European Review for Medical and Pharmacological Sciences 2014; 18: 1896-1903

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 ἰς (*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

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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]. Urinary 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 [^{3}H]myo-inositol is converted to [^{3}H]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 threefold 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 expected, 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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