Inositol: history of an effective therapy for Polycystic Ovary Syndrome

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Abstract. – Inositol is a physiological compound belonging to the sugar family. The two inositol stereoisomers, myo-inositol and D-chiroinositol are the two main stereisomers present in our body.

Myo-inositol is the precursor of inositol triphosphate, a second messenger regulating many hormones such as TSH, FSH and insulin. D-chiroinositol is synthetized by an insulin dependent epimerase that converts myo-inositol into D-chiro-inositol. Polycistic Ovary Syndrome (PCOS) is a metabolic and hormonal disorder and a common cause of infertility. Insulin resistance and the consequent hyperinsulinaemia contribute to hyperandrogenism development, typical marker of PCOS. In these patients myo and/or D-chiro-inositol administration improves insulin sensivity while only myo-inositol is a quality marker for oocytes evaluation.

Myo-inositol produces second messengers for FSH and glucose uptake, while D-chiroinositol provides second messengers promoting glucose uptake and glycogen synthesis. The physiological ratio of these two isomers is 40:1 (MI/DCI) and seems to be an optimal approach for the treatment of PCOS disorders.

Key Words:
Inositol, Polycistic ovary syndrome, PCOS.

Introduction

The history that has led to the widespread use of inositol compounds in the clinical gynecologic practice is a fascinating and complex tale.

In 1850 Johanes Joseph Scherer (1814-1869)[1,2] isolated from the muscle a hexahydroxycyclohexane that he named Inositol [from Ancient Greek stem of iç (*is, in-*, "sinew, fiber"), -ose (indicating a carbohydrate), -ite ("ester"), -ol ("an alcohol")], as it formally belongs to the sugar family[3]. The structure of this hexahydroxycyclohexane allows the formation of 9 different stereoisomers. Among them, myo-inositol is by far the most distributed in biological systems and represents the most interesting form from a metabolic and functional point of view. Indeed, myo-inositol is currently thought as a prebiotic molecule[4], given the prominent functions inositol and inositol-derivatives support in several biological systems.

Later, in 1850, inositol was found the main component of phytates, i.e. salts of the inositol hexaphosphoric acid. The discovery of phytate dates from 1855 to 1856 when Hartig[5] first reported small round particles in various plant seeds similar in size to potato starch grains. Those particles were rich in phosphorous, calcium and magnesium, but without proteins or lipids. As that substances have been not detected neither in meat or dairy products, they were named 'phytin', in order to outline its plant origin. To explain the high phosphorous, calcium and magnesium contents of phytin, several molecular structures were under controversial debate for many years. Eventually, in 1914, Anderson[6] presented the molecular structure of myo-inositol-1,2,3,4,5,6-hexakis dihydrogen phosphate, also called phytic acid, which was confirmed by various modern analytical methods[7,8].

Inositol (and its derivatives: salts, phosphates and associated lipids) are found in many foods (especially fruits and beans)[9]. In plants, inositol is generally represented in the form of hexaphosphate, and phytic acid or its salts (phytates).

Myo-Inositol was once considered as a member of the vitamin B complex; however, it cannot be considered a 'true' essential nutrient, given that it can be synthesized by the human body. However, it is still a matter of controversy if such biosynthesis may provide amounts considered adequate for good health from glucose.

Myo-inositol is synthesized by both prokaryotes and eukaryotes cells. Myo-inositol is basically incorporated into cell membranes as phosphatidyl-myo-inositol, the precursor of inositol

triphosphate that acts as second messenger regulating the activities of several hormones such as FSH, TSH and insulin. In addition, inositol is an important component of the structural lipids specifically phosphatidyl-inositol (PI) and its various phosphates, including the phosphatidyl-inositol phosphate (PIP) lipids[10].

After the original discovery by Scherer, many researchers have started to study the role of inositol in different organs and tissues, namely highlighting its relevant role in ensuring a proper cell shape and oocyte fertility.

In 1964, Eisenberg et al[11], and Eisenberg and Bolden[12] reported that testes are rich of free inositol; few years later, Voglmayr and Amann[13], Lewin and Beer[14], and Ghafoorunissa[15] showed that the prostate, the epididymis and seminal vesicles contain a large amount of myo-inositol. The seminal fluid is one of the richest sources of inositol, since concentration of inositol in seminal fluid is almost three times higher than that found in plasma[16,17]. These preliminary findings provided the first indirect proof relating inositol-based molecules to germ-cell (spermatozoa and oocytes) physiology.

Over the last six decades, Joseph Larner has tirelessly pursued scientific studies on insulin action mechanisms, providing new insights into the cause, diagnosis, and cure of non-insulin-dependent diabetes mellitus[18]. In 1974, Larner proposed the existence of different intracellular chemical mediators of insulin, and hypothesized that, after the binding of insulin to its receptor, different intracellular pathways could be selectively triggered according to the specific mediator involved[18]. In 1988 Larner et al[19] came to the conclusion that the two inositol stereoisomers, myo-inositol and D-chiro-inositol, are chemical mediators of insulin, acting through different mechanisms. Both D-chiro-inositol and myo-inositol have similar structures, differing in the stereochemistry of only one hydroxyl group[20]. Natural sources for these inositols are endogenous biosynthesis and dietary intake. Myo-inositol is synthesized from glucose-6-phosphate in two steps. First, glucose-6-phosphate is isomerised to myo-inositol-1-phosphate, which is then dephosphorylated by an inositol monophosphatase enzyme giving free myo-inositol[21]. In vivo, D-chiro-inositol is synthetized by an epimerase that converts myo-inositol into D-chiro-inositol. Larner first demonstrated a decreased D-chiro-inositol content in urine as well as tissues of human subjects and animals with type 2 diabetes[20,22]. Uri-nary decrease in D-chiro-inositol was accompanied by an increase in myo-inositol content[20,22]. Additional investigations demonstrated that the altered inositol excretion patterns in human[23] and monkey urine[20,22] were specifically related to the underlying insulin resistance, rather than to the diabetes type. To explain the altered pattern of urine inositol excretion observed under insulin resistance, i.e., increased myo-inositol whereas D-chiro-inositol decreases, Larner postulated a defect in the epimerization process, that physiologically enacts the conversion of myo-inositol to D-chiro-inositol. He showed that [³H]myo-inositol is converted to [³H]chiro-inositol in vivo in rats[24], and in vitro in fibroblasts[25], and that this process is stimulated by insulin. Larner also demonstrated that the conversion of [(3)H]myo-inositol to [(3)H]chiro-inositol in vivo was markedly decreased in insulin sensitive tissues (liver, muscle, and fat) of Goto-Kakizaki (GK) rats, (inbreeding Wistar rats selected for insulin resistance), compared to Wistar controls[26]. In a follow-up study, Sun et al[27] (Larner's group) analyzed GK and Wistar rat tissues for total myo-inositol and D-chiro-inositol content. They also developed an epimerase enzyme assay to measure myo-inositol conversion to D-chiro-inositol, as well as a bioassay to measure epimerase activity in cytosolic extracts of tissues of GK and Wistar control rats. Their results showed a consistent decreased total D-chiro-inositol/myo-inositol in kidney, liver, and muscle in GK rats compared to controls; additionally, Sun et al[27] provided evidence for a myo-inositol to D-chiro-inositol epimerase activity in rat liver cytosol. Importantly, they demonstrated that epimerase bioactivity was significantly decreased in cytosolic extracts of muscle, liver, and fat from GK type 2 diabetic rats, versus Wistar controls. On the basis of these data, Larner's group hypothesized that a decreased myo-inositol to D-chiro-inositol epimerase activity may play a role in explaining the decreased D-chiro-inositol/myo-inositol ratio observed in urine and tissues of both animals and humans.

Around the same period when Larner was publishing those results, a gynecological disorder of wide clinical and social interest, the Polycystic Ovary Syndrome (PCOS), was for the first time linked to insulin resistance, and namely to hyperinsulinemia.

PCOS, the most common cause of infertility, is associated to ovarian dysfunction, metabolic and hormonal impairments, and menstrual irregularity, affecting up to 10% of the total female

population in reproductive age. In 2003, the European Society of Human Reproduction and Embryology (ESHRE), and the American Society for Reproductive Medicine (ASRM) sponsored a Consensus Meeting in Rotterdam, in order to reach a general agreement on the diagnostic criteria for that syndrome. The current definition requires at least two of the following clinical manifestations: chronic ovulatory disorder (oligo-ovulation to anovulation, and amenorrhea), presence of polycystic ovaries on ultrasound examination, and hyperandrogenism, either clinically established or confirmed by laboratory tests[28].

The pathogenesis of PCOS is still largely unknown, although various etiological factors are suspected to be involved. In the past decade, increasing compelling evidence has been accumulated supporting the central role of insulin resistance and/or compensatory hyperinsulinemia in the PCOS pathogenesis[29,30]. Indeed, hyperinsulinaemia, secondary to insulin resistance, is very common in PCOS patients, occurring in approximately 80% of women with PCOS and central obesity, as well as in 30%-40% of lean women diagnosed with PCOS[31,32]. Insulin resistance and subsequent hyperinsulinemia contribute both directly and indirectly to hyperandrogenism development[33,34]. Insulin directly stimulates the ovary theca cells to produce greater amount of androgens, and to inhibits hepatic synthesis of sex hormone-binding protein (SHBG), thus indirectly increasing the levels of circulating free androgens. Moreover, theca cells in PCOS patients present a greater sensitivity to insulin action on androgen secretion. Noteworthy, the insulin resistance observed in PCOS patients predisposes to the development of type 2 diabetes mellitus, especially when a family history of diabetes mellitus is recorded, and if patients are obese[35]. The importance of insulin resistance in PCOS is further underscored by the fact that insulin-sensitizing compounds such as metformin, pioglitazone and troglitazone, have been proposed as treatment for PCOS-associated hyperinsulinemia[30,36,37]. It is worth noting that metformin may antagonize some hyperandrogenic signs, by reducing total and free testosterone concentrations[38,39]. However, commonly used insulin-sensitizing drugs, by inducing gastrointestinal side effects, could likely reduce patients' compliance[40], and therefore it is unlikely they can be used in routine clinical practice.

The discovery that the impairment in the insulin signalling could be due to a defect in the inositolphosphoglycans (IPGs) second messenger pathway[20,41] opened a new horizon in the clinical management of PCOS. IPGs are known to have a role in activating enzymes that control glucose metabolism[42,43]. In PCOS women, a defect in tissue availability or altered metabolism of inositol or IPGs mediators may contribute to insulin resistance[44].

In 1998, the Insmed Pharmaceuticals Company took out a patent claiming the effectiveness of D-chiro-inositol for the treatment of PCOS. That patent originated from the promising data provided by the first clinical trial which assessed the effectiveness of D-chiro-inositol in the treatment of PCOS, published in *The New England Journal of Medicine*[45]. In particular, this clinical study measured steroids in serum and performed oral glucose-tolerance tests before and after the oral administration of 1200 mg of D-chiro-inositol or placebo once daily for six to eight weeks in 44 obese PCOS patients. The results showed that D-chiro-inositol administration to PCOS patients was able to improve insulin sensitivity and to reduce serum free testosterone levels compared to the placebo group. Additionally, diastolic and systolic blood pressure, and plasma triglyceride concentrations were decreased in patients treated with D-chiro-inositol. Ovulation occurred in 19 out of 22 women (86%) who received D-chiro-inositol , as compared to 6 out of 22 (27%) in the placebo group[45]. These promising results laid the foundations for follow-up studies. In 2002, Nestler and Allan published an additional clinical study in which they tested whether administration of D-chiro-inositol would affect the concentration of circulating insulin and androgens, and the frequency of ovulation in lean PCOS patients. Those results extended and confirmed earlier findings by showing that, in lean PCOS women, D-chiro-inositol reduced serum insulin and androgens, and improved some PCOS-associated metabolic abnormalities (increased blood pressure and hypertriglyceridemia)[46]. However, when higher doses of D-chiro-inositol were used, the earlier results were not confirmed. Namely, no improvement in insulin sensitivity was reported in women who received a high dose of D-chiro-inositol. Furthermore, the release of D-chiro-inositol—containing inositolphosphoglycan (IPG) did not improve in several women in the high dose D-chiro-inositol group, suggesting that these women had a functional defect in D-chiro-inositol-containing inositolphosphoglycan release, rather than a simple nutritional deficiency

of D-chiro-inositol[47]. According to those data, the Insmed Pharmaceutical company decided to stop utterly clinical trials with D-chiro-inositol. Yet, these achievements highlighted the crucial difference emerging when different D-chiro-inositol dosages were used. Indeed, as stressed by the study of Cheang et al[47], clinical efficacy was achieved when treating patients with 2400 mg of D-chiro-inositol, thus leaving open the possibility that this high dose could be responsible of the paradoxical lack of efficacy.

Thankfully, the interest of the scientific community for the potential use of inositols in the clinical practice was not restricted to the D-chiro stereoisomer. In 1992, Chiu et al[48] published a study about the role of myo-inositol in vitro human fertilization (IVF). That study had a three-fold aim: (1) correlate the embryotrophic properties, assayed by post-implantation embryo culture, and the inositol levels of sera of IVF patients with different pregnancy outcomes following IVF; (2) the monitoring of between-cycle variations in embryotrophic properties and inositol levels of serum samples obtained from patients during normal and treated cycles, and (3) the investigation of the effects of replenishing myo-inositol in serum samples which have previously been found to be non-supportive of mouse embryogenesis[48]. The study reported an elevated level of inositol in serum samples of patients having successful IVF pregnancies, thus indicating a possible involvement of inositol in both the early in vitro phase of IVF and the maintenance of normal embryonic development. These findings are consistent with the observation of the teratogenic effect of diabetic patients' serum containing low myo-inositol levels, which causes dysmorphogenesis in cultured rodent embryos[49,50]. Furthermore, using the preimplantation mouse embryo assay to determine the trophic activity of the culture media, the authors[48] showed that the serum of patients having successful IVF pregnancies and containing high concentrations of myo-inositol, allowed the development of embryos with a greater number of somites. Ten years later, another work[51] from the same group examined whether the myo-inositol content in human follicular fluid was associated with better oocyte quality. A total of 53 patients treated with IVF was recruited. Follicular fluid and serum samples were collected and divided into two groups: group A consisted of follicular fluid associated with matured and fertilized oocytes, while group B was from follicles with immature and unfertilized oocytes. As ex-

pected, a statistically significant correlation between myo-inositol concentration in the follicular fluid and the quality of oocytes retrieved was found, thus suggesting that higher follicular concentrations of myo-inositol plays a role in follicular maturity, providing, inter alia, a 'quality' marker for oocytes evaluation[51].

Meanwhile, an Italian research group headed by Unfer[52] concluded a clinical study on the use of myo-inositol in PCOS patients. Twenty-five PCOS patients were enrolled in this study and continuously administered with myo-inositol combined with folic acid twice a day. During an observation period of 6 months, ovulatory activity was monitored with ultrasound scan and hormonal profile, and the numbers of spontaneous menstrual cycles and eventually pregnancies were assessed. On the basis of the obtained results, the authors[52] proposed the effectiveness of myo-inositol in restoring spontaneous ovarian activity, and consequently fertility in PCOS patients.

These results were later confirmed during follow-up investigations[53-56], highlighting how daily supplementation with myo-inositol, besides improving hormonal profile and restoring ovulation, induces regular menses in both lean and obese PCOS patients. Interestingly, when the effect of myo-inositol on oocyte quality in PCOS patients undergoing intracytoplasmic sperm injection (ICSI) cycles was evaluated, the amount of recombinant FSH (rFSH) administered and the number of days of stimulation were found to be significantly reduced in the myo-inositol group compared to the placebo group. Furthermore, in PCOS patients treated with myo-inositol and folic acid, but not folic acid alone, reduced germinal vesicles and degenerated oocytes at ovum pick-up were observed[57].

Additionally, Rizzo et al[58] evaluated the efficacy of a treatment with myo-inositol plus folic acid plus melatonin compared with myo-inositol plus folic acid alone on oocyte quality in PCOS women who underwent IVF cycles. Their results further supported the beneficial efficacy of myo-inositol and folic acid in improving fertility and suggested that the concomitant supplementation of melatonin can ameliorate oocyte quality and pregnancy outcomes in women with poor oocyte quality history[58].

Bearing in mind the positive relationship between follicular myo-inositol levels and better oocyte quality reported by Chiu et al[51], these findings conflicted with Nestler's theory that hypothesized a decreased myo-inositol to D-chiro-inositol

epimerase activity as a crucial factor in PCOS pathogenesis. Indeed, on the basis of Nestler's theory, the administration of myo-inositol would be expected to be ineffective in PCOS patients. Furthermore, it was still unclear why D-chiro-inositol, apparently so effective in preliminary studies[45,46], resulted to be ineffective at higher doses[47]. To solve this paradox, the effects of myo-inositol and D-chiro-inositol on oocyte quality in euglycemic PCOS patients were compared. Results showed that the total number of oocytes retrieved did not differ in the two treatments groups. However, the number of mature oocytes was significantly increased and the number of immature oocytes decreased in the myo-inositol compared to the D-chiro-inositol group. Furthermore, myo-inositol-treated patients showed an increase in the mean number of top quality embryos and in the total number of pregnancies compared to D-chiro-inositol-treated patients[59]. These findings can be explained by the fact that, unlike tissues such as muscle and liver, ovaries never become insulin resistant[60-62]. Therefore, the authors speculated that PCOS patients with hyperinsulinemia likely present an enhanced myo-inositol to D-chiro-inositol epimerization in the ovary[63]; this would result in an increased D-chiro-inositol/myo-inositol ratio (i.e., overproduction of D-chiro-inositol), which in turn would lead to myo-inositol deficiency in the ovary[63]. This myo-inositol depletion could eventually be responsible for the poor oocyte quality observed in PCOS patients[64]. Furthermore, it is likely that the putative myo-inositol deficiency in the ovary would also impair the FSH signaling, resulting in an increased risk of ovarian hyperstimulation syndrome for PCOS patients[63]. There is ample indirect evidence supporting this theory: it is well known that patients with elevated levels of insulin need a higher number of FSH IU when undergoing ovary stimulation protocols[65]; moreover, it has been found that myo-inositol supplementation in PCOS patients (preferably 3 months before ovary stimulation) reduces the amount of rFSH administrated during IVF cycles[57,59,66], with a direct impact on the possibility to achieve pregnancy[67]. Further support is provided by the data collected by Isabella and Raffone[68], who showed that increasing doses of D-chiro-inositol produce "ovary toxicity", characterized by a negative impact on oocyte quality, and a progressive reduction in the ovary response to FSH and negatively impacting oocyte quality. Interestingly, this negative effect was observed at the same dose of D-chiro-inositol as the one tested by Cheang et al[47].

A convincing proof arrived from the dosage of myo-inositol and D-chiro-inositol in the follicular fluid of PCOS patients vs. healthy subjects. The study demonstrated that follicular fluid from spontaneous cycles of healthy patients contains high concentrations of myo-inositol and low concentrations of D-chiro-inositol while in PCOS patients, the ratio of the two molecules is completely opposite. Therefore, such findings supported the "DCI paradox", accordingly to which "ovaries in PCOS patients likely present an enhanced myo-inositol to D-chiro-inositol epimerization that leads to a myo-inositol tissue depletion that could eventually be responsible for the poor oocyte quality characteristic of these patients"[69].

These findings explain the link between myo-inositol and FSH. Moreover, it is now clear why Nestler's results were not confirmed when higher doses of D-chiro-inositol were tested[47]: ovary of PCOS patient, very rich in D-chiro-inositol, does not longer require that molecule. On the contrary, by using D-chiro-inositol it is possible to counteract insulin-resistance (i.e., the ovary is never insulin-resistant) by reducing insulin levels which may also indirectly benefit the ovary.

One has to ask what should be the proper dose needed to ensure clinical efficacy without compromising ovarian function. To address this question, the plasma physiologic ratio of Myo/D-chiro-inositol was firstly identified, and then the clinical effectiveness of a product specifically designed according to those premises has been verified[70]. The physiological plasma ratio of the two isomers resulted approximately 40:1, and since the therapeutic dosage of myo-inositol ranges between 2 and 4 grams/die, LO.LI Pharma produced a new product containing 2 grams of myo-inositol and 50 mg of D-chiro-inositol. Modern technologies enabled manufacturing the product as soft gel capsules, which allow a comparable pharmacokinetics profile, even reducing the dose to a third of the original powder-base drug (i.e. 550 mg of myo-inositol and 13.8 mg of D-chiro-inositol, patented)[70]. From this innovative formulation scientists expected to obtain a two-fold effect: (1) an action on liver, mainly exerted by D-chiro-inositol, aimed at reducing insulinemic levels; (2) a selective effect on the ovary, where myo-inositol is thought to counteract the increased D-chiro-inositol levels, and hence re-establishing FSH sensitivity. Those results were later confirmed by Nordio et al[71], so far providing a further milestone in the promising story of inositol.

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PCOS

Myo-inositol in patients with polycystic ovary syndrome: A novel method for ovulation induction

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Abstract
Background. Polycystic ovary syndrome (PCOS) is often characterized by chronic oligo- or anovulation (usually manifested as oligo- or amenorrhea), and hyperandrogenism. In addition, 30–40% of PCOS women have impaired glucose tolerance, and a defect in the insulin signaling pathway (inositol-containing phosphoglycan mediators) seems to be implicated in the pathogenesis of insulin resistance. PCOS patients are subfertile as a consequence of such ovulatory disorders and often need drugs, such as clomiphene citrate or follicle-stimulating hormone, for ovulation induction, which increases the risk of multiple pregnancy and ovarian hyperstimulation syndrome. We hypothesized that the administration of an isoform of inositol (myo-inositol), belonging to the vitamin B complex, would improve the insulin-receptor activity, restoring normal ovulatory function.
Materials and methods. Twenty-five PCOS women of childbearing age with oligo- or amenorrhea were enrolled in the study. Ovulatory disorder due to PCOS was apparently the only cause of infertility; no tubal defect or deficiency of male semen parameters was found. Myo-inositol combined with folic acid (Inofolic®) 2 g twice a day was administered continuously. During an observation period of 6 months, ovulatory activity was monitored with ultrasound scan and hormonal profile, and the numbers of spontaneous menstrual cycles and eventually pregnancies were assessed.
Results. Twenty-two out of the 25 (88%) patients restored at least one spontaneous menstrual cycle during treatment, of whom 18 (72%) maintained normal ovulatory activity during the follow-up period. A total of 10 singleton pregnancies (40% of patients) were obtained. Nine clinical pregnancies were assessed with fetal heart beat at ultrasound scan. Two pregnancies evolved in spontaneous abortion.
Conclusion. Myo-inositol is a simple and safe treatment that is capable of restoring spontaneous ovarian activity and consequently fertility in most patients with PCOS. This therapy did not cause multiple pregnancy.

Keywords: *Myo-inositol, polycystic ovary syndrome, ovulation induction*

Introduction

Polycystic ovary syndrome (PCOS) is a medical condition that causes irregular menstrual cycles, chronic anovulation most often manifested as oligo- or amenorrhea, and androgen excess, with the typical ovarian ultrasound features [1]. It is the most common cause of ovulatory disorders and female infertility, and affects approximately 6–10% of women in childbearing age [2]. However, its pathogenesis is still poorly understood.

Recently, many investigators have focused on the impaired glucose tolerance that affects 30–40% of patients with PCOS [3]. Insulin plays a direct role in the pathogenesis of hyperandrogenemia in PCOS, acting synergistically with luteinizing hormone to enhance the androgen production of theca cells [4]. An inositol phosphoglycan molecule containing D-chiro-inositol (DCI) is known to have a role in activating enzymes that control glucose metabolism [5]. Indeed, a defect in tissue availability or altered metabolism of DCI or inositol phosphoglycan

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mediators has been found in PCOS women and may contribute to their insulin resistance [6,7].

Isoforms of inositol belong to the vitamin B complex. Epimerization of the six hydroxyl groups of inositol results in the formation of up to nine stereoisomers, including myo-inositol (MI) and DCI. MI is widely distributed in nature whereas DCI, the product of epimerization of the C1 hydroxyl group of MI, is relatively rare [8].

Elevated concentration of MI in human follicular fluid appears to play a role in follicular maturity and provides a marker of good-quality oocytes [9]. Furthermore, experiments on mouse oocytes showed that supplementation of MI in the culture medium increased meiotic progression of germinal vesicles by enhancing the intracellular $Ca2^+$ oscillation [10].

Thus we hypothesized that the administration of MI, a precursor of DCI, would improve insulin activity and restore ovulatory function and fertility in amenorrheic women with PCOS.

Materials and methods

A total of 25 women, 28 to 38 age years of age, with PCOS defined by oligo- or amenorrhea (six or fewer menstrual cycles during a period of 1 year), hyperandrogenism (hirsutism, acne or alopecia) or hyperandogenemia (elevated levels of total or free testosterone) and typical ovarian features on ultrasound scan, were enrolled in the study.

All patients attended our IVF Unit for infertility that had lasted for more than 14–16 months. Other medical conditions causing ovulatory dysfunction, such as hyperprolactinemia or hypothyroidism, or androgen excess, such as adrenal hyperplasia or Cushing's syndrome, were excluded by hormonal tests. All women underwent assessment of tubal patency and all male partners were evaluated with two different semen sample analyses, without finding any defect. Anovulation was ascertained by weekly plasma progesterone concentration < 2.5 ng/ml. Thus, at the end of diagnostic procedures, it was determined that the most likely cause of the couple's subfertility was ovulation dysfunction only.

PCOS women were treated orally with MI 2 g plus folic acid 200 μg (Inofolic®; Loli Pharma, Rome, Italy) as soluble powder, twice daily, continuously, until the end of the study or a positive pregnancy test. Patients were instructed to register their menstrual bleeding throughout the follow-up period of 6 months. Furthermore, in order to evaluate the restoration of spontaneous ovarian activity, weekly determination of serum progesterone and testosterone levels, as well as transvaginal ultrasound scan documenting the presence of follicular growth or luteal cyst, were performed after the first menstrual cycle. Pre- and post-treatment hormone

concentrations were statistically compared using the two-tailed *t* test.

Moreover, eventual pregnancies were confirmed by a positive test for plasma β-human chorionic gonadotropin and ascertainment of a fetal heart beat on ultrasound scan.

Results

Baseline clinical and biochemical features of the PCOS patients are reported in Table I. The outcome of treatment is shown in Tables I and II.

After a mean of 34.6 ± 5.5 days of MI administration, 22 out of the 25 women (88%) had a first menstrual cycle. Eighteen of these 22 patients presented monthly menstruations during the follow-up period. All of them maintained spontaneous ovulation activity, documented by follicular growth and increased serum progesterone concentrations in the luteal phase (mean 10.5 ± 1.8 ng/ml). Furthermore, after treatment with MI, these women showed significantly decreased concentrations of serum total testosterone (95.6 ± 8.5 vs. 45.2 ± 6.7 ng/dl; $p = 0.003$) and free testosterone (1.0 ± 0.8 vs. 0.38 ± 0.1 ng/dl; $p = 0.005$). The length of successive cycles was improved to 31.7 ± 3.2 days.

Two out of the 22 women showed only a follicular development on ultrasound without progesterone elevation during weekly blood sampling, while two women did not have any further ovarian activity after the first cycle.

During the observational period of 6 months a total of ten biochemical pregnancies occurred. Nine of the ten were singleton pregnancies documented at ultrasound scan, while one of them was a biochemical abortion. One out of the nine pregnancies

Table I. Clinical and biochemical features of the patients.

	Baseline	After myo-inositol
Age (years)	32 ± 4	
Body mass index (kg/m²)	28.5 ± 2.4	
Follicle-stimulating hormone (mUI/ml)	4.5 ± 2.8	
Luteinizing hormone TSH (mUI/ml)	6.3 ± 3.1	
Prolactin (ng/ml)	19.1 ± 2.7	
Thyroid-stimulating hormone	1.78 ± 0.85	
Serum progesterone (ng/ml)	1.8 ± 0.7	10.5 ± 1.8
Serum total testosterone (ng/dl)	95.6 ± 8.5	45.2 ± 6.7*
Serum free testosterone (ng/dl)	1.0 ± 0.8	0.38 ± 0.1†
Serum androstenedione (ng/dl)	230 ± 35	205 ± 28

Significant difference compared with baseline: *$p = 0.003$; †$p = 0.005$.

Table II. Outcome of treatment with myo-inositol.

No. of patients treated	25
No. of patients with menstrual cycle after treatment (% of patients)	22 (88)
No. of patients with restored monthly ovulation (% of patients)	18 (72)
No. of pregnancies	10
No. of pregnancies/no. of treated patients (%)	40
No. of pregnancies/no. patients with restored monthly ovulation (%)	55
No. of abortions (% of pregnancies)	2 (20)
Multiple pregnancy	0

evolved in a spontaneous abortion at 7 weeks of gestation. No multiple pregnancy was noted.

Discussion

PCOS is one of the most common endocrine disorders affecting women. Insulin resistance and hyperinsulinemia are strictly inherent to the phenotype of a high proportion of women with PCOS. A defect in insulin action has been suspected, particularly as consequence of a deficiency of DCI, a component of inositol phosphoglycan.

Chronic anovulation is often the main cause of infertility in patients of reproductive age. It is well known that ovulation induction is a complex issue owing to the increased risk of ovarian hyperstimulation syndrome and multiple pregnancy [11,12]. Clomiphene citrate, an antiestrogen, is the common first-choice drug in women with newly diagnosed PCOS, while insulin-lowering medications represent novel therapies for restoring spontaneous ovulation [13,14]. The efficacy of metformin is still debated, both alone and in association with clomiphene citrate [15,16]. Metformin treatment is associated with a higher incidence of side-effects such as nausea, vomiting and other gastrointestinal disturbances [17].

DCI administration increases the action of insulin in patients with PCOS, thereby improving ovulatory function and decreasing serum testosterone concentration [6,18,19]. MI, a precursor of DCI, is widely distributed in nature whereas DCI is relatively rare [7]. MI is present in human follicular fluid, where elevated concentrations appear to play a positive role in follicular maturity and provide a marker of good-quality oocytes [9]. Supplementation of MI in culture medium increased meiotic progression of germinal vesicles in mouse oocytes by enhancing the intracellular Ca^{2+} oscillation [10]. However, no data exist on therapy with MI in anovulatory women of reproductive age.

Our study demonstrated that MI oral supplementation restores spontaneous ovulation and menstrual cycles, and increases progesterone secretion in the luteal phase, in most infertile patients with PCOS. The present results are in line with other studies evaluating insulin-sensitizing agents in monotherapy or in association with clomiphene citrate [7,13,14, 16–18], suggesting the positive effect that MI plays on spontaneous ovarian activity. Furthermore, we found that MI therapy is able to reduce serum testosterone, both total and free, as already demonstrated with DCI. All pregnancies obtained in the follow-up period were singleton, and there was no increased incidence of abortion.

In conclusion, MI is a simple and safe treatment that is able to restore spontaneous fertility in most patients with PCOS.

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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

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 stirred 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/m²)	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-inositol 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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Myo-inositol in a new pharmaceutical form: a step forward to a broader clinical use

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EXPERT OPINION

Myo-inositol in a new pharmaceutical form: a step forward to a broader clinical use

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Objective: Dose-dependent side effects related to myo-inositol (MI) oral administration represent a significant shortcoming for its clinical use. Aiming to search for a pharmaceutical form able to be better absorbed, the pharmacokinetic (PK) profile of the new manufactured MI soft gelatin capsule form was evaluated and compared with the commercially available MI powder.

Research design and methods: A single-dose relative trial, consisting of four phases, was performed on 20 healthy volunteers who received different doses of MI powder and MI soft gelatin capsules. PK profiles related to the two pharmaceutical forms were obtained by analysis of MI plasma concentration, and the respective MI bioavailability was compared.

Results: The administration of MI powder and MI soft gelatin capsules resulted in a different bioavailability. MI soft gelatin capsule form showed similar PK parameters compared with three times higher doses of MI in powder form.

Conclusions: MI soft gelatin capsules displayed an improved bioavailability, allowing to substantially reduce the administered dose and to minimize the dose-dependent side effects. Considering the number of conditions in which MI supplementation is recommended, this evidence could support a broader use of MI in clinical practice.

Keywords: bioavailability, myo-inositol, PCOS, pharmacokinetics, PMDD, psychiatric disorders, soft gelatin capsules

Expert Opin. Drug Deliv. [Early Online]

1. Introduction

Myo-inositol (MI) is a precursor for many inositol-containing compounds that play critical and diverse roles in signal transduction, membrane biogenesis, vesicle trafficking and chromatin remodeling [1].

Indeed, many studies support the notion that MI is one of the precursors for the synthesis of phosphatidylinositol polyphosphates (PIPs) that are a source of several second messengers including diacylglycerol, which regulates some members of the protein kinase C family, inositol-1,4,5-triphosphate, which modifies intracellular calcium levels, and phosphatidylinositol-3,4,5-phosphate, involved in the signal transduction [1-3].

MI is a component of cell membranes and plays an important role in cell morphogenesis and cytogenesis, lipid synthesis, structure of cell membranes and cell growth. Related to all of these signaling pathways, MI regulates a variety of cellular processes including gametogenesis, fertilization, cell proliferation, cell development, secretion, contraction and neural activity [4,5].

Several trials highlighted that the administration of MI at high dose could result in mild gastrointestinal side effects such as diarrhea and nausea, which led to reduced patients' compliance [6]. Dose-dependent side effects are a strong shortcoming for MI clinical use, and in literature an incidence of about 5% gastrointestinal side effects

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Table 1. Pharmacokinetic parameters after oral administration of 0.6 g or 1.2 myo-inositol (MI) in soft gels and 2 g or 4 g of MI in powder.

Parameter	Mean (SD)					
	MI soft gels 0.6 g	MI powder 2 g	p-Value	MI soft gels 1.2 g	MI powder 4 g	p-Value
C_{max} (μmol/l)	31.5 (3.2)	36.3 (3.2)	NS	41.5 (3.5)	45 (3.5)	NS
T_{max} (min)	180 (6)	183(10)	NS	120 (7)	122 (12)	NS
$AUC_{(0-1440)}$	34809.5 (5509.3)	37452.2 (5219.9)	NS	40702.4 (6089.2)	44325.6 (6529.9)	NS

Data are tabled as mean ± SD.

$AUC_{(0-1440)}$: (μmol·min/l) area under the plasma concentration–time curve to the last measured concentration; C_{max}: (μmol/l) maximum observed plasma concentration during the 0 - 1440 min dosing interval; SD: Standard deviation; T_{max}: Time to peak concentration

is reported for doses higher than 12 g. Therefore, the development of different MI pharmaceutical forms that are able to improve the absorption rate and reduce side effects represents one of the main challenges in the clinical practice.

It is well known that oral absorption can be influenced by a variety of factors such as the physiochemical properties, formulation and dose of the compound, as well as the physiology and pathology of the gastrointestinal tract (GIT).

Nowadays, common approaches used to enhance oral absorption are based on the chemical modification of the molecule (salt, amorphous form and prodrug) and/or on the use of a vehicle in which the compound is soluble and remains soluble upon contact with the GIT environment.

More recently, the development of soft gelatin (soft gel) capsule form offered several advantages such as improving swallowability, masking odors and unpleasant taste and protecting the encapsulated compound against oxygen and light.

A soft gel is a single unit dosage form, consisting of a liquid or a semi-solid fill enveloped by a hermetically sealed shell. Its use is ideal to deliver compounds with sufficient solubility in a pharmaceutically acceptable non-aqueous vehicle, thereby removing any dissolution-rate-limiting steps and yielding to a faster, uniform and enhanced absorption [7].

Indeed, several studies were reported that highlight the advantages of this pharmaceutical form in terms of bioavailability and compliance above all.

In a recent published paper, the authors compared the clinical effectiveness of MI in premenstrual dysphoric disorder; in particular, they compared in a double blind randomized placebo controlled trial two different MI pharmaceutical forms: 12 g of MI in powder and 3.6 g of MI in soft gel capsules. After a placebo wash-out phase, the authors reported no difference in the effectiveness of these two pharmaceutical forms concerning GI side effects: indeed, no GI events were reported in the soft gel-treated group while one event was described in the powder-treated group [8].

Based on this evidence, aiming to minimize the dose-dependent side effects occurred after MI administration, we tested a new MI soft gelatin capsule formulation and we compared its bioavailability with the commercially available powder form. A single-dose relative bioavailability trial was conducted and the absorption parameters were evaluated.

2. Materials and methods

2.1 Patients and methods

The study involved 20 volunteers, 8 men and 12 women, enrolled at the AGUNCO Obstetrics and Gynecology Center (Rome, Italy). Subjects were evaluated on the basis of medical history, physical examination and laboratory screenings, and subjects who were found in poor general health were excluded. Volunteers were aged between 18 and 35 years, with a body mass index (BMI) ranging between 21 and 25 kg/m^2; additional demographic data were weight in the range of 55 – 86 kg and height 165 – 187 cm.

Before entering the trial a written informed consent was obtained from the volunteers, and the study was approved by the ethical committee.

Based on previously reported clinical studies, subjects received different doses of MI in two different pharmaceutical forms: soft gel capsule (patent pending) or powder. Pharmacokinetic (PK) parameters were evaluated based on the analysis of the MI plasma concentration. Blood samples were collected by venous puncture at pre-dose (0), and at 30, 60, 90, 120, 180, 300, 420, 540 and 1440 min post administration.

The study consisted of four different phases:
Phase I: 0.6 g of MI soft gel capsules was administrated
Phase II: 2 g of MI powder was administrated
Phase III: 1.2 g of MI soft gel capsules was administrated
Phase IV: 4 g of MI powder was administrated

Each phase was separated by a washout period of 15 days.

MI quantification was performed by Chelab Pharma Division using gas chromatography-mass spectrometry (GC-MS) analysis after extraction with organic solvents and derivatization.

Injection (1.0 μl) was performed in a split-less mode at 270°C and a capillary column Agilent 122-5532 DB-5 ms (0.25 mm × 30 m × 0.25 μm) was used. Total run-time was 15 min : oven at 70°C from 0 to 1 min; 20°C/min to 150°C; 10°C/min to 240°C; 4 min at 320°C post run. The
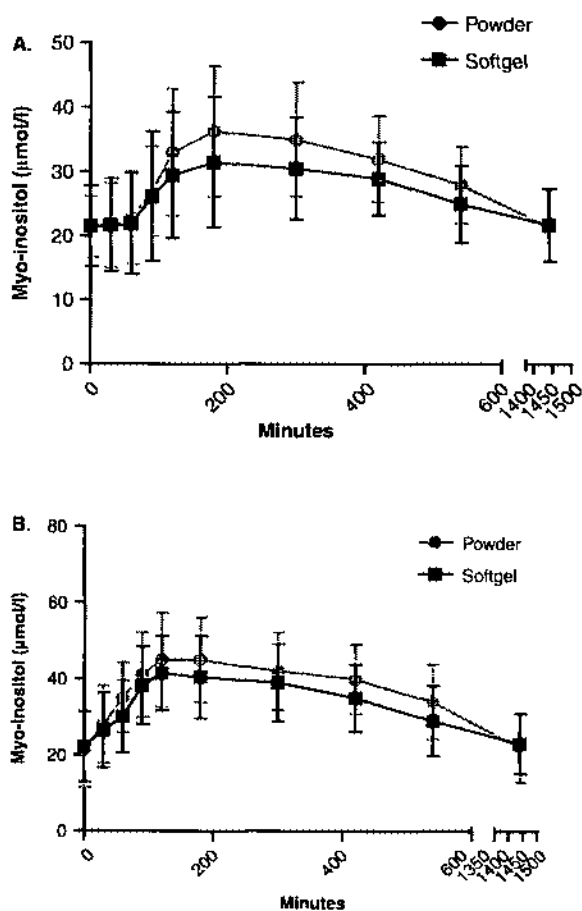
Figure 1. A. Comparison of the plasma concentration–time data profile for Phase I and Phase II (myo-inositol (MI) soft gel capsules 0.6 g and MI powder 2 g). **B.** Comparison of the plasma concentration–time data profile for Phase III and Phase IV (MI soft gel capsules 1.2 g and MI powder 4 g).

flow rate was fixed to 1.2 ml/min and results were analyzed by an MS 5973 Network Series detector in sim mode.

2.2 PK parameters

C_{max} and T_{max} were obtained directly from the plasma concentration, while area under the curve ($AUC_{(0-1440)}$) was calculated by trapezoidal method between 0 and 1440 min (see Table 1).

Data set were compared using one-way ANOVA with Bonferroni multi-comparison correction test.

3. Results

All the enrolled subjects completed the trial. The analysis of MI plasma concentration allowed us to obtain interesting results about the PK parameters of the two formulations. Data showed MI plasma concentration related to the four phases of the study.

In particular, in Figure 1A MI plasma concentrations after administration of 0.6 g of MI soft gel capsules or 2 g of MI powder were comparable; indeed, both pharmacological forms showed very similar PK parameters (Table 1). A similar result was observed after administration of 1.2 g and 4 g of MI soft gelatin capsules or MI powder, respectively (Figure 1B and Table 1).

In both cases the new MI form (soft gel capsules) was promptly absorbed showing a similar MI plasma concentration curve compared with the higher dose of MI powder.

Furthermore, there was no difference in the PK parameters between genders (data not shown).

Subjects that received MI soft capsules did not report any adverse effects while one subject reported a mild GI discomfort after administration of 4 g of MI powder.

4. Discussion

In this present study we have shown that the administration of MI in soft gelatin capsule form results in similar PK parameters as with a three times higher dose of MI in powder form.

MI is widely used in clinical practice due to its role in different cellular processes including cell proliferation, fertilization and neural activity.

Indeed, several clinical trials have already shown that MI plays a crucial role in treating PCOS symptoms such as hyperandrogenism, hyperinsulinaemia and oligo-anovulation (for review see [9]). Furthermore, a crucial role of MI in preventing the ovary hyperstimulation syndrome, during assisted reproductive technology protocol, has also been proposed [10,11].

A number of clinical trials also have suggested that MI administration provides effective results in the treatment of psychiatric disorders such as panic [12], depression [13] and more recently PMDD [8]. In order for MI to be effective, high doses (12 – 30 g) were administered occurring in mild GI discomforts, which reduced the patient's compliance [8]. The clinical use of several pharmacologically active compounds is tempered by their PK shortcomings and the dissolution in a vehicle of soft gelatin capsules often resulted in an enhanced bioavailability.

It is reported that many compounds encapsulated in soft gels are better absorbed compared with other conventional oral pharmaceutical forms. Among all, diclofenac potassium liquid-filled soft gelatin capsules were demonstrated to be more rapidly and consistently absorbed than the commercially available diclofenac potassium tablets, resulting in a shorter and more consistent time to onset of analgesia [14]. Similarly, ibuprofen in soft gel is absorbed faster than both film-coated tablet and liquid (prepared from effervescent ibuprofen tablet) [15]. In addition, cyclosporine, when administrated in soft gel, reaches therapeutic blood levels that are not achievable from the oral solution form [16]. Another example is the soft gelatin capsule containing levothyroxine dissolved in glycerin, that showed the most consistent dissolution pattern when compared with two different tablet formulations (the

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generic levothyroxine sodium by Sandoz, Inc. and Synthroid® by Abbott) [17].

Based on these evidences, MI was recently manufactured in a new soft gel capsule form.

In the present study, the PK profile of two MI pharmaceutical forms (powder and soft gel capsules) was evaluated. The concentration of the investigated molecule was measured in blood, followed by a single-dose administration to 20 healthy volunteers. The study was divided into four phases in which the subjects received different doses of MI soft gel capsules or MI powder: each phase was analyzed and the main PK parameters were calculated and compared.

Results clearly showed that despite the difference in MI dosage in the two forms, the PK of one capsule containing 0.6 g of MI was equivalent to the PK of 2 g of MI in powder. These results were confirmed also when two capsules and a double dose of MI in powder were administrated.

Therefore, the new MI form in soft gel capsule results in an improved GI absorption that allows to substantially reduce by one-third the administered dose compared to the powder form, overcoming the dose-dependent GI side effects previously described and thus improving the patients' compliance.

Nevertheless, study limitations are present. Indeed, the present results need to be confirmed on a larger and homogeneous population and a direct dosage comparison needs to be performed. However, the strength of the present paper is that we provide a possible explanation and a rationale for the clinical evidence that two different doses administered in the two different pharmacological forms have the same clinical effectiveness [8].

5. Conclusions

Development of soft gelatin (soft gel) capsules is of growing interest and several studies report the ability to perform a uniform, faster and enhanced absorption compared to other oral forms [14-17].

Dose-dependent side effects are an effective shortcoming for the oral administration of MI and the use of different pharmaceutical forms to improve the oral absorption represents one of the main challenges in the clinical practice.

Indeed, MI was recently manufactured in a new soft gelatin capsule form and in order to compare its absorption characteristics with the commercially available MI powder, a single-dose relative trial was conducted.

MI soft gelatin capsules showed an improved bioavailability that substantially reduces the administered dose, thereby representing a clinical advantage for the treatment of psychiatric disorders and other conditions in which the MI supplementation is recommended.

Declaration of interest

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Metabolic and hormonal effects of myo-inositol in women with polycystic ovary syndrome: a double-blind trial

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Abstract. – To investigate the effects of treatment with Myo-inositol (an insulin sensitizing drug), on circulating insulin, glucose tolerance, ovulation and serum androgens concentrations in women with the Polycystic Ovary Syndrome (PCOS). Forty-two women with PCOS were treated in a double-blind trial with Myo-inositol plus folic acid or folic acid alone as placebo. In the group treated with Myo-inositol the serum total testosterone decreased from 99.5±7 to 34.8±4.3 ng/dl (placebo group: from 116.8±15 to 109±7.5 ng/dl; $P=0.003$), and serum free testosterone from 0.85±0.1 to 0.24±0.33 ng/dl (placebo group: from 0.89±0.12 to 0.85±0.13 ng/dl; $P=0.01$). Plasma triglycerides decreased from 195±20 to 95±17 mg/dl (placebo group: from 166±21 to 148±19 mg/dl; $P=0.001$). Systolic blood pressure decreased from 131±2 to 127±2 mmHg (placebo group: from 128±1 to 130±1 mmHg; $P=0.002$). Diastolic blood pressure decreased from 88±1 to 82±3 mmHg (placebo group: from 86±1 to 90±1 mmHg; $P=0.001$). The area under the plasma insulin curve after oral administration of glucose decreased from 8.54±1.149 to 5.535±1.792 μU/ml/min (placebo group: from 8.903±1.276 to 9.1±1.162 μU/ml/min; $P=0.03$). The index of composite whole body insulin sensitivity (ISIcomp) increased from 2.80±0.35 to 5.05±0.59 mg-2/dl-2 (placebo group: from 3.23±0.48 to 2.81±0.54 mg-2/dl-2; $P<0.002$). 16 out of 23 women of Myo-inositol group ovulated (4 out of 19 in placebo group). Treatment of PCOS patients with Myo-inositol provided a decreasing of circulating insulin and serum total testosterone as well as an improvement in metabolic factors.

Key Words:

Polycystic Ovary Syndrome, PCOS, Myo inositol, Metabolic syndrome.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age[1-3]; its aetiology remains unknown[4]. Ovulatory disorders represent a major cause of infertility, and the oligoovulation and anovulation with polycystic ovary syndrome (PCOS) are common cause of infertility, which is the most common endocrinopathy of reproductive aged women affecting 6-10% of the population.

The current definition of PCOS requires the presence of two of the following three conditions: (i) oligo- and/or anovulation; (ii) clinical and/or biochemical signs of hyperandrogenism that may be associated with hirsutism; and (iii) polycystic ovaries – and the exclusion of other aetiologies. Other features of PCOS are acne, seborrhea, obesity, insulin resistance, impaired glucose tolerance and type 2 diabetes mellitus, dyslipidaemia, cardiovascular disease and infertility. Furthermore, endocrine and metabolic alterations as elevated serum concentrations of testosterone, insulin, luteinizing hormone (LH) and prolactin are prevalent in PCOS population. These last may have a profound implications for the long-term health of patients.

In young women with PCOS, insulin resistance may occur with high frequency. In fact many studies revealed that it is intrinsic to the syndrome and affects 30 to 40% of patients with PCOS[5]. Some studies showed that insulin resistance in the PCOS may be linked to abnormal ovarian steroidogenesis by means of altered insulin signal transduction[6,7].

Inositol phosphoglycan molecule is known to have a role in activating enzymes that control

glucose metabolism[8-10]. A defect in tissue availability or altered metabolism of inositol or inositol phosphoglycan mediators, as in PCOS women, may contribute to insulin resistance[11-12]. Isoform of inositol belongs to the vitamin B complex. Epimerization of the six hydroxylgroups of inositol leads to the formation of up to nine stereo isomers, including Myo-inositol (MYO) and D-chiro-inositol (DCI). Elevated concentrations of MYO in follicular fluid appear to play a role in follicular maturity and provide a marker of good quality oocytes[13-15]. Furthermore, experiments on mouse oocytes showed that an adding of MI to the culture increases the meiotic progression of germinal vesicle by enhancing the intracellular Ca^{2+} oscillation[16].

Women with the PCOS could be respond favourably to treatment with insulin-sensitizing drugs. Previous studies have shown that the use of metformin, troglitazone or Myo-inositol reduces serum androgens, and improves ovulation in women with the PCOS. In those studies, administration of metformin to patients showed a reduction in circulating and a decrement serum total and free testosterone concentrations[17].

A recent study outlines a deficiency of Myo-inositol in insulin resistance in women with the PCOS and the administration of Myo-inositol reduces serum insulin, decreases serum testosterone, and enhances ovulation[18].

The aim of this study was to investigate the metabolic and hormonal effects of MI in PCOS patients.

Material and Methods

This study was a double-blind trial (subjects and investigators).

42 patients, 18 to 40 years of age, were selected to study. They were PCOS affected with oligomenorrhea, high serum free testosterone level and/or hirsutism.

Women were observed by pelvic, ultrasonography and PCOS was found[19].

13 of the 42 women were taking some drugs (oral contraceptives, insulin-sensitizing agents and others) during two months before the study.

After randomization, 23 women received 4 gr of Myo-inositol plus 400 mcg of folic acid (Inofolic®) and 19 women received 400 mcg folic acid (Fertifol®) alone as placebo. The treatment was made for 12-16 weeks.

Seven women had impaired glucose tolerance (plasma glucose concentration >140 mg/dl, <200 mg/dl two hours after oral ingestion of 75 g of dextrose). Four of them was assigned to receive Myo-inositol (Inofolic®) and three were assigned to receive placebo (Folic acid only).

The study was approved by the Institutional Review Boards and each woman gave written informed consent.

When we started the study, the patients were in the follicular phase of the menstrual cycle (serum progesterone concentration lower than 2.5 ng/ml). On the first day blood pressure, weight, height, waist to hip ratio were measured. In the morning, 8:30, 8:45 and 9:00 am., sex hormone binding globulin and serum steroids were obtained. At 9:00 a.m., 75 g of dextrose were administered and plasma glucose and insulin were measured after 30, 60, 90, 120 minutes.

How to take drugs (Myo-inositol or placebo orally once a day) was explained to the patients as well as not to change usual habits both for food, sport and lifestyle.

The serum progesterone was measured weekly and if the relevant results were over 8 ng/ml the ovulation was supposed

After 6 weeks of drugs (day 49), women in the follicular phase (serum progesterone concentration <2.5 ng/ml) repeated all the baseline measurement.

Statistical Analysis

The results are reported as mean values ± SE.

The areas under the response curves by the trapezoidal rule were used to evaluate the plasma glucose and insulin concentration after the oral administration of glucose.

The oral glucose tolerance test (OGTT), by the use of the index of composite whole-body insulin sensitivity (ISIcomp), was used to determine the insulin sensitivity. This methodology was developed by Matsuda and De Fronzo[20]: ISIcomp=10.000/square root of ([fasting glucose × fasting insulin] × [mean glucose × mean during OGTT]).

To analyze the difference in ovulation rates between the women who received the Myo-inositol and those who received placebo the Fisher exact test was used. The results of the other variables were obtained by comparing the changes from to baseline to the end of the study in both the groups. The distribution of the changes in the two groups was first tested for normality with use of the Wilks-Shapiro test, and then the distri-

Table I. Baseline characteristics.

Variable	Myo-inositol N = 23	Placebo N = 19
Age	28.8 ± 1.5	27.1 ± 1.4
Waist to hip ratio	0.88 ± 0.02	0.87 ± 0.02
BMI (kg/m²)	22.8 ± 0.3	22.5 ± 0.3
Menstrual period/yr	3 ± 1	3 ± 1
Free testosterone (ng/dl)	0.85 ± 0.11	0.89 ± 0.12
Androstenedione (ng/dl)	267 ± 19	271 ± 21
DHEAS (µg/dl)	366 ± 47	384 ± 63
Total testosterone (ng/dl)	99.5 ± 6.9	116.8 ± 14.7
17 beta estradiol (pg/ml)	45 ± 2.5	70 ± 6.7
Sex hormone binding globulin (nmol/L)	144 4 ± 18.6	147 ± 14.5
Total cholesterol (mg/dl)	210 ± 10.4	195 ±7.35
Triglycerides (mg/dl)	195 ± 20.2	166 ± 20.6
ISIcomp (mg-2/dl-2)	2.80 ± 0.35	3.23 ± 0.48
Glucose AUC (mg/dl/min)	12.409 ± 686	12.970 ± 802
Insulin AUC (µU/ml/min)	8.549 ± 1.149	8.903 ± 1.276
Fasting insulin (µU/ml)	32.5 ± 4.1	30.8 ± 7.3
Fasting glucose (mg/dl)	87.6 ± 3.5	84.9 ± 5.8
Systolic blood pressure (mmHg)	131 ± 2.3	128 ± 1.3
Diastolic blood pressure (mmHg)	88 ± 1.0	86 ± 7.0

DHEAS = dehydroepiandrosterone; AUC = area under the curve during 2 hours, 75 g oral glucose tolerance test; ISIcomp = index of composite whole body insulin sensitivity.

butions were compared with each other by using the Student to-tailed unpaired or the Wilcoxon rank sum test.

P values <0.05 were considered significant.

Results

The women of the two groups were similar for baseline characteristics (age, BMI, waist to hip ratio, plasma lipids, and other) (Table 1). No significant differences were recorded in the two groups for fasting plasma insulin, plasma glucose, areas under the curve for insulin and glucose during the OGTT, and frequency of glucose tolerance.

There was a slight change in BMI in both study groups (Table II).

There was not a statistically significant modification of waist to hip ratio in both groups.

There was a decrement in systolic pressure in Myo-inositol group (from 131±2 to 127±2 mmHg) while an increment in placebo group (from 128±1 to 130±1 mmHg; P=0.002); similarly about the diastolic blood pressure, with

Table II. Anthropomorphic and lipid characteristics.

Characteristic	Myo-inositol group N = 23		Placebo group N = 19		P value for change comparison
	Baseline	After treatment	Baseline	After treatment	
Systolic blood pressure (mmHg)	131 ± 2	127 ± 2	128 ± 1	130 ± 1	0.002
Diastolic blood pressure (mmHg)	88 ± 1	82 ± 3	86 ± 7	90 ± 1	0.001
Triglycerides (mg/dl)	195 ± 20	95 ± 17	166 ± 21	148 ± 19	0.001
Total cholesterol (mg/dl)	210 ± 10	171 ± 11	195 ± 7	204 ± 9	0.001
BMI (kg/m²)	22.8 ± 0.3	22.9 ± 0.3	22.5 ± 0.3	22.4 ± 0.1	NS
Waist to hip ratio	0.88 ± 0.02	0.87 ± 0.02	0.87 ± 0.02	0.89 ± 0.01	NS

NS: not significant.

Table III. Plasma glucose and insulin sensitivity index measurements (for 6 to 8 weeks).

Characteristic	Myo-inositol group N = 23		Placebo group N = 19		P value for change comparison
	Baseline	After treatment	Baseline	After treatment	
Fasting insulin (μU/ml)	32 ± 4	26 ± 8	30.8 ± 7	38 ±7	0.20
Fasting glucose (mg/dl)	87.6 ± 4	81.6 ± 4	84.9 ± 6	88 ± 4	0.12
Glucose AUC (mg/dl/min)	12.409 ± 686	10.452 ± 414	12.970 ± 802	12.992 ± 793	0.04
Insulin AUC (μg/ml/min)	8.54 ± 1.149	5.535 ± 1.792	8.903 ± 1.276	9.1 ± 1.162	0.03
ISIcomp (mg^{-2}/dl^{-2})	2.80 ± 0.35	5.05 ± 0.59	3.23 ± 0.48	2.81 ± 0.54	< 0.002

AUC = Area under the curve during 2 hours. 75 g oral glucose tolerance test; ISIcomp = index of composite whole body insulin sensitivity.

decrement (from 88±1 to 82±3 mmHg) in Myo-inositol group and increment (from 86±7 to 90±1 mmHg) in placebo group respectively (P=0.001).

In the Myo-inositol group plasma triglycerides decreased by 52% (from 195±20 to 95±17 mg/dl) and total cholesterol decreased significantly (from 210±10 to 171±11 mg/dl).

The fasting plasma insulin concentration did not change significantly in either study group (Table III). The area under the plasma insulin curve decreased by 36% (from 8.54±1.149 to 5.535±1.792 μU/ml/min) while the same was not in the placebo group (from 8.903±1.276 to 9.1±1.162 μU/ml/min; P=0.03).

Likely was for the fasting plasma glucose concentration (from 87.6±4 to 81.6±4 mg/dl). The area under the plasma glucose curve during OGTT decreased in Myo-inositol group (from 12.409±686 to 10.452±414 mg/dl/min) while a slight increment was in placebo group (from 12.970±802 to 12.992±793 mg/dl/min; P=0.04).

The composite whole body insulin sensitivity index (ISIcomp) increased by 84% (from 2.80±0.35 to 5.05±0.59 mg^{-2}/dl^{-2}) in the Myo-in-ositol group and did not change in the placebo group (from 3.23± 0.48 to 2.81±0.54 mg^{-2}/dl^{-2}) (Table III). The change between two groups was significant (P<0.002).

Sixteen (69,5%) and four (21%) women ovulated in the Myo-inositol group and the placebo group respectively. The different is statistically significant (P=0.001).

The progesterone peak value was higher in the Myo-inositol group (15.1±2.2 ng/ml).

In the Myo-inositol group there was a decrement of serum total testosterone (from 99.5±7 to 34.8±4.3 ng/dl) and free testosterone concentrations (from 0.85±0.11 to 0.24±0.03 ng/dl) (Table IV).

An increase of serum sex hormone binding globulin was revealed for each groups (P=0.40).

There was an important decrement of the serum dehydroepiandrosterone sulphate in the Myo-inositol group (from 366±47 to 188±24 μg/dl; P=0.003) while it wasn't significant in the placebo group (from 384± 63 to 320±35 μg/dl; P=0.06).

The other serum sex steroid concentration did not change between two groups.

Table IV. Serum sex hormone (for 6 to 8 weeks).

Characteristic	Myo-inositol group N = 23		Placebo group N = 19		P value for change comparison
	Baseline	After treatment	Baseline	After treatment	
Total testosterone (ng/dl)	99.5 ± 7	34.8 ± 4.3	116.8 ± 15	109 ± 7.5	0.003
Free testosterone (ng/dl)	0.85 ± 0.11	0.24 ± 0.03	0.89 ± 0.12	0.85 ± 0.13	0.01
DHEAS (μg/dl)	366 ± 47	188 ± 24	384 ± 63	320 ± 35	0.06
SHBG (nmol/l)	144.4 ± 19	198 ± 24	147 ± 4	163 ± 26	0.40
Androstenedione (ng/dl)	267 ± 19	196 ± 26	271 ± 21	306 ± 41	0.09
Progesterone peak value (ng/ml)*	–	15.1 ± 2.2	–	6.6 ± 1.3	0.003

DHEAS= Dehydroepiandrosterone; SHBG= Sex Hormone binding globulin; *the highest progesterone concentration measured for an individual subject during the study.

Discussion

Insulin-sensitizing agents have been recently 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. In fact, almost all obese PCOS women and more than half of those of normal weight are insulin resistant, and therefore present some degree of hyperinsulinemia. For this reason the use of insulin sensitizers had been suggested in most patients with PCOS, as a treatment useful in the reduction of serum androgen levels and gonadotropins, and in the improvement in serum lipids, and prothrombotic factor plasminogen-activator inhibitor type 1. These therapies have also been associated with a decrease in hirsutism and acne, and with a regulation of menses and an improvement of ovulation and fertility.

Recently a defect in the insulin signal pathway (inositol-containing phosphoglycan mediators) had been discovered to be implicated in the pathogenesis of insulin resistance[8,12]. As consequence, the administration of different isoforms of inositol as D-Chiro-inositol (DCI) or myo-inositol (MYO) is newly demonstrated improving the physiological insulin-receptor activity, restoring spontaneous ovulatory function in most of PCOS women[14,15,18,21].

Aim of our study was to better focus on metabolic implication of a chronic treatment with MYO in PCOS patients.

We analyzed a total of 42 patients treated by Myo-inositol (N° 23) or placebo (N° 19). Myo-inositol increased insulin sensitivity, improved glucose tolerance and decreased glucose stimulated insulin release. In these patients there was a 66% decrement of serum total testosterone and 73% decrement of serum free testosterone concentrations. In addition there was a decrement in systolic and diastolic blood pressure. Plasma triglycerides and total cholesterol concentration decreased.

In women with the PCOS, insulin resistance may be related to a deficiency in Myo-inositol containing mediator of insulin action and the administration of the Myo-inositol improves insulin sensitivity.

In conclusion, Myo-inositol decreases serum androgen concentrations, reduces circulating insulin and improves glucose tolerance and other metabolic values altered associated with insulin resistance in women affected by Polycystic ovary syndrome.

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MYOINOSITOL: A REVIEW OF ITS USE IN PATIENTS WITH POLYCYSTIC OVARY SYNDROME

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ABSTRACT

Polycystic ovarian syndrome (PCOS) or stein - leventhal syndrome is one of the most common endocrine disorders affecting women of reproductive age. The diagnostic criteria of PCOS are Oligo ovulation and/or anovulation, excess androgen activity, polycystic ovaries on ultra sonogram. The estimated prevalence is 36% of women in India are suffering from PCOS and the Global prevalence is 2.2% to 26%. Recently, Myoinositol (MI) - a novel insulin sensitizer has been marketed for PCOS with infertility. Treatment with MI improves the ovarian function, oocyte quality, metabolic and hormonal parameters in PCOS. MI promotes weight loss in PCOS. It has been shown to reduce the systolic and diastolic blood pressure. MI is safe and effective drug and as such there are no side effects and drug interaction at clinically used doses.

KEYWORDS: Polycystic ovarian syndrome, Myoinositol, Ovulation and Oocyte quality, Metabolic dysfunction.

INTRODUCTION

Polycystic ovarian syndrome (PCOS) or stein - leventhal syndrome is one of the most common endocrine disorders, affecting up to 20% of women of reproductive age.[1] The exact prevalence of PCOS is not known as the syndrome is not defined precisely. The estimated prevalence in women of reproductive age is 5-10%. According to the new criteria of Rotterdam, the prevalence among the general female population will raise up to 10%.[2]

Global prevalence -2.2% to 26% roughly 1 in 15 women Worldwide, 36%of women in India are suffering from PCOS.[3]

ETIOLOGY AND PATHOPHYSIOLOGY

The increased ovarian androgen production seen in PCOS is a result of a series of complex biochemical processes which begins with disordered activity in the enzyme cytochrome P450c 17α, which catalyses 17-hydroxylase and 17/20 lyase activities.[4] the rate limiting step in androgen biosynthesis.[5] PCOS develops due to excessive luteinizing hormones (LH) by the anterior pituitary gland with increase in LH/FSH ratio and through high levels of insulin in blood and insulin resistance. Persistently high levels of LH will produce excessive amounts of androstenedione by causing increased cytochrome P450 activity. Insulin like Growth Factor-1(IGF-1) potentiates the expression of LH receptors and stimulates LH induced androgen production and the accumulation of androgens in the ovary.[6] IGF-1 also acts as an amplifier of the action of FSH.[7] There is a strong implication of gene sequences in the etiology of PCOS.[8] It is possible that a gene (CYP11A1,CYP17A1) may render the ovary susceptible to insulin stimulation of androgen secretion while blocking follicular maturation.[9] In male genetic predisposition is expressed as premature balding.

SYMPTOMS AND DIAGNOSTIC CRITERIA

PCOS is one of the leading causes of female infertility. The most common immediate symptoms are anovulation-oligomenorrhea, amenorrhea and ultrasound polycystic ovaries, excess androgenic hormones (Hirsutism, Acne, Alopecia and Seborrhea) and insulin resistance. Mood disorders including depression, anxiety, bipolar disorder and binge eating disorder also occur more frequently in women with PCOS.[10] Diagnostic criteria for PCOS have been offered by three groups.

A. The National Institutes of Health/National Institute of Child Health and Human Disease (NIH/NICHD) 199218; based on exclusion of other androgen excess or related disorders include all the following
1. Clinical and/or biochemical hyperandrogenism, 2. Menstrual dysfunction, B. The European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ ASRM)200419; based on exclusion of other androgen excess or related disorders includes two of the following 1. Clinical and/or biochemical hyperandrogenism, 2. Oligo-ovulation or anovulation, 3. Polycystic ovaries and C. The Androgen Excess and PCOS Society 200620: based on exclusion of other androgen excess or

related disorders includes all the following 1.Clinical and/or biochemical hyper-androgenism, 2. Ovarian dysfunction and/or polycystic ovaries.[11] The diagnostic criteria of PCOS is based on the 2003 Rotterdams Criteria, a. Oligo ovulation and/or anovulation, b.Excess androgen activity, c. Polycystic ovaries on ultrasonogram, after excluding other causes like congenital adrenal hyperplasia, cushings syndrome, hyperprolactinemia, thyroid disease, acromegaly and androgen secreting tumours of the ovary.[12]

PCOS among adolescents is an emerging problem that needs careful assessment, timely intervention, and appropriate treatment.[13,14] The first urban community-based study diagnosing PCOS and phenotypes among adolescent and young girls in India, showed the prevalence of PCOS among them was 22.5% by Rotterdam and 10.7% by Androgen Excess Society criteria. Non-obese were comprised 71.8% of PCOS diagnosed by Rotterdam criteria. Hyperinsulinemia was present among 19.2% of diagnosed PCOS cases. Obese girls with PCOS were more hirsute, hypertensive, and had significantly higher mean insulin and 2 hr post 75g glucose levels compared with non-obese PCOS. Moreover this prevalence is relatively higher than that reported by most studies, mainly due to use of different diagnostic criteria, study settings, age groups of the sample studied and hence cannot be compared.[13]

ASSOCIATION OF PCOS WITH OTHER CO-MORBIDITIES

Risk factors for PCOS in adults includes type 1 diabetes, type 2 diabetes and gestational diabetes. Insulin resistance affects 50%–70% of women with PCOS leading to a number of co-morbidities including metabolic syndrome, hypertension, dyslipidemia, glucose intolerance, and diabetes.[11,15,16,17] Impaired Glucose Tolerance (IGT) has been found to increase the risk of cardio vascular diseases(CVD), mortality and progression to diabetes mellitus in general population. Recent population based data noted a mortality rate of 5.5% over 5years for those with IGT versus 1.9% with normal glucose tolerance. Furthermore, lifestyle intervention, drugs like metformin and glitazones can prevent IGT progression to diabetes mellitus, strengthening the argument for early treatment of PCOS women with insulin sensitizers. Gestational diabetes have been associated with an increased prevalence of PCOS.[11]

MANAGEMENT

Treatment for PCOD includes diet control, weight loss, oral contraceptives, topical eflornithine hydrochloride for hirsutism, anti androgens, insulin sensitizing agents – Metformin, Rosiglitazone and pioglitazone and surgical drilling of ovarian cyst. Women with

PCOS could respond favourably to insulin sensitizing drugs. Previous studies have shown that the use of metformin, Pioglitazone or myoinositol reduces serum androgens, serum total and free testosterone concentrations and improves ovulation in women with PCOS. [18] Efficacy of metformin is still debated, either alone or in association with clomiphene citrate. Furthermore metformin treatment is associated with a higher incidence of side effects, such as nausea or vomiting and other gastrointestinal disturbance.[19] while the use of thiazolidinediones has been related to weight gain and more recently to cardiovascular events, fragility fractures and bladder cancer.[20]

Recently, Myoinositol (MI) - a novel insulin sensitizer has been marketed for PCOS with infertility cases. MI could be proposed as an alternative to metformin treatment because the former can affect insulin target tissues and cells and potentiate insulin effects without the side effects of metformin. Several studies have demonstrated treatment with MI is effective in reducing hormonal, metabolic and oxidative abnormalities in PCOS patients by improving Insulin Resistance.[21]

MYO-INOSITOL

Chemistry and source
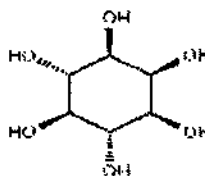
MI in 1850 Johanes Joseph Scherer (1814-1869) isolated from the muscle a hexahydroxycyclo- hexane that he named Inositol [from Ancient Greek stem ofiç (is, in-, "sinew, fiber"), -ose (in- dicating a carbohydrate), -ite ("ester"), -ol ("an alcohol")], as it formally belongs to the sugar family.[22] Inositol was found the main component of phytates, i.e. salts of the inositol hexaphosphoric acid. The discovery of phytate dates from 1855 to 1856 when Hartig first reported small round particles in various plant seeds similar in size to potato starch grains.[23] Those particles were rich in phosphorous, calcium and magnesium but without proteins or lipids. Inositol or cyclohexane-1,2,3,4,5,6-hexol is a chemical compound with formula $C_6H_{12}O_6$, a sixfold alcohol (polyol) of cyclohexane. MI is one of nine stereoisometric of a C6 sugar alcohol that belongs to vitamin B-Complex group.[24,25] Inositol is a carbohydrate, assayed at half the sweetness of table sugar (sucrose).[26] MI is the precursor of inositol triphosphate, a second messenger regulating many hormones such as

Thyroid Stimulating Hormone, Follicle Stimulating Hormone(FSH) and insulin.[27]

In 1988, Larner et al came to the conclusion that the two inositol stereoisomers.[28,20] MI and D-chiro-inositol (DCI), are chemical mediators of insulin, acting through different mechanisms. MI produces second messengers for FSH and glucose uptake, while D-chiroinositol provides second messengers for promoting glucose uptake and glycogen synthesis.[20,24] Both D-chiro-inositol and MI have similar structures, differing in the stereo-chemistry of only one hydroxyl group.[29] MI is synthesized from glucose-6-phosphate (G-6-P) in two steps. First, G-6-P is isomerised by an inositol-3-phosphate synthase enzyme to myoinositol 1-phosphate, which is then dephosphorylated by an inositol monophosphatase enzyme (called IMPase 1) to give free MI.[24] MI is a component of cell membrane and is an essential nutrient required by the human cells for the growth and survival in the culture.[27] In healthy adults, the serum concentration of MI remains within a range of 20-70 μmol/L . However, in new-born infants and fetuses, serum concentrations of MI are several times higher than in adults.[30] In humans most inositol is synthesized in the kidneys, in typical amounts of a few grams per day.[31,32] Certain studies were reported that testes, the prostate, the epididymis and seminal vesicles contain a large amount of MI.[30] The seminal fluid is one of the richest sources of inositol. In females MI is rich in ovarian fluid. MI is naturally present in a variety of plant foods. It is present in high concentration in fruits, beans, grains, and nuts. [20,24,33]

MECHANISM OF ACTION

MI is a precursor of D-chiro inositol. D-chiroinositol is synthetized by an insulin dependent epimerase that converts MI into D-chiro-inositol.

Deficiency of Inositol decreases
PI3 kinase activity
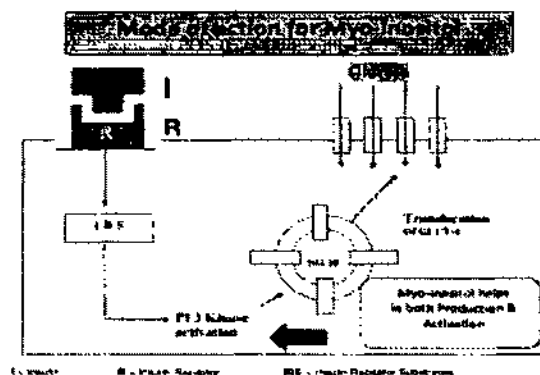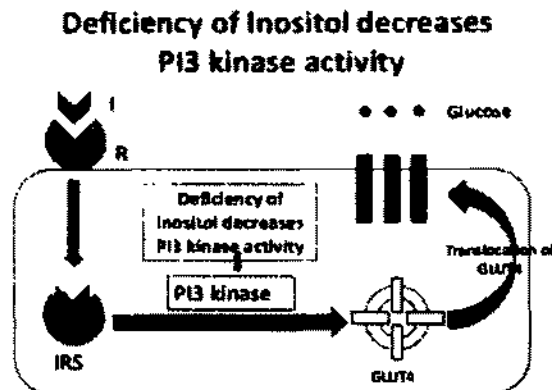
Insulin binds to its receptor forms a complex called Insulin Receptor Substrate (IRS). IRS Stimulates messenger called PI3 kinase. Activated PI3 kinase activates GLUT 4. Glucose is then taken up by GLUT4 through glucose channel for utilizing energy. Then IRS Complex breaks down releasing the receptor to go back to its original site. MI helps in both production and activation of PI3.[34]

USES

Inositol has been found to be an effective in treatment of PCOS, including insulin resistance, hyperandrogenism, and oligo-amenorrhea.[35,36] It has been implicated in insulin signal transduction.[37,38] Additionally MI supplementation improves features of dysmetabolic syndrome in post-menopausal women, including triglycerides, HDL cholesterol and diastolic blood pressure.[39] Other uses in panic disorder, Obsessive Compulsive Disorder(OCD), unipolar and bipolar depression, acute respiratory distress syndrome in premature infants and lithium induced psoriasis.[40,41] It is possibly safe for most adults sometimes it may cause nausea, tiredness, head ache, dizziness. Inositol can boost the effects of selective Serotonin Reuptake Inhibitors.[42]

DOSE, ADMINISTRATION AND DURATION

Dose is 200- 4000 mg once a day before breakfast for PCOS. MI is available in two types of dosage form one in powder form and another newer pharmaceutical preparation -soft gelatin capsule. A study of administration of MI powder and MI soft gelatin capsules resulted in a different bioavailability. Soft gelatin capsule form of MI showed similar pharmacokinetic parameters compared with three times higher doses of MI in powder form.[43] Many clinical trials underline the association between insulin tissue sensitivity increment and DCI or MI oral administration for at least 3 months.[44]

CLINICAL OUTCOMES OF THE MI ON PCOS

MI and Infertility/Ovulation and Oozyte quality

Several studies showed MI improves oocyte and embryo quality in PCOS. The prevalence of infertility caused mainly by anovulation in PCOS women varies between 35-94%.[45]Treatment with MI on PCOS with oligo, high testosterone, hirsutism cases showed, improves ovarian function and metabolic and hormonal parameters.[39,12] Another study showed in women with PCOS, 69.5% ovulated in MI group compared to 21% ovulated in placebo. Calcium signalling in oocytes has been extensively studied in various species because of inositols putative role in oocyte maturation and the early stages of fertilization.[46,47,48] and quality of the oocytes.[24,49] The presence of high levels of MI can indicate the well being of the follicle. The comparative study between MI and DCI showed, MI improved the oocyte and embryo quality than DCI in PCOS.[50] Another study showed though Myo and/or D-chiroinositol administration improves insulin sensitivity only MI is a quality marker for oocytes evaluation.[51] A study showed MI improved oocyte quality in patients undergoing Intra Cytoplasmic Sperm Insemination(ICSI), or with prior failed attempts at ICSI or diagnosed with PCOS or as poor responders.[52] Another study on patients undergoing ovulation induction for ICSI showed that, insulin lowering medications, particularly different isoforms of inositol, represent novel therapies for restoring spontaneous ovulation, with a potential positive effect also on human oocyte meiotic maturation.[53] The effects of MI in women with PCOS are well studied in a systematic review of randomized controlled trials study showed an overview on the clinical outcomes of the MI use as a treatment to improve ovarian function and metabolic and hormonal parameters in women with PCOS.[54]

In women with PCOS many ovulations are accompanied by elevated E2 and subnormal P concentrations, which may indicate a suboptimal follicular maturation and ovulation with a collection of high numbers of germinal vesicles and degenerated oocytes at ovum pick-up.[53] Its increased frequency of ovulation defined by luteal ratio, increase in ovulation rate evidenced by increase in E2 concentrations over the first week of treatment and shorter mean time to first ovulation.[55] Another study also confirmed that the association between concentration of MI with follicular volume, E_2 and better developmental of the oocytes suggests that higher level of MI in ovarian follicular fluid may be related to the wellbeing of the follicle and the quality of oocyte.[56] Study on PCOS with chronic anovulation and infertility undergoing assisted reproduction techniques showed number of follicles with a

diameter of more than 15 mm visible at ultra sound scan during stimulation and the number of oocytes retrieved at the pickup resulted significantly higher in MI group.[39]

Additionally, another study on PCOS women who underwent *in vitro* Fertilization (IVF) cycles, the efficacy of a treatment with MI + folic acid + melatonin compared with MI + folic acid alone on oocyte quality showed the beneficial efficacy of MI + folic acid in improving fertility and suggested that the concomitant supplementation of melatonin can ameliorate oocyte quality and pregnancy outcomes in women with poor oocyte quality history.[24] Furthermore, recently it was proposed as a preventing agent for folate resistant neural tube defects (NTDs).[31]

Melatonin is present in both male and female reproductive system. Increased level of melatonin in the ovarian follicular fluid and seminal fluid maintains the reproductive function. It plays role as an antioxidant and free radical scavanger which protects follicles from oxidative stress, rescuing them from atresia, leading to complete follicular maturation and ovulation and also in human seminal fluid contains melatonin, and spermatozoa express melatonin receptors; melatonin is able to stimulate flagellar motility of spermatozoa. Furthermore, it has been demonstrated that there is a direct correlation between melatonin concentrations in follicular fluid and oocyte quality.[57,58]

The dosage of MI and D-chiroinositol in the follicular fluid of PCOS patients verses healthy subjects, follicular fluid from spontaneous cycles of healthy patients contains high concentrations of MI and low concentrations of D-chiroinositol while in PCOS patients, the ratio of the two molecules is completely opposite. Therefore, such findings supported the "DCI paradox", accordingly to which "ovaries in PCOS patients likely present an enhanced MI to D-chiroinositol epimerization that leads to a MI tissue depletion that could eventually be responsible for the poor oocyte quality characteristic of these patients. Indeed, increasing DCI dosage progressively worsens oocyte quality and ovarian response.[59,60] Further support is provided by the data collected by Isabella and Raffone, who showed that increasing doses of D-chiroinositol produce "ovary toxicity", characterized by a negative impact on oocyte quality, and a progressive reduction in the ovary response to FSH and negatively impacting oocyte quality.[24]

Patients have abnormal menstruation patterns attributed to chronic anovulation. Some women have oligomenorrhea or secondary amenorrhea. Oligomenorrhea has been observed in 85-

90% of women with PCOS and as many as 30-40% of amenorrheic patients have PCOS. Dysfunctional uterine bleeding and infertility are the other consequences of anovulatory menstrual cycles. The menstrual irregularities in PCOS usually manifest around the time of menarche.[61] Several studies have reported MI capable of restoring spontaneous ovarian activity and consequent fertility[53] and improves ovulatory function.[35,62,53] A Study on PCOS less than 35years showed both metformin and MI can be considered as first line treatment for restoring normal menstrual cycles.[63]

MI and Pregnancy

Generally PCOS patients are subfertile as a consequence of ovulatory disorders and often need drugs, such as clomiphene citrate or follicle stimulating hormone, for ovulation induction, which increases the risk of multiple pregnancy and ovarian hyperstimulation syndrome. But treatment with MI therapy did not cause multiple pregnancy.[62]

A 2-year, prospective, randomized, open-label, placebo-controlled study was carried out in pregnant women with a family history of type 2 diabetes who were treated with 2 g MI plus 200 μg folic acid twice a day may reduce the incidence of Gestational Diabetes Mellitus and the delivery of macrosomia fetuses.[64]

MI and Insulin resistance

Several studies have reported that insulin resistance is common in PCOS women, regardless of the body mass index.[65] The prevalence of insulin resistance in PCOS ranges from 50%–70% and occurs Independent of obesity. The effect of obesity on insulin resistance is additive to that of PCOS.[66,67] MI increases whole body insulin sensitivity index[46] and a 12weeks study on PCOS showed improved glucose to insulin ratio and Homeostatic Model Assessment (HOMA) index[68] decreased serum free testosterone concentration, Dehydroepiandrosterone-Sulfate (DHEA-S) and increased Sex hormone-binding globulin(SHBG).[35,46,68] and reduced LH/ FSH ratio.[68] Some leading researchers conclude, "Myoinositol administration is a simple and safe treatment that ameliorates the metabolic profile of patients with PCOS, reducing hirsutism and acne"[69] through its actions of decreased testosterone and insulin levels, the participants who supplemented with MI experienced a reduction in hirsutism, and improvements in skin appearance. A 12 weeks study with 2 grams of MI + 200 mcg folic acid showed improved insulin sensitivity, androgen levels [70] and loss of weight.[55] A study on insulin resistant women with the PCOS showed oral administration of D-chiroinositol would improve insulin sensitivity.[35]

Recently a scientific literature mentioned a new molecule: the N-acetyl- cysteine (NAC) - a mucolytic drug acting as insulin sensitizer, represents an effective and safe strategy in the treatment of PCOS patients. This molecule appears to exert its beneficial effect both by increasing the insulin secretion by the beta cells of the pancreas and by inducing an increased sensitivity to the organism itself. A study conducted to evaluate the efficacy of NAC + Inositol + folic acid on ovulation rate and menstrual regularity in PCOS patients with and without insulin resistance suggested inositol and NAC may have additional noninsulin-related mechanisms of action that allow achieving benefits also in those patients with negative HOMA-index.[71]

Another study showed administration of MI on PCOS with micro polycystic ovaries at USG showed LH, prolactin, insulin levels and LH/FSH were reduced, insulin sensitivity results improved and menstrual cyclicity restored.[70]

MI on Obesity

Obesity is common in women who have PCOS. Obesity is present in nearly half of all women with PCOS, ranging from 30% to 60%.[72] Presence of obesity is a risk factor to amplify the consequences of PCOS and metabolic dysfunction like insulin resistance.[2] An increased early clinical and subclinical markers of atherosclerosis like endothelial dysfunction, impaired pulse wave velocity, increased carotid intima media wall thickness, presence of carotid plaque and increased coronary artery calcification observed in PCOS women are further exacerbated by obesity.[73] Previous studies showed that MI reduces plasma triglycerides, total cholesterol.[46,35] LDL cholesterol and increased HDL cholesterol.[55] Women ischemia Evaluation Study(WISE) highlighted that PCOS women undergo through an increased number of cardiovascular events. The combined therapy MI plus D-chiro-inositol improves the metabolic profile of PCOS women by significantly reducing total cholesterol, LDL, triglycerides, fasting insulin, fasting glucose and HOMA index and significantly increased HDL level, therefore reducing the cardiovascular risk.[73] and weight loss in MI group by reducing circulating leptin level.[55] But in another randomized controlled trial combined therapy with MI and D-chiro-inositol was more effective to reduce the risk of metabolic disease in PCOS in overweight patients compared to MI supplementation alone, after three months of treatment.[74] Treating women with MI has been shown to reduce systolic and diastolic blood pressure.[46,35]

Reactive oxygen species (ROS) has been considered to play a critical role in the success of different artificial reproduction techniques. ROS are produced within the follicle, especially during the ovulatory process, and it is believed that oxidative stress may be a cause of poor oocyte quality. High levels of oxidants, as H_2O_2, as been found in fragmented embryos.[75] Oxidative stress is involved in the pathogenesis and future complications of PCOS. This condition occurs when reactive oxygen species (ROS), which are intermediaries of a normal oxygen metabolism, are produced faster than the endogenous antioxidant defense systems can neutralize. Oxidation can lead to inter- and/or intramolecular cross-linking, thus inducing protein degradation, clustering and enzyme inactivation. A study on MI administration reduces oxidative stress in erythrocytes of patients with polycystic ovary syndrome. Previous studies have demonstrated that hyperglycemia increases ROS generation from peripheral blood leukocytes. The resulting oxidative stress may contribute to a pro-inflammatory state that induces IR and hyperandrogenism in women with this disorder and also increases the risk of cardiovascular disease.[21]

Safety

Several previous Studies suggested MI is a safe and effective drug. The safety data of the MI trials report mild side effects such as, nausea and one of flatus and mild insomnia only at 12 g/day or higher. Notably the dosage of 4 g/day of inositol commonly used in clinics is completely free of side effects.[31] Another study on pharmacokinetics and safety of a single intravenous dose of MI in preterm infants suggest supplemental inositol is safe and beneficial for preterm infants with respiratory distress. So for there is no evidence for MI drug interaction.[76]

A study on pharmacokinetics of inositol phosphates are synthesized from the parent molecule inositol, with daily dietary consumption of inositol estimated at one gram. Once inositol reaches the cells of the intestinal tract, it is phosphorylated to create inositol hexaphosphate, and then subsequently dephosphorylated to its lower forms (IP1-5), which play important roles in signal transduction. Independent of the route of administration, MI has been found to be absorbed almost instantaneously, transported intracellular and dephosphorylated into lower inositol phosphates. MI can reach targeted tumor tissue as early as one hour post-administration.[32]

Agents that affect the PI3K/AKT/mTOR pathway have potential as chemotherapeutic agents. Evidence shows that metformin and derivatives of MI also inhibits PI3K/AKT/mTOR

signaling. *In vitro* studies have confirmed that MI specifically inhibits this pathway in lung-cancer cell lines. In addition, the activity of AKT and PI3K has been evaluated in the patients who had received MI, and regression of dysplasia correlates with decreased PI3K activity. Combining inositol with budesonide, dexamethasone, N-acetyl cystine and/indole 3 carbinol increased efficacy even further. In several studies, oral inositol inhibited lung tumorigensis in mice exposed to carcinogen.[77]

MI deficiency in the ovary would impair the FSH signaling, resulting in an increased risk of ovarian hyperstimulation syndrome in PCOS patients. It is well known the patients with elevated levels of insulin need a higher number of FSH IU when undergoing ovary stimulation protocols. The physiological ratio of these two isomers is 40:1 (MI/DCI) and seems to be an optimal approach for the treatment of PCOS disorders. In order to ensure the proper dose and clinical efficacy without compromising ovarian function, certain modern technologies enabled manufacturing the product as soft gel capsules, by reducing the dose to a third of the original powder- base drug. From the above innovative formulation scientists expected to obtain a two-fold effect: 1. An action on liver, mainly exerted by D-chiroinositol, aimed at reducing insulinemic levels; 2. A selective effect on the ovary, where MI is thought to counteract the increased D-chiroinositol levels, and hence reestablishing FSH sensitivity.[24]

Development of soft gelatin capsules is of growing interest and several studies report the ability to perform a uniform, faster and enhanced absorption compared to other oral forms.[78,79]

CONCLUSION

In conclusion, by analyzing various studies on MI supplementation and combination with other drugs in the management of PCOS and insulin resistance cases suggested, MI is a safe and effective drug. It gives positive effect on the reproductive axis, other metabolic and hormonal parameters in women with PCOS. Limited studies are available regarding prevalence of PCOS in India, on MI- a novel insulin sensitizing agent. No study had been made till now pertaining to supplementation of inositol on PCOS treatment. It is clear that the underlying pathophysiology of PCOS is not fully understood. Further, as per the statement given by our Indian authors' PCOS is an emerging disorder during adolescence, screening and early intervention is necessary to improve the reproductive health of adolescents and prevent future morbidities. Hence, further studies are needed to explore the

prevalence, etiology, pathophysiology of PCOS, drug efficacy, safety, mechanism of action other than improving insulin sensitivity of target tissues in PCOS and risk associated PCOS cases.

With the present review, we aim to provide an overview on the clinical outcomes of the MI use as a treatment to improve ovarian function and metabolic and hormonal parameters in women with PCOS.

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The Rationale of the Myo-Inositol and D-Chiro-Inositol Combined Treatment for Polycystic Ovary Syndrome

Simona Dinicola, PhD[1], Tony T. Y. Chiu, PhD[2], Vittorio Unfer, MD[3], Gianfranco Carlomagno, PhD[3], and Mariano Bizzarri, PhD, MD[1]

Abstract

PCOS is one of the most common endocrine disorders affecting women and it is characterized by a combination of hyper-androgenism, chronic anovulation, and insulin resistance. While a significant progress has recently been made in the diagnosis for PCOS, the optimal infertility treatment remains to be determined. Two inositol isomers, myo-inositol (MI) and D-chiro-inositol (DCI) have been proven to be effective in PCOS treatment, by improving insulin resistance, serum androgen levels and many features of the metabolic syndrome. However, DCI alone, mostly when it is administered at high dosage, negatively affects oocyte quality, whereas the association MI/DCI, in a combination reproducing the plasma physiological ratio (40:1), represents a promising alternative in achieving better clinical results, by counteracting PCOS at both systemic and ovary level.

Inositols and their derivatives (salts, phosphates, and associated lipids) are found in many foods, especially fruits and beans. In plants, inositol (INS) is generally represented in the form of hexaphosphate, and phytic acid or its salts (phytates).[1] Inositol is a hexahydroxycyclohexane, chemically represented by a stereo isomeric family of 9 inositols, among which myo-inositol (MI) is the most widely distributed in nature.

INS was once considered as a member of the vitamin B complex, however, it cannot be considered a "true" essential nutrient, in order that the human body can synthesized it from glucose.[2] Indeed, INS is synthesized by both prokaryotic and eukaryotic cells, even if in mammals it is mainly obtained from dietary sources, as well inositol-6-phosphate. Within the cells INS is put in its free form or as phosphatidylinositol (phosphoinositides, PtdIns). Phosphatidylinositol can be phosphorylated to form phosphatidylinositol phosphate (PIP) and biphosphate (PIP2), which fulfill several relevant physiological roles.[3]

INS is basically incorporated into cell membranes as phosphatidyl-myo-inositol, the precursor of inositol triphosphate (Ins-1,4,5P3, InsP3), which acts as second messenger, regulating the activities of several hormones such as FSH, TSH, and insulin.[4] In addition, INS serves as an important component of the structural lipids phosphatidyl-inositol and its various phosphorylated derivatives, the phosphatidyl-inositol phosphate lipids.[5] The INS derivative inositol-3-phosphate is a second messenger formed by phospholipase-C (PLP-C) mediated cleavage of phosphatidyl-inositol-4,5-phosphate (PI-4,5-P2, PIP2), when cells are stimulated by growth factors or other hormones.[6] Following interaction of Ins-1,4,5P3 with its mitochondria-coupled receptors, INS derivatives participate in calcium regulation and further activate several protein phosphorylation processes via protein kinase C (PKC) (Figure 1).

In addition, membrane-bound phosphoinositides (glycosyl-phosphatidylinositol, GPI) anchors various proteins to the plasma membrane. Approximately 150 different GPI-anchored proteins have been identified, they belong to different molecules' families. Two

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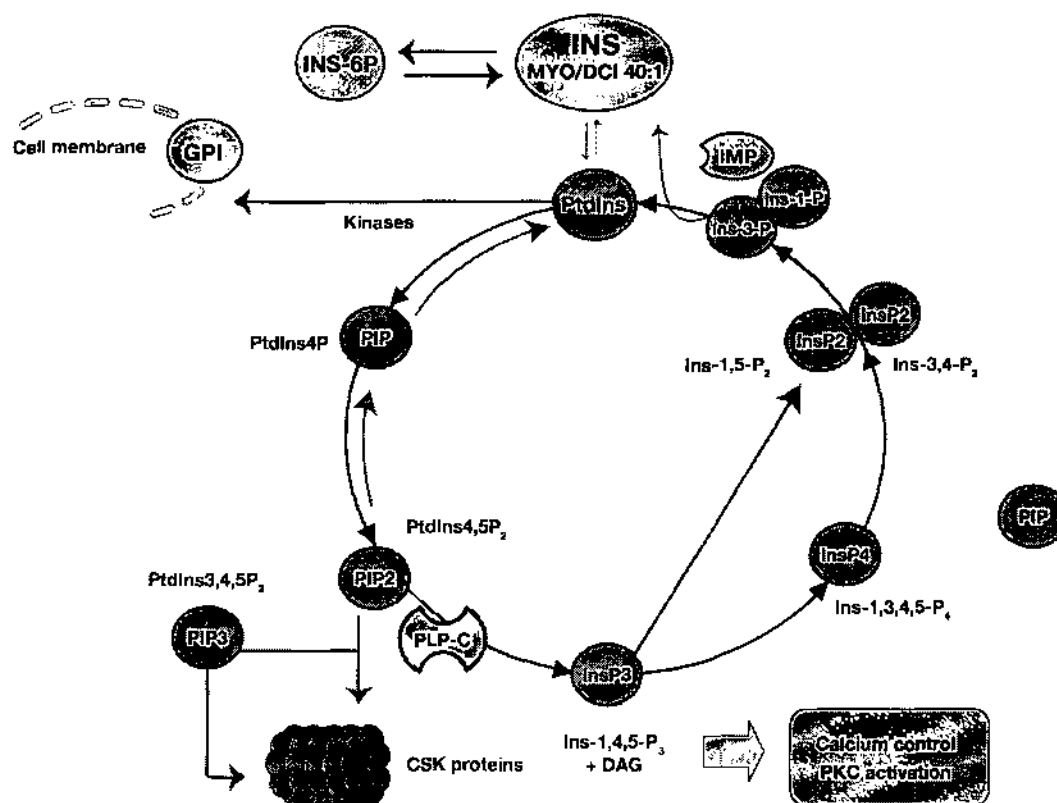Email: g.carlomagno@lolipharma.it

Figure 1. Intracellular inositol (INS) biochemical pathways. Ins-6-P, inositol exaphosphate (phytic acid); MYO, myo-inositol; DCI, D-chiro-inositol; PtdIns, phosphoinositides, phosphatidylinositol; PIP, PtdIns4P, phosphatidylinositol-4-phospate; PIP2, PtdIns4,5P2, phosphatidylinositol-4,5-biphospate; PIP3, PtdIns3,4,5P3, phosphatidylinositol-3,4,5-biphospate; GPI, glycosyl-phosphatidylinositol; PLP-C, phospholipase-C; CSK, cytoskeleton; InsP3, Ins-1,3,4-P3, inositol-1,3,4-phosphate; DAG, Di-acyl-glycerol; InsP4, inositol-1,3,4,5-phosphate; InsP2, inositol-1,5-phosphate and inositol-3,4-phosphate; Ins-3-P, inositol-3-phosphate; Ins-1-P, inositol-1-phosphate; IMP, inositol monophosphate phosphatise; PKC, protein kinase C.

distinct inositol phosphoglycan (IPG) putative mediators of insulin action have been separated and purified from GPI-proteins anchored on the extracellular side of cell membrane.[7,8] IPG-A (inositol phosphoglycan-AMP kinase inhibitor) containing MI and glucosamine, and IPG-P(Inositol phosphoglycan-phosphatase simulator) are constituted by D-chiro-inositol (DCI) and galactosamine.[9] Convincingly data obtained from different experiment suggest that IPGs are released in the extracellular space[10] and they hence actively re-enter the cell membrane through an ATP-dependent transport.[11]

Whereas intracellular INS pool is almost (>99%) constituted by MI in most tissues, significant differences have been noticed in the concentration of MI and DCI in fat, muscle and liver.[12] This different distribution reflects the distinct function, the two isomers are likely to play in those tissues, and their respective proportions are actively maintained as MI is enzymatically transformed into DCI through a NAD, NADH-dependent epimerase, accordingly to tissue requirement.

Biological Function of Inositol and Its Derivatives in Oocyte Biology

Increasing evidence indicates that INS, by itself or through its derivatives, plays a relevant role in several critical biochemical pathway including morphogenesis, cytoskeleton rearrangement, glucose metabolism, regulation of cell proliferation and fertility.[13,14]

MI is thought to exert a pivotal role namely in oocyte and spermatozoa development, as well as during fertility-related processes.[15]

The organs of the male reproductive tract are particularly rich in free MI.[16] High concentrations have been confirmed in the testis, epididymal, vesicular, and prostatic fluids of the rat.[17,18] Rat studies reveal that the MI concentration of the uterus and ovaries are under hormonal control. The INS concentration of female reproductive organs is much higher than in blood serum owing to their ability to concentrate MI from the blood stream.[19] Interestingly, the INS concentration in the uterine fluid was much lower than in seminal fluid of the

rat; it means that the surrounding MI concentration for the sperm is lowered when entering the uterus. These high MI concentrations in the male and female reproductive tracts suggest that inositol concentration in physiological fluids may significantly influence fertility. As far as results obtained on animal experimental models can hardly be transferred to human beings. Such data have been partially observed also in human studies and a clear-cut correlation has been found between MI serum levels and the outcome of pregnancy in vitro fertilization (IVF) treated patients: MI levels increased significantly during in IVF treatment when they are compared to natural cycle[20]; additionally, in the same setting, the embryo-trophic properties of the sera were examined by a post-implantation mouse embryo culture showing a strong correlation with IVF outcome.

Indeed, MI is essential in ensuring proper oocyte maturation. MI and PLP-C mediated InsP3 release and LH/FSH activity[21,22]; furthermore, through specific Ins-1,4,5P3 receptors (IP$_3$-R), inositol-3-phosphate participates in modulating intracellular Ca^{2+} release from mitochondria. In oocytes that mechanism involves a specific receptor subtype (IP$_3$-R1),[23] and it seems to play a pivotal role in oocyte maturation, namely during the final stages of oogenesis, when oocyte sensitivity to calcium fluctuations reaches the maximal value. Indeed, calcium release from rat oocytes is triggered by Ins-1,4,5P3 injection, leading then to oocyte maturation.[24] Moreover, a supplementation with MI can promote meiotic progression into fertilization-competent eggs, whereas depletion of MI intracellular stores desensitizes inositol-related pathways, reducing InsP$_3$ and releasing[25] proper calcium. Seemingly to what happen in the oocyte, in the zygote as well Ca^{++} oscillations may play a relevant physiologic role.

MI absorption into embryos is an ATP-dependent process,[26] leading to incorporation of INS into PtdIns and inositol phosphates[27]; as such, MI and its derivatives enhance bovine blastocyst development from in vitro culture with medium supplemented with MI[28] and actively participate in embryogenesis.[29] Unfortunately, it is not possible to drawn firm clinical conclusions, since the culture media were not the one used in the clinical settings. Yet, in "Colazingari et al," a new published paper, more relevant evidence is provided on the effect of MI in improving IVF outcomes. Indeed, by employing media routinely used into clinic and applying the protocol for embryo culture used for batch release, authors were able to demonstrate that culturing embryos in media enriched with MI, embryos have a more physiological cleavage rate and an increased number of expanded blastocyst formed by an higher number of blastomeres.[30]

InsP$_3$ receptors are indeed over expressed during the early stages of zygote development, and calcium fluctuations occurring in the cleavage stage of the embryo are likely to influence the pre-implantation embryo development.[31] Furthermore, it has been demonstrated that the proportion of fertilized oocytes with 2PN, the number of 2-cell stage embryos developed and the percentage of normality of the post-implantation embryos were significantly higher when germinal vesicles were cultured in a maturation medium containing MI compared with control medium. Moreover, phosphorylated derivatives of INS (Ins-1,4,5P3) participate in cytoskeleton regulation[32,33] and are required to accelerate oviductal transport of oocytes[34] (Figure 2). Moreover, a very preliminary report indicates that MI modulates the anti-mullerian hormone (AMH) serum levels.[35] AMH belongs to the Tgf-β superfamily and it is releases after FSH stimulation by the granulosa cells. In turn AMH decrease oocyte sensitivity to FSH and participates in regulating follicle maturation.[36] Indeed, poor serum AMH levels are considered a marker of diminished ovarian reserve (DOR).[37] It is worth of noting that MI supplementation significantly enhances AMH serum levels in patients affected by DOR, and then increase the likelihood of pregnancy.

Inositol is also needed to afford normal developmental processes. Fetuses require INS during gestation and have been proven to be able in concentrating it from maternal blood. At midgestation the MI concentration in mixed-umbilical cord serum was fivefold higher than the maternal serum concentration. At term, the serum MI concentration of the neonates had decreased, but it was still two- to threefold higher than in maternal blood.[38] Yet, MI promotes differentiation of the fetal lung[39] and prevents neural tube defects.[40] Given that MI uptake from embryonic cells is competitively inhibited by glucose, it has been suggested that congenital malformations, especially of the central nervous system and the heart, observed with high frequency in infants of diabetic mothers,[41] could be ascribed to hyperglycemia-induced tissue specific shortage of MI.[42,43] On the contrary, MI supplementation has been shown to reduce the birth prevalence of neural tube defects in streptozotocin induced diabetic Sprague–Dawley rats and Curly tail mice. A similar effect could be expected in human subjects.[44]

Inositol and Glucose Metabolism in PCOS Patients

Overall such results advocate a relevant physiological role sustained by INS and its metabolites in human reproduction, as claimed by "Beemster et al" seminal paper.[45] Namely, during the last decades, INS supplementation has been proposed as a novel treatment in women affected by polycystic ovary syndrome (PCOS).
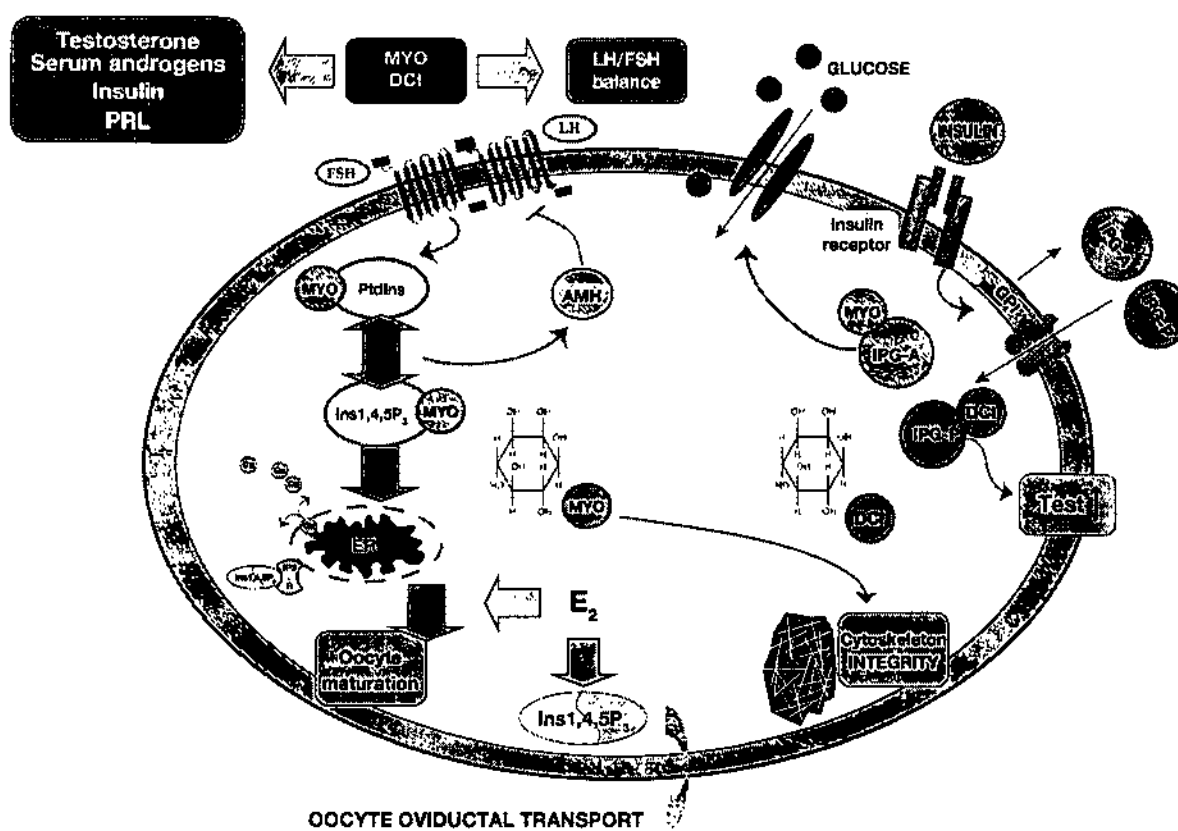
Figure 2. Inositol functions in the oocyte. E2, estradiol; Ins-1,3,4-P3, inositol-1,3,4-phosphate; IPG-P, inositol phosphoglycan-phosphatase simulator; IPG-A, inositol phosphoglycan-AMP kinase inhibitor; PtdIns, phosphoinositides, phosphatidylinositol; IP3-R, Inositol-1,3,4-phosphate-receptor; ER, endoplasmatic reticulum; CSK, cytoskeleton; MYO, myo-inositol; DCI, D-chiro-inositol; AMH, anti-mullerian hormone; GPI, glycosyl-phosphatidylinositol.

The early impetuses for these studies rely on the well-known correlation in between metabolic syndrome and PCOS, as well as the observed defects in INS metabolism in PCOS and the implication of INS in insulin signal transduction. It is widely acknowledged that both insulin insensitivity and metabolic syndrome are prominent features in a consistent proportion of patients affected by PCOS;[46–49] a disease characterized by chronic anovulation, hyperandrogenism, dyslipidemia, and infertility that affect barely 10% of women of reproductive age.[50] It is worth of noting that insulin signaling pathways involve inositol phosphoglycans. When insulin binds to its receptor, two distinct inositol phosphoglycans (IPG) are released by hydrolysis of glycosyl-phosphatidylinositol lipids located at the outer leaflet of the cell membrane. IPGs are then internalized and they affect intracellular metabolic processes, namely by activating key enzymes that control the oxidative and non-oxidative metabolism of glucose.[7]

Indeed, phosphoglycans formed by DCI (IPG-P) seem to play a relevant role in insulin signaling transduction and seems to be more effective in partially restoring insulin sensitivity and glycogen synthesis than phosphoglycan incorporating MI.[8]

Yet, MI supplementation has demonstrated to significantly improve features of dysmetabolic syndrome, including insulin sensitivity, impaired glucose tolerance, lipids levels and diastolic blood pressure.[51] In addition, it has been noticed that MI improve glucose tolerance in rhesus monkeys,[52] meanwhile Schofeld and Hackett demonstrated that MI-containing IPG from *P. falciparum* also had insulin-like effects in vitro and in vivo.[53] At least in part, such results could be explained by the transformation of MI to DCI occurring in peripheral tissues. Indeed, insulin resistance has been associated to reduced availability of DCI, documented by decreased urinary excretion of IPG-P in both animals[54] and diabetic patients,[55] and by lowered DCI levels in muscle from type 2 diabetes individuals.[55,56] In turn, hyperglycemia was reduced in diabetic rats or monkeys suffering from insulin resistance, by supplementing the diet with DCI.[7] Such discrepancies could presumably be explained considering

that while DCI is crucial for glycogen synthesis, MI increases glucose cellular uptake.[57]

On the other hand, it is well recognized that increased insulin sensitivity in PCOS patients by means of conventional antidiabetic drugs promotes a significant improvement in the ovulatory function and decreases serum androgen concentrations.[58–60] It is worth noting that Metformin increases the insulin stimulated release of DCI-phosphoglycans, thus evidencing the antidiabetic drug may enhance insulin sensitivity by restoring an inositol-based signaling.[60] In support of this hypothesis it was further documented that PCOS patients show increased DCI urinary clearance and consistent DCI urinary loss, presumably leading to a tissue depletion of DCI-phosphoglycans.[61] Once more, these data provided hints in hypothesizing a direct correlation between the availability of inositol phosphoglycans and insulin resistance, even if further studies are warranted in order to fully elucidate the mechanistic pathways linking inositol-derivatives to glucose metabolism.

Inositols and Ovary Function

Observations relating INS to glucose metabolism provided impetus to ascertain the usefulness, whether any, of DCI supplementation in PCOS clinical management. In his seminal paper, Nestler et al[62] demonstrated that in women with PCOS, DCI treatment at a dose of 1,200 mg/daily significantly reduced serum testosterone levels and improved both ovulation rate and metabolic parameters, such as blood pressure and triglycerides. Unfortunately that study provided no information on menstrual cycle regularity. Similar results have been subsequently recorded in lean PCOS women by the same group.[63] Those preliminary data prompted Nestler and colleagues to advance the results they obtained during the first trials, by increasing up to 2,400 mg the daily dose of DCI administered to PCOS patients.[64] That study was able to find a direct correlation between insulin-stimulated release of DCI-containing phosphoglycans and insulin sensitivity. Unexpectedly, that investigation was incapable in confirming the previous beneficial effects of DCI on ovary function, and the Authors did not offer any valuable hint to explain such a paradoxical outcome. A recent investigation performed on PCOS patients treated with increasing DCI doses (from 300 to 2,400 mg/day) has provided a compelling confirmation that by increasing DCI dosage paradoxically worsens oocyte quality and ovarian response in non-obese and non-insulin resistant PCOS women.[65] Namely, total r-FSH units increased significantly in the two groups receiving the higher doses of DCI, while the number of immature oocytes was significantly increased in the three groups treated with the highest doses of DCI. Additionally, the number of grade I

embryos was significantly reduced by DCI supplementation.

It is intriguing that those disappointing data are in some way mirrored by results obtained by treating PCOS patients with Metformin: the antidiabetic drug decreases the follicles number and worsens their quality,[66] even if Metformin significantly increases the insulin-stimulated release of DCI-phosphoglycans.[61]

Such contradictory results could likely be explained by considering the different function in each INS isomer plays in distinct tissues. Indeed, a specific MI/DCI ratio has been observed within each tissue: high DCI (even if always lower than MI concentration) is generally observed in glycogen storage tissues (fat, liver, muscle), whereas tissues characterized by high consumption rate of glucose (brain, heart) present low DCI levels.[67] Oocytes are characterized by high glucose consumption along the oxidative pathway: thus, by impairing sugar availability oocyte quality would likely be compromised . Indeed, reduced availability of glucose in both oocytes and follicular cells caused by defective transportation of glucose it is suspected to occur in PCOS.[68] In turn, energy impairment promotes alternative pathways to utilize fatty acid and amino acids for energy as a compensatory mechanism to deal with energy requirement.[69] Moreover, in PCOS women, genes involved in the glucose uptake pathway are down-regulated at ovarian level[70,71] and energy supplementation is required in achieving higher oocyte quality and better outcome after IVF in PCOS patients.[70] Those data highlight how is important to maintain a proper glucose metabolism for oocyte development. Undoubtedly, both DCI and MI are required to fulfill such function in cooperating with insulin. Yet, MI seems to play a more important role in oocyte, as suggested by the fact that almost the 99% of intracellular INS pool is constituted by MI.[72] DCI, instead, is produced from MI through a NAD-dependent epimerase whenever it is required. The epimerase conversion of MI to DCI is under insulin control: in type 2 diabetes patients, the reduced tissue insulin sensitivity leads to reduced epimerase activity and hence DCI synthesis.[55] However, ovary never displays insulin resistance, unlike other tissues.[73] Thus, increased insulin levels as those recorded in insulin-resistant patients, are likely to increase the activity of ovary epimerase, raising in that way the DCI intracellular production, meanwhile MI levels were progressively reduced. Thereby, in hyperinsulinemic PCOS patients DCI levels paradoxically increase in the ovary. In turn, DCI increase may promote glycogen and testosterone synthesis, impairing the oocyte maturation. Eventually, that mechanism would lower oocyte's MI content, and the MI depletion will in turn negatively affect oocyte quality.

Such imbalance may shed light in the pathogenesis of PCOS at cellular level and helps elucidate the theory

known as "the DCI paradox in the ovary."[74] That hypothesis has recently received a preliminary confirmation by two independent investigations. A study authored by Larner and coworkers has found a significant increase in the epimerase activity in the theca cells obtained from ovary of PCOS women, leading to a dramatic reduction in the MI/DCI ratio.[75] Another research, by investigating the INS concentration in follicular fluid obtained from PCOS women has observed a significant decrease in the MI/DCI ratio: while normal MI/DCI ratio is nearly 100:1, in follicular fluid of PCOS women that value is only 0.2:1.[72] The remarkable abatement in MI levels may thus likely contribute in explaining at least some of the observed dysfunction of ovary in PCOS.

Indeed, whereas DCI administration has been demonstrated to improve the systemic consequences of insulin resistance, namely by modulating insulin effects of endocrinology balance in non-ovarian tissues, DCI has probably only a marginal effect on oocyte function. In turn, a DCI overload, as such obtained by administering 600 mg/day or more, may have likely worsen INS imbalance into ovary cells, determining a dramatic decrease in the MI/DCI intracellular ratio. Additionally, high release of DCI-phosphoglycans, under insulin stimulation, enhances de novo testosterone biosynthesis from ovarian theca cells, thus raising serum androgen levels.[76] Those effects would explain why the promising results obtained by Nestler and coworkers during the first study have not been confirmed in the second one.

Indeed, it is of outmost importance to ensure the proper MI concentration at the ovary level. MI-based phospho-glycans (IPG-A) are required to facilitate oocyte glucose uptake.[7] Furthermore MI improves oocyte response to FSH, as indicated by the reduced requirement in rFSH IU administered during IVF cycles.[77,78] MI supplementation restores spontaneous ovulation and increases progesterone release during the luteal phase in all but few PCOS patients.[79] Therefore, it is worth of noting that a MI deficiency in the ovary would likely impair the FSH signal, resulting in an increased risk of ovarian hyperstimulation syndrome for PCOS patients. MI exerts in addition other appreciable systemic effects, by improving the reproductive axis functioning in PCOS patients through the reduction of the hyper-insulinemic state which affects LH secretion.[80] In that study, following 12 weeks of MI treatment, serum hormone levels were normalized and menstrual cycle was restored in amenorrheic patients. Moreover, PCOS patients submitted to ICSI, the associated treatment of MI and folic acid, but not folic acid alone, significantly decreases the cancellation rate, it reduces oocyte degeneration and germinal vesicles at ovum pickup, thus increasing the success probability of the therapy.[77] Indeed, serum and follicular fluid concentration of MI has been proven to be directly associated with oocyte maturation and fertility outcome in IVF-treated patients.[81]

Inositols and PCOS Treatment

Usefulness of MI supplementation has since been assessed by several reports. Morgante et al have evidenced that MI supplementation in insulin resistant-PCOS patients produces significant results, given the significant reduction in cancellation rate (0% vs. 40%) and the consequent improvement in clinical pregnancy rate (33.3% vs. 13.3%) obtained with INS treatment.[82] Gerli et al[83] conducted a randomized, double-blind, placebo-controlled trial of 283 PCOS patients treated with MI. In that study, frequency of ovulation (40%) was increased by almost twofold in women who received MI, versus the control group. Moreover, aiming to elucidate the systemic beneficial effects associated to MI therapy, additional studies were able to show that MI treatment lowered lipids,[84] insulin and androgen levels, increased insulin sensitivity, reduced blood diastolic pressure, and was effective in treating acne[21] and hirsutism.[85] Overall, those data demonstrated that MI is equally effective than DCI in normalizing metabolic and endocrine features commonly associated to insulin resistance and PCOS.

Yet, normalizing insulin resistance is not enough for restoring a proper ovulatory function, as suggested by a recent study comparing MI supplementation versus Metformin.[86] Sixty PCOS patients were treated with MI 4 g plus Folic acid, and 60 PCOS patients with Metformin 1500 mg/day. Among the patients treated with Metformin, 50% restored spontaneous ovulation activity. Pregnancy occurred spontaneously in 11 (36.6%) of these patients. In the MI group, 65% of patients restored spontaneous ovulation activity, ovulation occurred after a mean of 14.8 days from the day 1 of the menstrual cycle; in this latter group pregnancy occurred in 18 (48.4%) patients, showing a positive trend in increase.

Overall, those data evidence that MI supplementation provides significant benefit in PCOS management (Table 1), even most papers suffer for the lack of proper randomization and/or are flawed by few statistical inconsistencies.

However, by considering both the systemic and the ovary hallmarks of PCOS, INS supplementation should preferably include both the isomers: MI and DCI. Given that physiological values of the MI/DCI ratio, evaluated both in the plasma as well as in the follicular fluid, range from 40:1 to 100:1, it seems reasonable that INS should be administered jointly respecting a proportion that should reflect the natural balance among the two stereoisomers. Therefore, as proposed by a recent paper,[87] the combined administration of MI and DCI in the physiological plasma ratio (40:1), could be considered as a first line approach in

Table 1. Eligible RCTs Where MI Have Been Evaluated for the Treatment of PCOS Patients

Ref.	Study Design	Duration	Intervention	No. of Subjects	Inclusion Criteria	Exclusion Criteria	Assessment of the Response	Results
Genazzani et al[80]	Randomized, controlled vs. folic acid	12 weeks	MYO 2 g FA 200 mg/day	N = 20, Treatment: 10, Placebo: 10	Presence of micropolycystic ovaries at ultrasound; mild to severe hirsutism and/or acne; oligomenorrhea or amenorrhea; absence of enzymatic adrenal deficiency and/or other endocrine disease; normal PRL levels (range 5–25 ng/mL); no hormonal treatment for at least 6 months before the study	Not described	LH, FSH, PRL, E2, A, 17OHP, T, insulin, cortisol, OGTT[a] for insulin, glucose, C-peptide determinations, vaginal ultrasound examination Feriman-Gallway score, BMI, HOMA	LH, PRL, T, insulin levels, LH/FSH resulted significantly reduced. Insulin sensitivity resulted significantly improved. Menstrual cyclicity was restored in all amenorrheic and oligomenorrheic subjects
Morgante et al[82]	Randomized, controlled trial vs. placebo	4 weeks	Inositol 1,500 mg	N = 30, Treatment: 15, Placebo: 15	Clomiphene-failure patients with PCOS, with insulin resistance evaluated by Homeostatic Model Assessment Index	Not described	HOMA, FSH (IU/L), LH (IU/L), PRL (ng/mL), 17-OHP, DHEAS, number of follicles >15 mm in diameter, number of follicles >18 mm in diameter, E2 levels on day of hCG administration (pg/mL), cancellation rate (%), clinical PR (%)	The total number of follicles >15 mm and >18 mm in diameter, and the peak E2 levels were significantly lower in the inositol group compared with the control group. In addition, although the cancellation rate was significantly lower in the inositol group, the clinical PR was not significantly higher, compared with the control group
Gerli et al[83]	Double-blind, placebo-controlled vs. placebo	16 weeks	Inositol 200 mg/day	N = 283, Treatment: 136, Placebo: 147	Age: <35 years women with oligomenorrhea and PCOS ovaries. Ovaries were described as polycystic (PCOs) about the criteria of Adams et al[b]	Patients with significant hyperprolactinemia, abnormal thyroid function tests and congenital adrenal hyperplasia	Ovarian activity was monitored using serum E2, P and LH. Ovulation frequency was calculated using the ratio of luteal phase weeks to observation weeks. Inibin-b, fasting glucose, fasting insulin, or insulin AUC, VLDL, LDL, HDL, total cholesterol, triglycerides, BMI	Ovulation frequency significantly higher in the treated group vs. the placebo. follicular maturation was rapid: the circulating concentration of E2 increased only in the inositol group during the first week of treatment. Significant weight loss was recorded in the inositol group, whereas in the placebo group was recorded an increase of the weight. A significant increase in circulating high-density

Table I. Continued

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Ref.	Study Design	Duration	Intervation	No. of Subjects	Inclusion Criteria	Exclusion Criteria	Assessment of the Response	Results
Minozzi et al[84]	Open-label clinical study	12 months	MYO 4 g/day	N = 155	PCOS	Women with secondary endocrine disorder, those wishing to conceive during the next 12 months and those with contradictions to oral contraceptive use	Clinical and anthropometric measurement included: age, (BMI), serum levels of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, apolipoprotein B (apoB), lipoprotein(a) [Lp(a)], fasting glucose level, fasting insulin level, insulin resistance, measured by homeostasis model assessment (HOMA-IR), testosterone (T), sex hormone-binding globulin (SHBG), D4-androstenedione (A), dehydroepiandrosterone (DHEAS), leutenizing hormone (LH), and hirsutism score, evaluated by using a modification of the Ferriman–Gallwey (FG) score	lipoprotein was observed only in the inositol-treated group. A higher reduction of FG score in OCP plus MI therapy group vs OCP alone therapy group. OCP plus MI significantly decreased hyperinsulinemia vs the OCP group. Androgens serum levels decreased in both groups, but significantly more in OCP plus MI group. The lipid profile was improved in the OCP plus MI group, by reducing low-density lipoprotein cholesterol levels and enhancing high density lipoprotein cholesterol levels
Minozzi et al[85]	Open-label clinical study	6 months	MYO 2 g/twice a day	N = 46	Women with mild to moderate hirsutism, evaluated using a modification of the Ferriman–Gallwey score	Hyperprolactinemia, hypothyroidism, adrenal hyperplasia and Cushing's syndrome. Patients who had taken hormonal medications, including contraceptive pills, for the past 6 months	Hirsutism scores Serum concentrations of total cholesterol, HDL cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, apolipoprotein B, lipoprotein(a), glucose, insulin, testosterone, sex hormone-binding globulin (SHBG), Δ4-androstenedione, cortisol, dehydroepiandrosterone sulphate (DHEA-S), LH, FSH and E2 were measured within the first 5 days of the menstrual cycle	No changes in BMI were observed. The hirsutism decreased after therapy. Total androgens, FSH and LH concentrations decreased, oestradiol concentrations increased. There was a slight non-significant decrease in total cholesterol concentrations, an increase in HDL cholesterol concentrations and a decrease in LDL cholesterol concentrations. No significant changes were observed in serum triglyceride, apolipoprotein B and lipoprotein(a) concentrations.

(Continued)

Table I. Continued

Ref.	Study Design	Duration	Intervation	No. of Subjects	Inclusion Criteria	Exclusion Criteria	Assessment of the Response	Results
								Insulin resistance, analysed by homeostasis model assessment, was reduced significantly after therapy
Raffone et al[86]	Randomized controlled vs. metformin	Until the end of the study or positive pregnancy test	MYO 4 g FA 400 mg	N = 120, Treatment: 60, Placebo: 60	PCOS <35 years defined by Rotterdam Criteria	Other medical condition causing ovulatory dysfunction, tubal defects, semen parameters defects	Restoration of spontaneous ovulation and menstrual cycles and increasing rate pregnancy, ovarian activity, serum progesterone dosage, progesterone levels, β-hCG plasma level	Among the patients treated with metformin, 50% restored spontaneous ovulation activity. Pregnancy occurred spontaneously in 11 (36.6%) of these patients. In the MI group, 65% of patients restored spontaneous ovulation activity, ovulation occurred after a mean of 14.8 days from the day 1 of the menstrual cycle; in this latter group pregnancy occurred in 18 (48.4%) patients, showing a positive trend in increase
Nordio and Proietti[87]	Randomized controlled	6 months	2 g MYO powder 550 mg of Myo plus 13.8 mg of D-chiro-inositol in soft gel capsule	N = 50, Treatment: 24, Combined therapy: 26	Overweight women with PCOS	Diabetic subjects, smokers and alcohol users were excluded from the study	Plasma glucose and insulin concentrations Serum progesterone Ovulation function	The combined administration of MI and DCI reduces the metabolic and clinical alteration of PCOS and reduces the risk of metabolic syndrome
Costantino et al[103]	Double-blind, randomized controlled vs. folic acid	12–16 weeks	MYO 4 g FA 400 mg/day	N = 42, Treatment: 23, Placebo: 19	Presence of oligomenorrhea, high serum-free testosterone level and/or hirsutism presence of micropolycystic ovaries at ultrasound	Not described	Systolic/diastolic blood pressure, triglycerides, cholesterol, BMI, plasma glucose and insulin sensitivity, total/free T, DHEAS, SHBG, A, progesterone peak value	MI increased insulin sensitivity, improved glucose tolerance and decreased glucose stimulated insulin release. There was a decrement in serum total T and serum-free T concentrations. In addition, there was a decrement in systolic and diastolic blood pressure. Plasma triglycerides and total cholesterol

(Continued)

Table 1. Continued

Ref.	Study Design	Duration	Intervation	No. of Subjects	Inclusion Criteria	Exclusion Criteria	Assessment of the Response	Results
Papaleo et al[104]	Prospective, randomized, controlled vs. folic acid	During ovulation induction for ICSI	MYO 4 g FA 200 mg/day	N = 60, Treatment: 30, Placebo: 30	Age: <40 years PCOS women diagnosed by oligoamenorrhea, hyperandrogenism orhyperandrogenemia and typical features of ovaries on ultrasound scan	Other medical conditions causing ovulatory disorders: hyperinsulinemia, hyperprolactinemia, hypothyroidism, or androgen excess, such as adrenal hyperplasia or Cushing syndrome	Number of morphologically mature oocytes retrieved, embryo quality, pregnancy and implantation rates. Total number of days of FSH stimulation, total dose of gonadotropin administered, E2 level on the day of hCG administration, fertilization rate per number of retrieved oocytes, embryo cleavage rate, live birth and miscarriage rate, severe ovarian hyperstimulation syndrome	concentration decreased Total r-FSH units and number of days of stimulation were significantly reduced in the myo-inositol group. Peak E2 levels at hCG administration were significantly lower in patients receiving myo-inositol. The mean number of oocytes retrieved did not differ in the two groups, whereas in the group cotreated with myo-inositol the mean number of germinal vesicles and degenerated oocytes was significantly reduced, with a trend for increased percentage of oocytes in metaphase II
Gerli et al[105]	Double-blind, randomized, controlled vs. folic acid	16 weeks	MYO 4 g FA 200 mg/day	N = 92, Treatment: 45, Placebo: 47	Age: <35 years Women with oligomenorrhea, amenorrhea and PCOS ovaries. Ovaries were described as polycystic (PCOs) about the criteria of Adams et al[b]	Patients with significant hyperprolactinemia, abnormal thyroid function tests, and congenital adrenal hyperplasia	Ovarian activity was monitored using serum E2, P, LH. ovulation frequency was calculated using the ratio of luteal phase weeks to observation weeks. Inibin-b, fasting glucose, fasting insulin, or insulin AUC, VLDL, LDL, HDL, total cholesterol, triglycerides, BMI	Beneficial effect of MYO treatment upon ovarian function, anthropometric measures and lipid profiles

LFA, folic acid; PRL, prolactin; E2, estradiol; A, androstenedione; 17OHP, 17-hydroxy-progesterone; T, testosterone; P, progesterone; OGTT, oral glucose tolerance; BMI, body mass index; LH, luteinizing hormone; FSH, follicle stimulatig hormone; DHEAS, dehydroepiandrosterone; SHBG, sex hormone binding globulin; EAUC, area under the curve of OGTT; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

[a]OGTT was performed sampling 15 minutes before, and 30, 60, 90, 120, and 240 minutes after the oral assumption of 75 g of glucose.

[b]Adams J, Polson JW, Franks S. Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. Br Med J 1986; 293: 355–359.

PCOS overweight patients, being able to reduce the metabolic, hormonal and clinical alteration of PCOS.

Conclusion

PCOS is one of the most common endocrine disorders affecting women. It represents the most common cause of female infertility and it is characterized by a combination of hyperandrogenism, chronic anovulation and irregular menstrual cycle.[88] A significant number of patients suffers also from metabolic syndrome and insulin resistance, even if such features are currently not entirely understood.[89,90]

While a significant progress has recently been made in the diagnosis for PCOS,[91-93] the optimal infertility treatment in such cases remains to be determined.[94]

Recently, several clinical studies have highlighted the usefulness of INS supplementation in PCOS treatment. MI is readily taken up following oral ingestion[95] and it is has been proven to be safe even after high dose consumption.[96]

However, despite the relatively high number of reports, only few of them fulfill the criteria of randomized clinical trial. Those studies have been extensively reviewed elsewhere.[97,98] Among 70 studies focusing on PCOS treatment by means of different pharmaceutical composition incorporating INS, 21 were considered eligible as they involve MI.[97] Yet, only six of them were randomized controlled clinical trials (level of evidence I_b), involving more than 300 PCOS patients. Remarkably, in all the studies analyzed, no side effects were reported at the doses of both 2 and 4 g/day, thus resulting in a high patient compliance. The 4 g/day treatment regimen is useful to treat the symptom spectrum, resulting in a more complete and effective treatment. Overall, those studies indicate that MI supplementation improves several of the hormonal disturbances of PCOS, providing so far a level I_a evidence of MI effectiveness. MI mechanisms of action appear to be mainly based on improving insulin sensitivity of target tissues, resulting in a positive effect on the reproductive axis (MI restores ovulation and improves oocyte quality) and hormonal functions (MI reduces clinical and biochemical hyperandrogenism and dyslipidemia) through the reduction of insulin plasma levels.

These systemic hallmarks of PCOS are significantly affected by both DCI and MI supplementation. However, DCI treatment, mostly when it is administered at high dosage (ie, 600 mg or more), exerts disappointing effects on ovary functions.[65] Oocyte physiology, among other factors, is likely to be dependent on a fairy balance in between MI and DCI. Indeed, MI is an important constituent of follicular microenvironment, playing a determinant role in both nuclear and cytoplasmic oocyte development. Perhaps, the content of MI in follicular fluids may represent a more appropriate physiological indicator than follicular volume for monitoring the status of the developing follicles. Follicles that containing good quality oocytes have higher concentrations of MI in follicular fluids, probably due to the intricate relationship between MI and inositol phosphates in the PtdIns cycle activation for oocyte maturation.

Additionally, MI by improving glucose uptake may improve both oocyte energy status and oocyte quality. Moreover, during ovarian stimulation MI reduces FSH-IU necessary for ovarian stimulation. Altogether, this evidence hints that MI exerts several beneficial effects that improve the pregnancy chance.[99]

A further confirmation of the link among MI and oocyte quality has been provided by two recent studies performed on both PCOS and non-PCOS women.[100] In a prospective randomized open-label, pilot clinical trial involving one hundred non-PCOS women undergoing multiple follicular stimulation for in vitro fertilization, the addition of MI promotes the oocyte's meiotic maturation and reduces the number of gonadotropin cycles of treatment, while maintaining clinical pregnancy rate. Even if this study is underpowered to evaluate IVF outcomes like implantation and clinical pregnancy, a trend in favor of increased incidence of implantation in the group pre-treated with MI was observed. Another clinical investigation,[101] focused on one hundred PCOS women undergoing IVF-ET. Patients were treated with MI combined with DCI in the physiological ratio (40:1), or with DCI alone (500 mg). Significant better results were observed in the MI + DCI treated group, given that patients treated with this combination required lower dosages of FSH for a shorter period of time and showed an improvement in both oocyte quality and pregnancy rate.

Therefore, since the attention of the scientific community has recently been drawn on the relevance of the MI/DCI ratio for a proper ovary function, a treatment based on the association MI/DCI, in a physiological plasma range (ie, 40:1) seems to be the most effective approach, as recently stated by an international Consensus Conference held in Florence (December 2013).[102] Moreover, the recent data by Larner's group provided a molecular mechanistic basis supporting that statement, highlighting the "utility of both MI and D-chiro-inositol as effective agents in treatment. Certainly, a balance between the two inositols is required for normal physiological function and regulation of the MI to chiro-inositol epimerase opens a new avenue for upcoming studies."[75]

Undoubtedly, further studies are warranted to fully elucidate the molecular pathways triggered by myo- and D-chiro-inositol underlining their beneficial effects and to provide a well-grounded rationale for INS supplementation in PCOS patients.[103-105]

Declaration of Conflicting Interests

G.C. and V.U. are Lo.Li. Pharma employees.

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Letter to the Editor

Polycystic ovary syndrome: a vitamin deficiency? Floating a new pathogenesis hypothesis

Dear Editor,

Polycystic ovary syndrome (PCO'S) is a medical condition that causes irregular menstrual cycle, chronic anovulation most often manifested as oligo-amenorrhea, and androgen excess with the typical ovarian ultrasound features[1]. It is the most common cause of ovulatory disorders and female infertility and affects approximately 6-10% of women in childbearing age[2]. However, its pathogenesis is still poorly understood.

Recently many investigators focused both on impaired glucose tolerance, that affects 30 to 40% of patients with PCO'S[3], and insulin resistance, that is manifested in a significant proportion of women with PCO'S.

Insulin plays a direct role in the pathogenesis of hyperandrogenemia in the polycystic ovary syndrome, acting synergistically with luteinizing hormone to enhance the androgen production of theca cell[4]. Since the report by Burghen et al[5] in 1980 where PCOS was associated with hyperinsulinemia, it has become clear that this syndrome has major metabolic as well as reproductive morbidities. The recognition of this association has also instigated extensive investigation on the relationship between insulin and gonadal function. An inositol phosphoglycan molecule containing D-chiro-inositol is known to have a role in activating enzymes that control glucose metabolism[6]. A defect in tissue availability or utilisation of D-chiro-inositol (DCI) or inositolphosphoglycan (IPG) mediators may contribute to insulin resistance[7,8].

In turn, this association has led to the treatment of women with PCOS with insulin sensitizing agents such as troglitazone[9], inositol[8,10,11] and metformin[12].

Myionositol) (MI) and D-chiro-inositol (DCI), are isoforms of inositol and belong to the vitamin B complex. MI is widely distributed in nature whereas DCI, the product of epimerization of C_1 hydroxyl group of MI, is relatively rare[13]. We speculate that PCOS should be a clinic manifestation of a genetically determined vitamin (myionositol) deficiency. If that pathogenesis hypothesis would be true, the simply supplementation of myionositol should be the first line treatment of PCOS patients. Currently, in support of our hypothesis, clinical data show that all metabolic, endocrinology and ovarian changes observed in PCOS patients can be reversed by oraly supplement of myionositol (Table I).

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Table I.

Study	Patients	Treatment	Period	Other treatment product	Results	Conclusions
"Efficacy of Myo-inositol in the treatment of cutaneous disorders in young women with polycystic ovary syndrome" Martino M. Zacchè et al, Gynecol Endocrinol 2009; 25: 508-513	50	2 g/day	3+6 months	200 mcg folic acid	After 3 months of Myo administration, plasma LH, testosterone, free testosterone, insulin and HOMA index resulted significantly reduced. No significant changes were observed in plasma FSH and androstenedione levels. Both hirsutism and acne decreased after 6 months of therapy.	Myo-inositol administration is a simple and safe treatment that ameliorates the metabolic profile of patients with PCOS, reducing hirsutism and acne.
"Myo-inositol administration positively affects hyperinsulinemia and hormonal parameters in overweight patients with polycystic ovary syndrome" Genazzani et al, Gynecol Endocrinol, 2008; 24: 139–144	20	2 g/day	12 weeks	200 mcg folic acid	After 12 weeks of Myo administration plasma LH, PRL, T, insulin levels and LH/FSH resulted significantly reduced. Insulin sensitivity, expressed as glucose-to-insulin ratio and HOMA index resulted significantly improved after 12 weeks of treatment. Menstrual cyclicity was restored in all amenorrheic and oligomenorrheic subjects. No changes occurred in the patients treated with folic acid.	Myo administration improves reproductive axis functioning in PCOS patients reducing the hyperinsulinemic state that affects LH secretion.
"Myo-inositol in patients with polycystic ovary syndrome: a novel method for ovulation induction" Papaleo E et al, Gynecol Endocrinol 2007; 23: 700-703	25	2 g/day (twice a day)	6 months	200 mcg folic acid twice a day	Twenty-two out of the 25 (88%) patients restored at least one spontaneous menstrual cycle during treatment, of whom 18 (72%) maintained normal ovulatory activity during the follow-up period. A total of 10 singleton pregnancies (40% of patients) were obtained. Nine clinical pregnancies were assessed with fetal heart beat at ultrasound scan. Two pregnancies evolved in spontaneous abortion.	Myo-inositol is a simple and safe treatment that is capable of restoring spontaneous ovarian activity and consequently fertility in most patients with PCOS. This therapy did not cause multiple pregnancy.
"Myo-inositol is an effective first-line approach for inducing ovulation in women with polycystic ovary syndrome" Papaleo et al, Gynecol Endocrinol 2008: 24 (Suppl 1): Issn: 0951-3590 OP 4	51	2 g/day (twice a day)	6 months	200 mcg folic acid twice a day	Forty-two out of 51 patients restored at least one spontaneous menstrual cycle during treatment while nine patients showed Myo-inositol resistance. Thirty-four out of 42 women maintained monthly ovulatory activity during the follow-up period corresponding at 66.6% of patients with PSO. A total of 18 pregnancies were obtained.	Comparing results with data presented in literature. Myo-inositol is a simple and safe treatment that is capable to restore a spontaneous ovarian activity and consequently fertility in most patients with polycystic ovary syndrome. This therapy did not cause multiple pregnancy.

Table I. *(Continue)*

Study	Nr. Patients	Treatment	Period	Other treatment product	Results	Conclusions
"Myo-inositol in the treatment of obesity and insulin resistance in PCOS adolescent girls: a randomized, controlled trial" Nigro et al, Gynecol Endocrinol 2008; 24 (Suppl 1): Issn: 0951-3590 OP 6	49	2 g/day (twice a day)	6 months	200 mcg folic acid twice a day	Myo-inositol had a greater treatment effect over placebo for weight, body mass index, waist circumference, se abdominal adipose tissue and fasting insulin. Si improved in 55% of subjects while on Myo-inositol and 17% of subjects while on placebo ($P=0.21$).	Myo-inositol therapy for obese insulin-resistant young PCOS patients results in significant improvement in body composition and fasting insulin.
"The effects of Myo-inositol on ovarian stimulation and in vitro fertilization: a pilot study" Gerli et al, Gynecol Endocrinol 2008; 24 (Suppl 1): Issn: 0951-3590 OP 28	104	4 g/day	3 weeks	400 mcg folic acid then recombinant FSH	Bayesian analysis showed probabilities of 0.05 that Myo-inositol reduces FHS requirement by at least 12% and of that 10% oocytes are collected after Myo-inositol co-treatment.	Co-administration of Myo-inositol is therefore likely to increase the number of oocytes collected after ovarian stimulation in insulin-resistant women with PCOS and reduce the requirement for FSH.
"Metabolic and hormonal effects of Myo-inositol in women with polycystic ovary syndrome: a double-blind trial" D. Costantino et al, Eur Rev Med Pharmacol Sci 2009; 13: 105-110	42	4 g/day	12-16 weeks	400 mcg folic acid	There was a decrement in systolic pressure in Myo-inositol group (from 131 ± 2 to 127 ± 2 mmHg) while an increment in placebo group (from 128 ± 1 to 130 ± 1 mmHg; $P=0.002$); similarly about the diastolic blood pressure, with decrement (from 88 ± 1 to 82 ± 3 mmHg) in Myoinositol group and increment (from 86 ± 7 to 90 ± 1 mmHg) in placebo group respectively ($P=0.001$). In the Myo-inositol group plasmatriglycerides decreased by 52% (from 195 ± 20 to 95 ± 17 mg/dl) and total cholesterol decreased significantly (from 210 ± 10 to 171 ± 11 mg/dl). Sixteen (69,5%) and four (21%) women ovulated in the Myo-inositol group and the placebo group respectively. The different is statistically significant ($P=0.001$). There was an important decrement of the serum dehydroepiandrosterone sulphate in the Myo-inositol group (from 366 ± 47 to 188 ± 24 µg/dl; $P=0.003$) while it wasn't significant in the placebo group (from 384 ± 63 to 320 ± 35 µg/dl; $P=0.06$).	Myo-inositol decreases serum androgen concentrations, reduces circulating insulin and improves glucose tolerance and other metabolic values altered associated with insulin resistance in women affected by Polycystic ovary syndrome.

Table I.

Study	Patients	Treatment	Period	Other treatment product	Results	Conclusions
"Myo-inositol may improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial" Papaleo E et al. Fertil Steril 2009: 91:1750-1754	30+30	4 g/day	12 months	400 mcg folic acid	Total r-FSH units (1,958 ± 695 vs. 2,383 ± 578) and number of days of stimulation (11.4±0.9 vs. 12.4±1.4) were significantly reduced in the Myo-inositol group. Furthermore, peak E(2) levels (2,232±510 vs. 2,713±595 pg/mL) at hCG administration were significantly lower in patients receiving Myo-inositol. The mean number of oocytes retrieved did not differ in the two groups, whereas in the group cotreated with Myo-inositol the mean number of germinal vesicles and degenerated oocytes was significantly reduced (1.0 ± 0.9 vs. 1.6 ± 1.0), with a trend for increased percentage of oocytes in metaphase II (0.82 ± 0.11% vs. 0.75 ± 0.15%).	These data show that in patients with PCOS, treatment with Myo-inositol and folic acid, but not folic acid alone, reduces germinal vesicles and degenerated oocytes at ovum pick-up without compromising total number of retrieved oocytes. This approach, reducing E(2) levels at hGC administration, could be adopted to decrease the risk of hyperstimulation in such patients.
"Randomized, double blind placebo-controlled trial: effects of Myo-inositol on ovarian function and metabolic factors in women with PCOS" S. Gerli et al, Eur Rev Med Pharmacol Sci 2007: 11: 347-354	92	4 g/day	14 weeks	400 mcg folic acid	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.

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