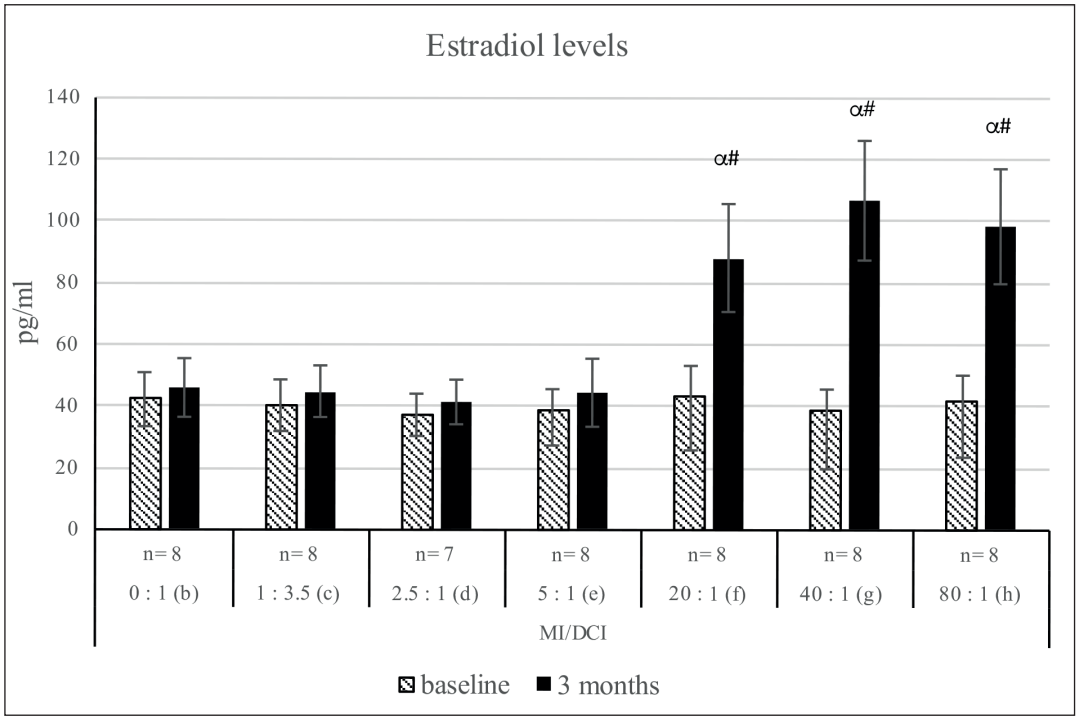

Figure 4. E2 levels at two different timepoints in patients administered with various MI/DCI ratio. Treatment dose: 2 g (total MI + DCI) twice a day. Values are expressed as mean ± SD. *p*: value at the 3rd month *vs.* baseline and between treatments at the same time. The level of statistical significance was set at $p \leq 0.05$. The significant difference between means was calculated: α = vs baseline, same formulation, and # = vs b, c, d, e, same time.

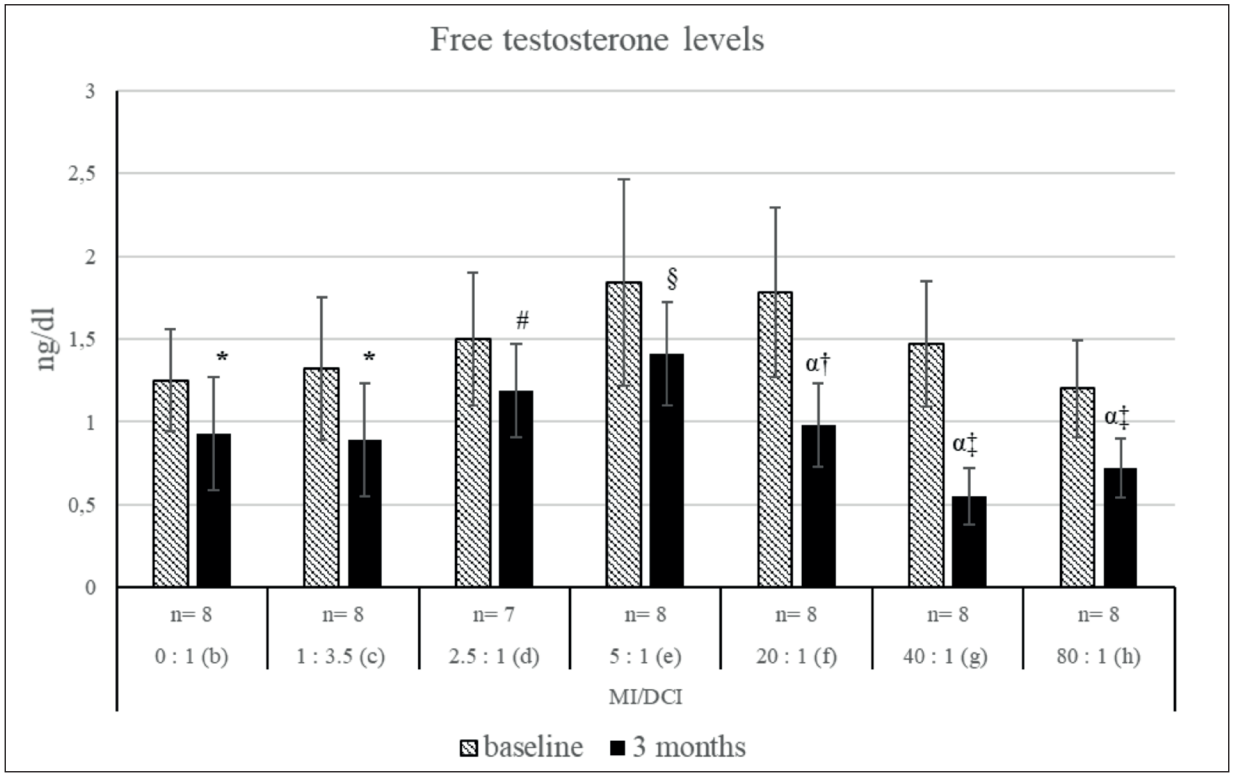

Figure 5. Free testosterone levels at two different time points in patients administered with various MI/DCI ratio. Treatment dose: 2 g (total MI + DCI) twice a day. Values are expressed as mean ± SD. *p*: value at the 3rd month *vs.* baseline and between treatments at the same time. The level of statistical significance was set at $p \leq 0.05$. The significant difference between means was calculated *vs.*: α = *vs. baseline*, same formulation; * = *vs. e, g* same time; # = *vs. g, h* same time; § = *vs. b, f, g, h* same time; † = *vs. e, g, h* same time; ‡ = *vs. b, c, d, e, f* same time.

therapy for most of the parameters analyzed, and mainly for the primary outcome (ovulation). On the contrary, it seems to work as a hindrance to MI efficacy. We initially intended to enroll more patients; however, the study was terminated for ethical reasons because the differences in the primary outcome and in some secondary outcomes (progesterone, SHBG, estradiol) were found highly significant.

It is remarkable that the these data in human agree with the findings by Bevilacqua et al[21] in a mouse model of PCOS, where PCOS was induced in C57BL/6N female mice by placing them at a continuous light cycle (24 hr) for 10

Table III. HOMA Index values at two different timepoints in patients administered with various MI/DCI ratio. Treatment dose: 2 g (total MI + DCI) twice a day.

MI/DCI	N.	HOMA index t 0	HOMA index 3rd month
0:1	8	5.05 ± 1.51	3.05 ± 0.85$^{\alpha}$
1:3.5	8	5.84 ± 1.47	2.89 ± 0.64$^{\alpha}$
2.5:1	7	4.45 ± 1.29	2.71 ± 0.76$^{\alpha}$
5:1	8	4.94 ±1 .53	2.88 ± 0.89$^{\alpha}$
20:1	8	4.21 ± 1.18	2.51 ± 0.73$^{\alpha}$
40:1	8	4.49 ± 1.45	2.45 ± 0.68$^{\alpha}$
80:1	8	4.62 ± 1.27	2.98 ± 0.81$^{\alpha}$

Values are expressed as mean ± SD. *p*: value at the 3rd month vs. baseline and between treatments at the same time. The level of statistical significance was set at $p \leq 0.05$. The significant difference (α) between means was found only *vs*. baseline of the same formulation.

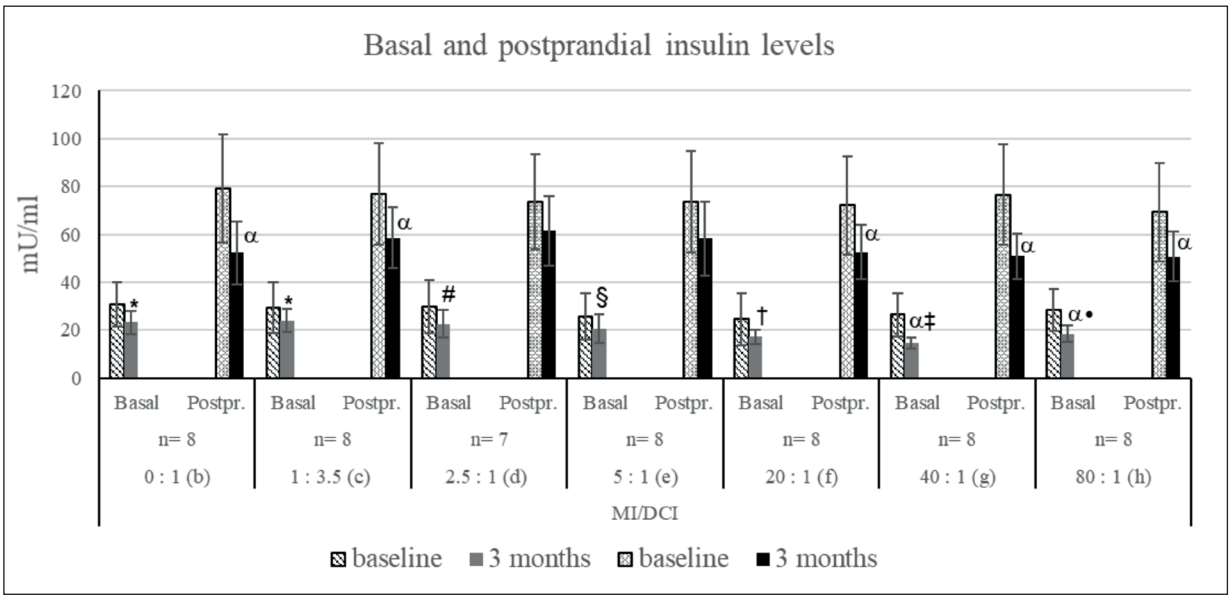

Figure 6. Basal and postprandial insulin levels at two different time points in patients administered with various MI/DCI ratio. Treatment dose: 2 g (total MI + DCI) twice a day. Values are expressed as mean ± SD. *p*: value at the 3rd month *vs.* baseline and between treatments at the same time. The level of statistical significance was set at $p \leq 0.05$. The significant difference between means was calculated *vs.*: α = *vs. baseline*, same formulation; * = *vs. f, g, h* same time; # = *vs. f, g* same time; § = *vs. g* same time; † = *vs. b, c, d* same time; ‡ = *vs. b, c, d, e, h* same time; • = *vs. b, c, g* same time.

weeks. The authors treated the groups with a combination of MI and DCI for a total of 420 mg/kg in 2 ml drinking H_2O/day at one of the following respective ratios: 5:1; 20:1; 40:1; 80:1. Control mice received plain drinking water. It was found that mice suffering from PCOS showed a faster recovery of physiological ovarian phenotype as well as a normal reproductive function when treated with a formulation of MI/DCI in the ratio of 40:1, with respect to water alone or to other MI/DCI formulations. In addition, a positive, although less pronounced effect was obtained with the 80:1 MI/DCI formula. Mice treated accordingly also showed a faster gain of body weight, which correlated with physiological features. In addition, it is impressive how the ratio of theca/granulosa cell layer thickness was fully restored in both the 40:1 and 80:1 MI/DCI treated animals. Yet, the 40:1 formula resulted the most effective in restoring a normal uterus, regular structure of ovaries and follicles, and fertility observed as time of delivery after mating. An impressive normalization of the ratio of theca/granulosa cell layer thickness (TGR) was only obtained by treating animals with the 40:1 formula. Concerning this point, it is important to remind that the alteration of theca cell layer is a hallmark of PCOS, as demonstrated by Gilling-Smith et al[22]. Therefore, the achievements of the preclinical and the clinical studies essentially reinforce each other. To explain the disappointing outcomes due to the highest DCI dosages (0:1, 1:3.5, 2.5:1, and 5:1 MI/DCI ratios), we have to keep in mind the overstimulation of epimerase activity in the PCOS ovary, mentioned before. In this context, an exogenous intake of DCI is harmful. The additional increase of this stereoisomer amplifies its toxic effect in the ovary, where DCI induces a progressive weakening of the response to FSH (only MI is involved in FSH signaling) and harmful effects on oocyte quality. Several works demonstrated the pivotal role played by MI in female fertility. Of note, MI physiologically represents almost 99% of the intracellular content of inositols in oocytes[14]. The experience deriving from some clinical researches should be carefully considered. Cheang et al[23] demonstrated that the reduction in the efficiency of insulin signaling, characterizing the PCOS patients, could ensue from a defect in the IPG insulin second messenger pathway. Such condition is consistent with the insulin-mimetic role of IPGs in activating enzymes that control glucose metabolism. To test if DCI could be able to ameliorate the hor-

monal status and restore ovulation, 1200 mg of DCI or placebo were administered once a day by oral route for 6-8 weeks to 44 obese PCOS women. The therapy improved insulin sensitivity and decreased circulating free testosterone levels, furthermore it induced ovulation in 19 of 22 treated women (86%), whereas only 6 of 22 women (27%) ovulated in the placebo group[24]. These findings were in accordance with those obtained by Iuorno et al[25]. After this success, the Authors designed a large multi-centre placebo-controlled trial with 2400 mg DCI in PCOS women, which means a double dose of DCI with respect to that previously used. However, the results were disappointing, and after the failure of the study this therapeutic regimen was left. The lack of efficacy in the last trial was related to the high dose of DCI administered, which evidently exerted a negative effect on the ovary. It was speculated that DCI is very useful at systemic level for decreasing hyperglycaemia, but it shows detrimental consequences at ovarian level when given orally at the dose used (2400 mg). This evidence prompted to state that the two stereoisomers elicit opposite effects at the same dose. In other words, the increase of MI administered is beneficial, whereas the parallel growth of DCI is harmful. A recent study[26] offered a direct proof supporting the essential importance in keeping and preserving the physiologic concentrations between MI and DCI in the ovary, namely in the follicular fluids, to obtain the highest blastocyst quality. It was shown that the endogenous MI and DCI levels constitute a central factor and their alteration leads to a several decrease in blastocyst quality. Specifically, while MI induced favorable effects on all parameters analyzed, DCI rise over certain limits caused a deterioration of blastocysts. The author stated that MI and DCI are, respectively, "high quality" and "low quality" biomarkers for blastocysts (and also for oocytes). The study identified a threshold (MI/DCI content in follicular fluids = 70:1) for blastocysts quality. Values of about 70:1 or higher (ideal ratio: 100:1) relate to good quality blastocysts and a successful IVF. On the bases of this framework, the authors suggested to use the ratio between MI and DCI in follicular fluids as a promising marker for evaluating in advance the blastocyst performance in IVF. Furthermore, they recommended to pre-treat women undergoing IVF with MI to improve ART outcome[26].

The above data are consistent with our findings that demonstrated the necessity to strictly regulate the administration of the two inositol stereoisomers and strongly support the therapeutic use of MI and DCI at the 40:1 ratio.

Although the results of our trial are convincing, the presence of a limited number of patients obviously requires new supports obtained with much more subjects.

Conclusions

This first study in humans, using different ratios of MI and DCI in PCOS, achieved very clear results, in agreement with the previous one carried out in mice by Bevilacqua et al[21]. Both the preclinical and the clinical study support the 40:1 MI/DCI ratio as the best one for PCOS treatment aimed at restoring ovulation in these patients. Furthermore, it was demonstrated that DCI activity is beneficial mainly at a specific ratio with MI, whereas the increase of DCI causes the loss of the beneficial effects at reproductive level.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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