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The Combined therapy with myo-inositol and D-Chiro-inositol reduces the risk of metabolic disease in PCOS overweight patients compared to myo-inositol supplementation alone

M. NORDIO, E. PROIETTI*

Department of Medical Physiopathology, Sapienza University of Rome, Rome (Italy)
*Hospital San Giovanni Evangelista, Cardiology Department and UTIC, Rome (Italy)

Abstract. – *Background:* **PCOS is the main cause of infertility due to metabolic, hormonal and ovarian dysfunctions. Women affected by PCOS often suffer of insulin resistance and of a compensatory hyperinsulinemia. These conditions put the patients at risk of developing several metabolic disorders. Both myo-inositol (MI) and D-chiro inositol (DCI) glycans administration has been reported to exert beneficial effects at metabolic, hormonal and ovarian level. Beside these common features, MI and DCI are indeed different molecules: they belong to two different signal cascades and regulate different biological processes.**

Aim: **In this study, we aim to verify whether the two molecules have a synergistic action by acting on their specific cellular pathways. The effectiveness in reducing the risk of metabolic syndrome as well as in enhancing the ovarian functions of a combined therapy with MI and DCI was compared to a mono therapy in a randomized controlled trial.**

Methods: **Fifty overweight women with PCOS were enrolled and divided in two groups to receive MI and DCI (MI+DCI group) or MI alone (MI group) for a period of six months. Baseline measurements were repeated at three months (T1) and at the end of the treatment (T2).**

Results: **At the end of the treatment, both MI and MI+DCI groups showed an improvement of the metabolic parameters and no significant differences were found. As expected, the combined supplementation with MI and DCI resulted to be more effective, compared to the MI group, after three months of treatment.**

Conclusions: **The combined administration of MI and DCI in physiological plasma ratio (40:1) should be considered as the first line approach in PCOS overweight patients, being able to reduce the metabolic and clinical alteration of PCOS and, therefore, reduce the risk of metabolic syndrome.**

Key Words:

PCOS, Myo-inositol, D-chiro-inositol, Metabolic syndrome.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age[1-3]. It is characterized by menstrual abnormalities, clinical or biochemical hyperandrogenism, multiple abnormal cysts and enlarge ovaries. Furthermore, due to its feature, PCOS is the main cause of infertility due to the menstrual dysfunction.

Insulin resistance and the compensatory hyperinsulinemia affect some 65-70% of women with PCOS, among whom 70-80% are obese (BMI > 30) and 20-25% are of normal weight (22 < BMI < 25)[4]. This condition is a risk factor for developing both type 2 diabetes mellitus (DM) and cardiovascular diseases. Therefore, once a diagnosis of PCOS is given, it is necessary to monitor the patient for these potential risks. Several studies have shown that an altered insulin signal transduction in PCOS patients may cause insulin resistance, that in turn induces abnormal ovarian steroidogenesis[5,6].

Overweight women with PCOS seem to respond favourably to the treatment with insulin-sensitizing drugs, such as metformin or troglitazone (TZDs), which reduce serum androgens concentration and restore ovulation. However, several limitations have been linked

Corresponding Author: Maurizio Nordio, MD; e-mail: maurizionordio1@gmail.com

to their use. Indeed, metformin induces predominantly gastrointestinal discomforts consisting of bloating, nausea and diarrhoea. TZDs have significant practical limitations; in particular, they induce weight gain and more recently their use has been associated with increased coronary artery disease and myocardial infarction[7].

Inositol is a six-carbon polyol which has been characterized as an insulin sensitizer; indeed, inositolphosphoglycan (IPG) mediators play a key-role in multiple cellular processes that control glucose metabolism[8,9]. Several studies suggest that a deficiency in tissue availability or an altered metabolism of IPG mediators may contribute to insulin resistance[10,11]. Epimerization of the six hydroxyl-groups of inositol leads to the formation of up to nine stereoisomers, including myo-inositol (MI) and D-chiro-inositol (DCI). Both IPGs were insulin mimetic when administered *in vivo* enhancing the physiological insulin-receptor activity and reducing glucose levels in serum. More recently, the administration of MI and DCI has been related to an improvement of ovulation in most PCOS women[12-14]. Indeed, MI supplementation reduces serum insulin levels, decreases serum testosterone, and restores ovulation[15]. Despite the similar role of MI and DCI on insulin resistance, an elevated concentration of MI in follicular fluid appears to play a specific role in follicular maturation and provides a marker of good oocyte quality[16-18].

Moreover, experiments on mice oocytes have shown that exposure to MI positively affects the meiotic progression of germinal vesicle by enhancing the intracellular Ca^{2+} oscillation[16,19]. On the other hand, it is well known that DCI glycans administration is related to the activation of the glycogen sintethase[20].

The MI/DCI physiological ratio is specific in each tissue and the enzymatic conversion of the two isomers is affected by the condition of insulin resistance[21,22]. Up to now, no data have been reported on MI/DCI ratio in the ovary.

In order to take advantage of the unique feature of both inositols stereoisomers, MI and DCI, we investigated the effects of a new combined treatment. In particular, in this study we compared the effects of MI supplementation alone versus a combined MI and DCI therapy in reducing the metabolic syndrome risk as well as the improvement of the clinical features in PCOS overweight women.

Material and Methods

Subjects

Fifty women with PCOS (BMI > 27 kg/m^2, mean age 28 years old, range 18-41) were recruited for the study.

PCOS was diagnosed according to the criteria established by the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) in Rotterdam in 2003: (1) oligo-anovulation, (2) hyperandrogenism (clinical or biochemical) and (3) presence of 12 or more follicles in each ovary measuring 2-9 mm in diameter, and/or increased ovarian volume (> 10 ml)[23,24].

Diabetic subjects, smokers and alcohol users were excluded from the study.

The Institutional Ethical Committee approved the protocol, and all patients gave a written informed consent before entering the study.

Protocol

Patients were randomly assigned to two groups: in the MI group, 24 women received 2 g of myo-inositol in powder (Inofolic® Lolipharma, Rome, Italy) and in the MI+DCI group, 26 women received 550 mg of myo-inositol plus 13,8 mg of D-chiro-inositol in soft gel capsule (Inofolic® Combi, Lolipharma patent) twice a day.

The new combined therapy was manufactured according to the physiologic ratio of the two isomers in the body (40:1 data not shown). The new pharmaceutical form (soft gel capsule) is able to improve the gastrointestinal absorption allowing to substantially reduce by one third the administered dose compared to the powder form[25].

The treatments were performed for 6 months.

At pre-treatment blood pressure, weight and height were measured and waist to hip ratio (WHR) and BMI were calculated through standard equation. In the morning, sex hormone binding globulin, serum steroids and lipid profile levels were measured. All the patients underwent an oral glucose tolerance test (OGTT) and plasma glucose and insulin were measured after 20, 30, 60, 120 minutes. Seven women showed impaired glucose tolerance (plasma glucose concentration > 140 mg/dl, < 200 mg/dl two hours after oral ingestion of 75 g of glucose). Four of them were assigned to MI group and three to the combined therapy MI+DCI. The incremental in-

sulin ($AUC_{insulin}$) and glucose ($AUC_{glucose}$) areas under the curve (AUCs) were calculated by the trapezoidal method.

The homeostasis model assessment (HOMA) was used as index of insulin resistance for each patient.

The patients were also asked not to change usual habits both for food, sport and lifestyle.

Serum progesterone was measured weekly and ovulation was confirmed when the revealed levels were over 10 ng/ml.

Baseline measurements were repeated at two time points: after three months (T1) of treatment and at the end of the study (T2).

Statistical Analysis

Data are reported as mean values ± standard deviation (SD).

To compare the two groups the unpaired *t*-test (parametric distribution) was used. The significance of differences between the pre- and post-treatment measures (at first and third month) were analyzed using one-way ANOVAs.

The source of the detected significances was determined by Bonferroni correction for repeated measures. *p* values less than 0.05 were considered statistically significant.

Results

Statistical analysis revealed no significant differences between groups at baseline (age, BMI, waist to hip ratio, hormonal and lipids profile levels) (Table I).

No change of waist to hip ratio and BMI was detected in both groups after the treatment (Table II).

After six months (T2) no significant differences were found between the two groups. As expected, a number of significant changes were observed at T1 ($p < 0.03$) At this time point, both plasma glucose and insulin concentrations showed a significant reduction in the MI+DCI group while no relevant changes were reported in the treatment with MI alone (Figure 1, Table III).

Significant differences were also observed in the serum sex hormone levels. In particular, compared to the MI group, the decrement of total testosterone and the increment of the serum sex hormone binding globulin were more relevant in MI+DCI group both at T1 and at T2 (Table IV).

Finally, in both groups there was a striking improvement of the ovulation function and all the women ovulated after treatment.

Table I. Baseline characteristics.

Variable	MI n = 24	MI+DCI n = 26
Age	28.2 ± 1.5	27.9 ± 1.4
Waist to hip ratio	0.88 ± 0.02	0.87 ± 0.02
BMI (kg/m^2)	27.7 ± 2.3	27.5 ± 2.9
Menstrual periods/yr	3 ± 1	3 ± 1
Free testosterone (ng/dl)	0.87 ± 0.11	0.85 ± 0.14
Androstenedione (ng/dl)	271 ± 14	263 ± 15
DHEAS (µg/dl)	369 ± 52	365 ± 62
Total testosterone (ng/dl)	97.2 ± 19.2	95.4 ± 10.7
17 beta estradiol (pg/ml)	79 ± 3.5	82 ± 2.6
Sex hormone binding globulin (nmol/L)	149 ± 20	145 ± 16
Total cholesterol (mg/dl)	200 ± 9.4	210 ± 8.5
Triglycerides (mg/dl)	195 ± 20.2	166 ± 20.6
Lower density cholesterol-C (mg/dl)	130 ± 13.5	129 ± 11.8
Glucose AUC (mg/dl/min)	16919 ± 1057	17229 ± 668
Fasting glucose (mg/dl)	96.2 ± 11.8	94.8 ± 12.5
Insulin AUC (µU/ml/min)	12610 ± 670	12985 ± 517
Fasting insulin (µU/ml)	12.3 ± 3.7	12.8 ± 4.1
HOMA index	2.4 ± 1.2	2.7 ±1.1
Systolic blood pressure (mmHg)	129 ± 2.5	131 ±1.6
Diastolic blood pressure (mmHg)	87 ± 2.6	88 ± 3.3

DHEAS = dehydroepiandrosterone; AUC = area under the curve during 2 hours, 75 g oral glucose tolerance test; HOMA-IR = homeostatic model assessment.

Table II. Anthropomorphic characteristics.

Characteristic	MI group n = 24		MI+DCI group n = 12	
	Baseline	T2	Baseline	T2
Systolic blood pressure (mmHg)	129 ± 2.5	127 ± 2	131 ± 1.6	128 ± 1.2
Diastolic blood pressure (mmHg)	87 ± 2.6	82 ± 1*	88 ± 3.3	80 ± 2*
BMI (kg/m^2)	27.7 ± 2.3	27.3 ± 2.1	27.5 ± 2.9	26.9 ± 2.4
Waist to hip ratio	0.88 ± 0.02	0.87 ± 0.02	0.89 ± 0.02	0.87 ± 0.04

*$p < 0.05$, respect baseline group.

Discussion

In the present study, we show that a combined MI and DCI therapy in physiological ratio was able to restore hormonal and metabolic parameters in overweight PCOS women earlier than the treatment with MI alone.

Insulin-sensitizing agents have been suggested as the therapy of choice for polycystic ovary syndrome (PCOS), since insulin resistance and associated hyperinsulinemia are recognized as important pathogenetic factors of the syndrome[26,27].

Indeed, almost all obese PCOS women and more than 50% of normal weight are insulin resistant, presenting some degree of hyperinsulinemia.

The administration of two different stereoisomears of inositol, D-chiro-inositol (DCI) and myo-inositol (MI), has been related to improved physiological insulin-receptor activity. Unlike other insulin sensitizer agents, no important side effects have ever occurred after MI or DCI administration. Only a few cases of gastrointestinal discomforts, such as nausea and diarrhea episodes, were reported after administration at high dosages (> 18 g), and therefore their use is considered safe in long-term therapy[28].

In 1999 Nestler et al[12] performed a clinical study in which DCI was administered in obese hyperinsulinemic PCOS women. The oral administration of 1200 mg/die of DCI was directly

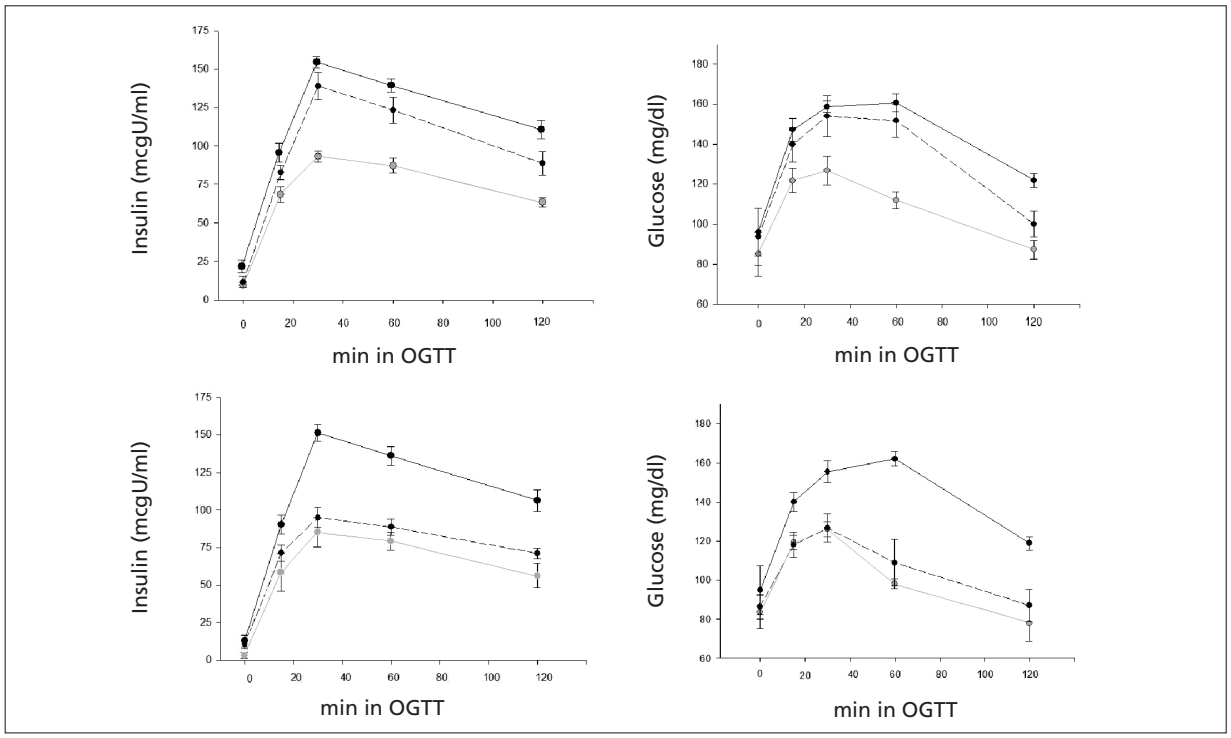

Figure 1. Serum glucose and insulin concentrations during oral glucose tolerance test (OGTT) at baseline (*black*), at T1 (*dashed*) and at T2 (*grey*) of treatment with MI alone or MI+DCI.

Table III. Plasma glucose and insulin sensitivity index measurements.

Characteristic	MI group n = 24			MI+DCI group n = 26		
	Baseline	T1	T2	Baseline	T1	T2
Fasting insulin (μU/ml)	12.3 ± 3.7	11.7 ± 3.5	9.6 ± 1.9 **	12.8 ± 4.1	10.1 ± 2.9*††	9.2 ± 2.1**
Insulin AUC (μg/dl/min)	13718 ± 579	12586 ± 647	8800 ± 469**	13832 ± 730	9870 ± 847†*	8588 ± 422**
Fasting glucose (mg/dl)	96.1 ± 11.8	93.2 ± 10.9	85.2 ± 10.9**	94.8 ± 12.5	85.9 ± 7.2*††	83.6 ± 8.6**
Glucose AUC (mg/dl/min)	16919 ± 1057	16209 ± 447	11580 ± 401**	17229 ± 668	12358 ± 515*†	10690 ± 513**
HOMA-IR	2.4 ± 1.2	2.2 ± 1.3	1.9 ± 2.1 **	2.7 ± 1.1	1.82 ± 0.12 *	1.5 ± 0.28**

AUC = Area under the curve during 2 hours, 75 g oral glucose tolerance test; HOMA-IR = homeostasis model assessment.
*$p < 0.05$; **$p < 0.01$ respect baseline group; †$p < 0.05$, ††$p < 0.03$ respect control treatment group.

Table IV. Serum sex hormone before and after treatment.

Characteristic	MI group n = 24			MI+DCI group n = 26		
	Baseline	T1	T2	Baseline	T1	T2
Total testosterone (ng/dl)	97.2 ± 19.2	60.3 ± 12.7*	40.1 ± 9.5**	95.4 ± 10.7	50.4 ± 10.2*	32.7 ± 10.0**
Free testosterone (ng/dl)	0.87 ± 0.11	0.65 ± 0.09*	0.24 ± 0.03**	0.85 ± 0.14	0.44 ± 0.08*	0.23 ± 0.02**
DHEAS (μg/dl)	369 ± 52	320 ± 31	196 ± 23**	365 ± 52	278 ± 32*	179 ± 27**
SHBG (nmol/l)	149 ± 20	160 ± 24*	202 ± 27*	145 ± 16	180 ± 17*	208 ± 20*
Androstenedione (ng/dl)	271 ± 14	250 ± 13*	198 ± 19**	263 ± 15	255 ± 14*	194 ± 15**

DHEAS = Dehydroepiandrosterone; SHBG = Sex hormone binding globulin: *$p < 0.05$, respect baseline **$p < 0.01$ respect baseline.

linked to improved insulin sensitivity and hormonal profile and regulation of menses.

These results were not confirmed in a second clinical trial when a double dose of DCI was administered in PCOS subjects suggesting a negative effect of DCI at ovarian level, as also suggested by the "D-chiro-inositol paradox"[13,29].

DCI is synthesized by an insulin-dependent epimerase that converts MI into DCI. Each tissue is characterized by a specific conversion rate, depending on the specific needs. In particular high DCI concentrations are present in tissues such as liver muscles and fat that are responsible of glycogen synthesis and storage[21,30].

When insulin resistance occurs, the conversion rate is affected, resulting in a decreased level of DCI in cells. Unlike tissues such as muscle and liver, ovaries never become insulin resistant[31,32].

On this basis, it can be speculated that in the ovary hyperinsulinemia leads to an enhanced MI to DCI epimerization resulting in an overproduction of DCI and in a reduction of MI[29].

MI deficiency is related to a poor oocyte quality and several recent studies highlighted the positive effects of MI supplementation in PCOS women. Unfer et al. have shown that the administration of 4 g/die of MI, beside improving the hormonal profile and restoring ovulation, is able to induce regular menses in both lean and obese PCOS patients[33,29]. Additional studies have also shown that MI supplementation, rather than DCI, is able to improve oocyte quality, reducing the amount of FSH administered during the *in vitro* fertilization (IVF) procedure[34]. Since MI and DCI regulate different biological processes, we suppose that the synergistic action of their concomitant administration may be more beneficial than MI alone on overweight PCOS women.

Results have shown that both treatments normalize the metabolic parameters and restore ovulation in overweight PCOS women. In particular, at the end of the treatment both the fasting insulin and glucose serum concentration level are significantly reduced. Nevertheless, compared to the treatment with MI alone, the combined therapy has shown significant changes on the metabolic profile after three months of treatment. On this basis, we speculate that DCI rapidly reduces the peripheral hyperinsulinemia while the presence of MI mainly improves the ovulatory function.

In conclusion, we suggest that a combined therapy of MI plus DCI may be the first line approach treatment in PCOS overweight patients who need to control insulin levels and increase ovarian MI content, reducing the risk of developing a metabolic disease.

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