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REVIEW

Inositols in the ovaries: activities and potential therapeutic applications

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ABSTRACT

Introduction: Myo-inositol (MI) and D-chiro-inositol (DCI) play a key role in ovarian physiology, as they are second messengers of insulin and gonadotropins. Ex-vivo and in-vitro experiments demonstrate that both isomers are deeply involved in steroid biosynthesis, and that reduced MI-to-DCI ratios are associated with pathological imbalance of sex hormones.

Areas covered: This expert opinion provides an overview of the physiological distribution of MI and DCI in the ovarian tissues, and a thorough insight of their involvement into ovarian steroidogenesis. Insulin resistance and compensatory hyperinsulinemia dramatically reduce the MI-to-DCI ratio in the ovaries, leading to gynecological disorders characterized by hyperandrogenism, altered menstrual cycle and infertility.

Expert opinion: Available evidence indicates that MI and DCI have very specific physiological roles and, seemingly, physiological MI-to-DCI ratios in the ovaries are crucial to maintain the correct homeostasis of steroids. Inositol treatments should be evaluated on the patients' specific conditions and needs, as long-term supplementation of high doses of DCI may cause detrimental effects on the ovarian functionality. In addition, the effects of inositol therapy on the different PCOS phenotypes should be further investigated in order to better tailor the supplementation.

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Myo-inositol; D-chiro-inositol; Polycystic Ovary Syndrome; Ovarian Steroidogenesis; Epimerase; Insulin Resistance

1. Introduction

Inositols are a group of small cyclic polyols. Out of nine possible stereoisomers, only five occur naturally, with myo-inositol (MI) representing the major component. MI is naturally part of the human diet, mainly found in nuts, seeds and cereals as phytate (myo-inositol hexaphosphate). Humans, however, endogenously transform glucose-6-phosphate in MI, which is then incorporated into cellular membranes as phosphatidylinositol. An insulin-dependent enzyme of the epimerase class converts MI into the isomer D-chiro-inositol (DCI), promoting the inversion of one of the chiral carbon atoms on the molecule. Epimerase activity is tissue specific, as MI-to-DCI ratios change in different surroundings. Because MI and DCI participate in a variety of cellular signaling pathways [1], one may argue that physiological ratios reflect the sensitivity of the organs and tissues to specific extracellular signals.

This Expert Opinion gathers the available information about the role of these two inositol isomers in the ovaries and points out that maintaining physiological ratios is crucial to avoid pathological states. Indeed, altered MI-to-DCI ratios characterize the ovarian tissues of women with gynecological diseases like Polycystic Ovary Syndrome (PCOS). In such cases, inositol supplementation may represent a suitable therapeutic approach to restore the physiological ratios and counteract the pathological features.

2. Inositol derivatives as insulin mediators

Insulin is a metabolic hormone with a dual activity. It controls glucose metabolism, stimulating cell uptake, and has a mitogenic function, promoting cellular proliferation. On the surface of plasma membrane, insulin binds to its receptor, a transmembrane protein with an intracellular tyrosine kinase catalytic domain [2]. Upon activation, the receptor undergoes autophosphorylation, starting a cascade reaction that promotes glucose transport via the phosphatidylinositol-3-kinase

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Article highlights

- Physiological content of myo-inositol (MI) and D-chiro-inositol (DCI) is specific of each tissue or organ, with the individual insulin response that regulates the local MI-to-DCI ratio. In case of hyperinsulinemia, tissues that do not develop insulin resistance, like the ovaries, become enriched in DCI.
- Inositols participate in the steroidogenesis, as they are second messengers of insulin and gonadotropins. Moreover, recent evidence suggested that DCI downregulates the expression of aromatase, reducing the conversion of androgens into estrogens.
- MI is currently used to treat infertility and menstrual irregularities in polycystic ovary syndrome (PCOS). Combination of MI and DCI in the 40:1 ratio appears to be the best option to improve the metabolic profile in overweight PCOS subjects. Treatment with DCI should be carefully evaluated in order to avoid the onset of unwanted side effects that may worsen PCOS symptoms.
- Elevated MI-to-DCI ratios in the follicular fluid are associated with good-quality blastocysts, supporting MI supplementation before assisted reproduction technology. Moreover, MI treatment during ovarian stimulation increases FSH sensitivity, reducing the total amount of gonadotropins required in the stimulation protocols.
- Reduced absorption of inositols may reduce the efficacy of the treatment. Alpha-lactalbumin modulates the opening of intestinal tight junctions, enhancing the absorption of MI and DCI even in 'inositol-resistant' subjects.

(PI3K) pathway. Insulin signal (Figure 1) also activates phospholipase C (PLC), which cleaves the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP_2) with release of the second messengers diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3) [3].

Earlier investigations proved that two distinct inositol-based compounds mediate the intracellular activity of insulin [4]. Subsequent insights clarified that such second messengers contain an amino sugar and either MI or DCI [5,6]. As mentioned, an insulin-dependent epimerase enzyme transforms MI into DCI, providing the appropriate ratio between the two isomers. Insulin resistance causes the activity of epimerase to decrease [7], as also confirmed by Kennington et al. [8], who reported a lower urinary excretion of DCI in patients with non-insulin-dependent type II diabetes mellitus, with respect to healthy subjects. They also observed a corresponding increase in MI excretion [8], which may depend on the competition for glucose carriers in the renal tubular region [9]. While there is quite general agreement that urinary excretion of MI increases with reduced insulin sensitivity, findinding on DCI clearance are more controversial [9–12]. Some aspects should be considered: *a)* urinary concentrations of DCI are much lower than those of MI, thus measurements may be more easily subject to fluctuations; *b)* the majority of the studies fail to take eating habits into account, when DCI mainly comes from dietary intake [10]; *c)* renal catabolism of inositols due to altered expression of the MIOX enzyme remains unaccounted for [13]; *d)* correlation between plasma concentrations of inositols and their urinary excretion remains widely underinvestigated. Some authors stated that combination of urinary concentration of MI (uMI) and DCI (uDCI) provides more useful and reliable information, either as index for insulin sensitivity [9] or as prognostic marker for type II diabetes [10]. Villeneuve at al. put forward the hypothesis that uDCI may be also dependent on insulin levels as they found increased uDCI in women with PCOS, while opposite results in men with hyperinsulinemia [14]. The authors concluded that excess insulin (more relevant in hyperinsulinemic men than in PCOS women) may induce renal absorption of DCI to counteract the reduced intracellular conversion of MI to DCI due to insulin resistance. Overall, based on available evidence, urinary excretion of inositols depends on several variables and hardly provides an accurate indication of the inositol status of the body.

Irrespective of fluctuations in the urinary excretion of inositols, insulin resistance causes reduced intracellular DCI content. Unlike most organs and tissues, the ovaries remain sensitive to insulin even in resistant subjects. Excess insulin stimulates the ovarian epimerase to enhance the conversion from MI to DCI [15], leading to altered and defective MI-to-DCI ratios.

As PCOS women often exhibit insulin resistance and a compensatory hyperinsulinemia [16], the majority of available evidence on ovarian inositol content and the effect of inositol therapy derives from studies and clinical trials on PCOS patients.

3. The role of inositols in ovarian steroidogenesis

In the ovaries, insulin stimulates the biosynthesis of androgens (Figure 1) [17]. Earlier work from the group of Nestler highlighted some mechanistic features of the insulin-induced androgen biosynthesis, reporting that inositolphosphoglycans (IPGs) are involved in the process as second messengers [18,19]. Exposure of freshly isolated human thecal cells to insulin produced a 10-fold increase in testosterone biosynthesis in the presence of INS-2, a synthetic analogue of DCI-based putative insulin mediator. *In-vitro* studies on cultured human thecal cells further demonstrated that INS-2 stimulates the production of testosterone even in the absence of insulin. The authors also showed that, when thecal cells were preincubated with antibodies that block the IPG signaling system, the activity of insulin was almost completely lost [18].

As DCI mediates the biosynthesis of androgens in the cells of the ovarian theca, it also likely modulates the production of estrogens in the granulosa cells. Indeed, *in-vitro* experiments on cultured human granulosa cells demonstrated that treatment with DCI downregulates in a dose–response manner the expression of two key steroidogenic enzymes, *aromatase* (CYP19A1), and *cytochrome P450 side-chain cleavage* (P450scc) [20]. The same study proved that the observed effect suppresses only the insulin boosting on gonadotropin signal. In fact, the stimulatory input of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) on the expression of the same steroidogenic enzymes remains unaffected by DCI treatment. Because of such observations, more recent parallelism arose between DCI and aromatase inhibitor drugs [21]. Cheang et al. provide clinical indications of DCI activities on ovarian steroidogenesis [22]. Their study involved hyperandrogenic PCOS women, treated with 2400 mg DCI/die (1200 mg, twice a day). After 6 months of supplementation, the patients

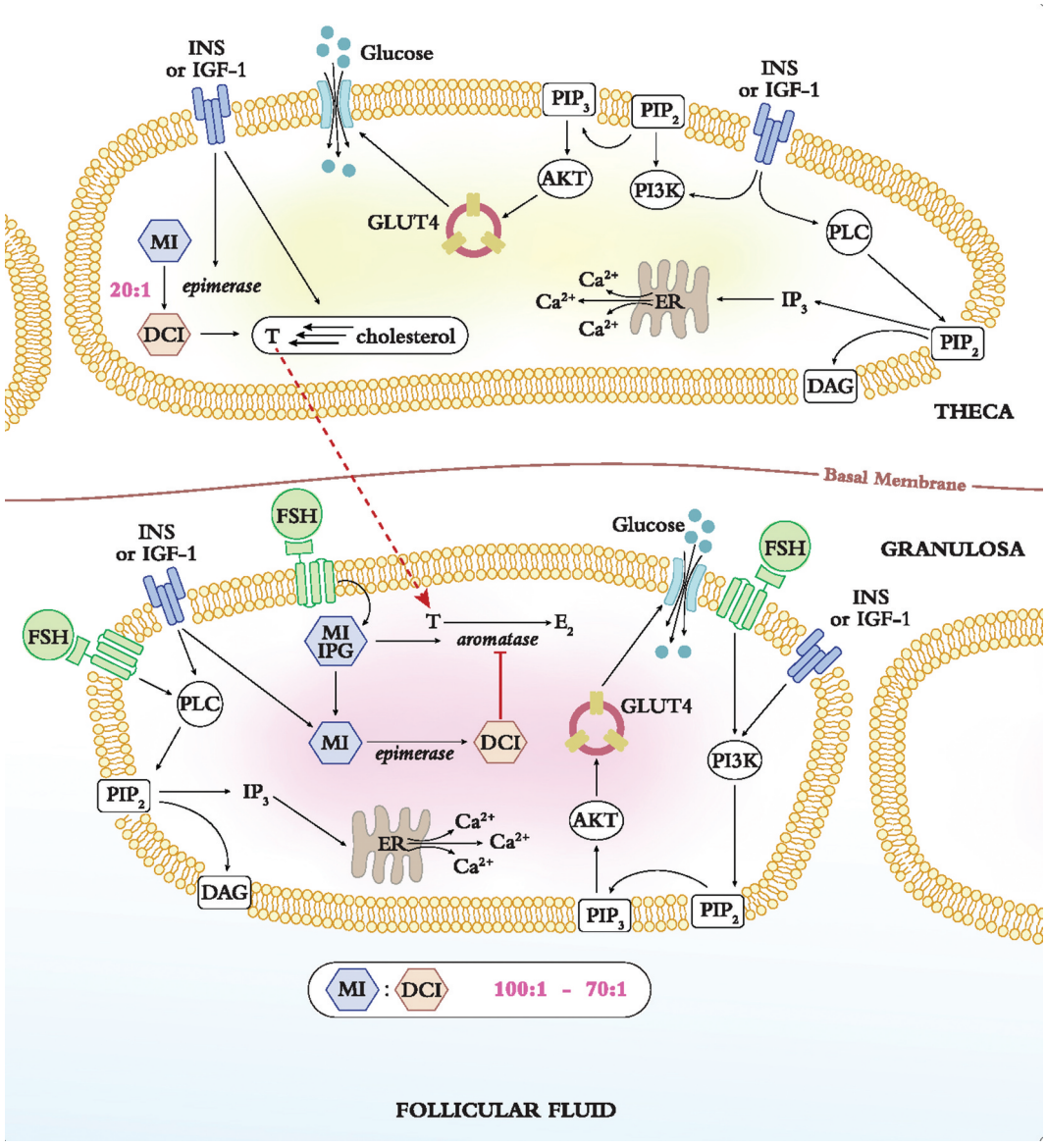

Figure 1. Inositol content and main inositol pathways in ovarian follicles.

displayed unvaried plasma testosterone levels, despite previous reports of lower doses of DCI able to decrease androgen production in PCOS women [23]. Moreover, recent pre-clinical evidence indicated that elevated doses of DCI may alter murine ovarian morphology, inducing a PCO-like or even a menopausal phenotype [24].

The literature features less direct evidence of the involvement of ovarian MI in steroid biosynthesis. MI is a well-known second messenger of FSH in the granulosa cells [25,26], and studies during artificial insemination procedures proved that MI supplementation enhances ovarian sensitivity to FSH [27]. FSH signal is crucial in the folliculogenesis process as it stimulates the granulosa cells of ovarian follicles to produce estradiol [28]. Indeed, FSH induces the up-regulation of aromatase expression [29], mainly activating the cAMP pathway [30]. More recent studies on rat granulosa cells demonstrated that activation of the IPG pathway is also necessary for aromatase overexpression [31], suggesting a role for MI in estrogen biosynthesis in the ovaries.

Even though further studies are necessary to provide a proper understanding of the regulatory activity of both MI and DCI in ovarian steroidogenesis, available data suggest a preferential role for DCI-based second messengers in stimulating ovarian androgen production in the thecal cells. On the other hand, MI-based second messengers are likely to partake in the FSH-induced estrogen biosynthesis in the granulosa cells.

4. Inositols in the ovaries

MI and DCI are essential for the physiological development and metabolism of ovarian follicles (Figure 1). In particular, MI orchestrates glucose metabolism and mediates the granulosa response to gonadotripins, especially FSH [26]. On the other hand, available information indicates that DCI is directly

involved in regulating the biosynthesis of steroids [32]. Interestingly, inositol ratios vary significantly in the different milieux of ovarian follicles, suggesting that specific regulation of the epimerase has a key role in follicular development. Moreover, inositol ratios are also supposed to determine the follicular fate during the selection of the dominant follicle [33]. As the ovaries preserve their insulin sensitivity even in resistant subjects, hyperinsulinemia abnormally enhances local epimerase activity [15], stimulating the transformation of MI into DCI and causing profound alterations in the ovarian physiology.

4.1. Thecal cells

Heimark et al. reported that MI-to-DCI ratio in thecal cells of healthy women is around 20:1 [34]. Follicular theca is the main site of ovarian androgen biosynthesis, which may justify higher DCI contents than those found in the plasma and in most organs and tissues. Earlier evidence, indeed, demonstrated that DCI-based phosphoglycans directly stimulate testosterone production from cultured human thecal cells [18]. Moreover, DCI reduces aromatase gene expression in a dose–response manner [20], thus providing a mechanistic rationale for the minimal physiological production of estrogens observed in the thecal cells [35].

In PCOS subjects, thecal MI-to-DCI ratio is about 4 times lower [34], likely due to the increased activity of epimerase under the stimulus of excess insulin. Unsurprisingly, hyperandrogenism is a hallmark of the syndrome [36], and one of the Rotterdam diagnostic criteria [37].

4.2. Granulosa cells

MI mediates the intracellular signaling of gonadotropins in the granulosa cells, inducing estrogen production and sustaining follicle development and final maturation. Despite the lack of information regarding the MI-to-DCI ratio in the granulosa cells, as well as evidence regarding a specific role of DCI, it is reasonable assuming a physiologically high MI content in the granulosa of healthy women. As granulosa cells express receptors for both insulin and insulin-like growth factor-1 (IGF-1) [38], hyperinsulinemia is likely to increase DCI content at the expense of MI, leading to the reduced ovarian sensitivity to FSH generally observed in PCOS women.

4.3. Follicular fluid

The follicular fluid provides a nourishing, metabolically active environment for the developing oocyte. Studies on patients undergoing *in-vitro* fertilization (IVF) procedures found that elevated MI concentrations in human follicular fluid correlate with better quality oocytes and higher grade blastocysts [39,40]. MI-to-DCI ratios within the range 70:1–100:1 are physiologically suited for proper oocyte maturation in the follicular fluid [40,41]. Insulin resistance in PCOS women increases the DCI content of follicular fluid and causes MI-to-DCI ratio to drop to 0.2:1 [42], with detrimental effect on oocyte growth [43].

5. Therapeutic application of inositols

Based on altered inositol values observed in patients with gynecological disorders, such as PCOS, therapeutic approaches aim to restore the physiological ratios (Figure 2). Indeed, proper supplementation proved to ameliorate the clinical picture and to relieve the symptoms, significantly improving the patients' quality of life [44]. Studies on murine model of PCOS provided indication that treatment with 40:1 MI-to-DCI ratio, physiologically found in plasma [45], constitutes the most effective strategy to restore ovarian physiological functions [46]. Nordio et al. later confirmed these data in a clinical trial on anovulatory, insulin-resistant PCOS patients [47].

Some insulin-resistant PCOS women feature both hyperandrogenism and hyperestrogenism, due to the activity of insulin both on the thecal cells and on the aromatase expression in the granulosa [48]. Excess estrogens blocks the release of FSH and GnRH through a negative feedback on either the hypothalamus and the anterior pituitary [49], preventing ovarian follicles to properly develop and reach the ovulatory stage. Insulin-sensitizing agents, such as metformin, may be effective in restoring ovulation in such cases [50], avoiding the side-effects of oral ovulation-inducing drugs that are normally employed in clinical practice [51–53].

Because of their role in insulin signaling, also inositols may be helpful in restoring the ovulatory function in PCOS women, either as individual isomers or in combination. Indeed, Kamenov et al. found that the administration of MI to anovulatory PCOS women was an effective and well-tolerated therapeutic option to induce ovulation and to reduce the number of patients that require clomiphene citrate administration [54]. Also, short-term treatment (6–8 weeks) with DCI proved to induce the ovulation in insulin-resistant PCOS women in a dose–response manner [55], decreasing insulin and systemic androgen levels [23]. However, administration of excessive amounts of DCI seemingly have unfavorable outcomes on the androgen status [22], raising concerns about the safety of treatments with DCI in PCOS women. Current evidence suggests that the administration of DCI should be tailored on the patients' characteristics, carefully evaluating the dosage and the duration of the treatment.

5.1. Polycystic ovary syndrome

Hyperinsulinemia in PCOS women alters physiological MI-to-DCI ratios in the ovaries, as the organ retains normal sensitivity to insulin. Excess insulin, indeed, stimulates the ovarian epimerase to convert MI into DCI, causing a pathologic increase in the DCI concentration at the expense of MI content. Such occurrence leads to decreased granulosa sensitivity to FSH and stimulates the thecal cells to produce

Inositol therapy

	PCOS		ART	
	Who	**What**	**Who**	**What**
MI (+ α-LA)	Lean PCOS women	• Increases FSH sensitivity • Induces ovulation *May be used to decrease the use of clomiphene citrate* • Reduces testosterone levels • Decreases HOMA index	PCOS women undergoing fertilization procedures	• Reduces the amount of FSH required for ovarian stimulation • Improves oocyte quality • Improves pregnancy rate
MI:DCI (+ α-LA)	Overweight/obese PCOS women (40:1 ratio)	• Increases FSH sensitivity • Induces ovulation • Reduces testosterone levels • Improves HOMA index and metabolic parameters		(70-100:1 ratio in the follicular fluid - prognostic marker for embryo quality and successful pregnancy)
DCI (+ α-LA)	Anovulatory women (short treatment)	• Decreases systemic insulin levels • Induces ovulation ⚠ High dosage or extended duration may worsen PCOS symptoms		(Hampers correct oocyte development)

Figure 2. Effects of inositol supplementation in PCOS and ART procedures.

androgens, contributing to the pathological feature of the syndrome.

Over the years, researchers have constantly reviewed the properties of inositols and the advances in the use of inositol therapy in PCOS [41,44]. They highlighted that MI helps restoring the fertility of PCOS women, enhancing the effect of either metformin or clomiphene citrate treatments, but the combination with DCI proved to be more effective in restoring physiological metabolic pathways and improving PCOS symptoms in case of obesity. Several authors also pointed out that DCI supplementation must be carefully evaluated, as inappropriate levels may alter ovarian physiology [44,56,57]. Bizzarri et al. proposed that MI may promote the rearrangement of the cytoskeleton in the follicles and that such activity may in part account for the observed effect of supplementation in PCOS [58]. MI proved also to potentiate the effect of oral contraceptive pills in the treatment of PCOS in teenagers, enhancing the antiandrogenic effect, counteracting the weight gain associated to hormonal treatments and improving the metabolic profile [59]. In short, supplementation with inositols improves the patients' quality of life by ameliorating the metabolic pathways and by relieving the symptoms related to PCOS [60].

A first meta-analysis on the efficacy of inositol therapy included nine randomized clinical trials (RCTs) with 247 cases and 249 controls [61]. The selected studies investigated supplementation of MI alone or in combination with DCI [59,62–69]. The authors found a statistically significant reduction of fasting insulin levels and Homeostatic Model Assessment (HOMA) index. They concluded that MI has beneficial effects in improving metabolic profile in PCOS women and a tendency in reducing hyperandrogenism. Zeng et al. confirmed such findings with a further meta-analysis that included 10 RCTs and a total of 573 patients [70]. They reported that MI therapy improves HOMA index and leads to increased estradiol levels, and concluded recommending the administration of MI to PCOS women with insulin resistance. Even though excluded from the official diagnostic criteria, insulin resistance is a quite common feature of women with PCOS, irrespective of their BMI [71–73]. Poor insulin response leads to hyperglycemia and compensatory systemic hyperinsulinemia, which induces the production of

free radicals and reactive oxygen species (ROS) that maintain a general inflammation state. Given the effect of MI on insulin sensitivity, Donà et al. demonstrated that supplementation reduces the oxidative damage on erythrocytes that they observed in lean PCOS patients [74]. The authors concluded suggesting MI as an alternative to metformin in the management of altered insulin states in women with PCOS.

Even though MI supplementation improves the clinical picture in PCOS women by increasing FSH sensitivity, the administration of MI and DCI in the 40:1 ratio, respectively, yields much better results in restoring the metabolic profile in overweight or obese patients [75]. Indeed, the integrated therapy combines the effect of DCI-mediated reduction of circulating insulin with the activity of MI in restoring ovarian FSH sensitivity and glucose metabolism [68,76–78]. Among various ratios investigated (i.e. 5:1, 20:1. 40:1, 80:1), MI-to-DCI 40:1 proved to yield the best results in recovering physiological morphology of ovarian follicles, reducing the ratio between the thickness of theca and granulosa in murine model of PCOS [46]. Clinical investigations confirmed these results in women with PCOS, with the 40:1 ratio able to restore ovulation better than the other ratios tested, or even the sole DCI [47]. Moreover, the co-administration improved the metabolic parameters of overweight patients with PCOS faster that the treatment with the sole MI [67]. Roseff and Montenegro noted that, given the evidence and the lack of side-effects, it is surprising how inositol therapy has yet to gain a foothold in gynecological practice, especially for insulin-resistant women with altered ovarian physiology [76].

5.2. Assisted reproductive treatment (ART) protocols

Inositol content of follicular fluid is crucial for correct oocyte maturation and, hence, for successfully achieving pregnancy. Chiu et al. found, indeed, that elevated concentrations of MI in human follicular fluid correlated with better oocyte quality, proposing the use of MI as marker of oocyte status [39]. Later, *in-vitro* experiments on murine oocytes demonstrated that MI is crucial for cellular maturation [79]. The authors found also a significant improvement in the post-implantation development of embryos. On the other hand, increasing concentrations of DCI in the follicular fluid seem to play a detrimental role on oocyte development [43,76], in line with the high MI-to-DCI ratios found in the follicular fluid of healthy subjects [40].

On these premises, Ravanos et al. investigated the inositol content in follicular fluid from eight healthy egg donors within an assisted fertilization program [40]. They found that elevated MI-to-DCI ratios correlated with higher quality blastocysts, suggesting that inositol ratio, rather than MI content, may represent a prognostic marker for embryo quality and successful pregnancy. The authors further suggested considering pretreatment with MI in order to improve the outcome of ART protocols. Ozay et al. provided stronger evidence in this regard with a clinical trial on infertile PCOS patients treated with MI [80]. With respect to a control group, MI supplementation significantly increased pregnancy rates and lowered both the doses of recombinant FSH used in stimulation protocols and the duration of the ovulation induction. A meta-analysis later confirmed such findings [27].

Recently, Facchinetti et al. reviewed the rationale for inositol supplementation in ART protocols and provided an update on the most recent applications, supporting the use of MI supplementation in assisted reproduction [81].

5.3. Therapeutic strategies to increase intestinal absorption of inositols

The details of inositol absorption across the intestinal barrier have yet to be fully elucidated. Passive diffusion through cell tight junctions seems likely at high concentrations of inositols [82], but active transport appears the main absorption mechanism. A specific protein called sodium/myo-inositol co-transporter-2 (SMIT2) mediates such process in the small intestine [83]. Competition experiments highlighted that DCI competes for binding to SMIT2, reducing the absorption of MI when the two isomers are administered together [82]. SMIT2 has also a certain affinity toward glucose and other monosaccharides, indicating that dietary carbohydrates may reduce the intestinal absorption of inositols.

Pharmacokinetic studies demonstrated that the plasma concentration of MI increases after oral administration, and peaks in the range 90–240 minutes, with differences ascribable to the initial dose [82,84,85]. Saturation of transporters seems to occur as well [85], suggesting that inositol treatment should ideally be provided in more than a single daily administration in order to achieve full coverage. Moreover, administration of elevated doses of inositols may result in reduced absorption. Indeed, while single administrations up to 4 g of MI largely proved safe and devoid of unwanted side effects, single administrations over 12 g cause gastrointestinal side effects that impair inositol absorption [85].

The effect of inositol supplementation may be impaired by a reduced intestinal absorption, which occurs in almost 40% of PCOS women [54]. In addition to these 'inositol-resistant' subjects, dietary habits may contribute to reduce the absorption and the endogenous production of MI [76,82,86].

Monastra et al. found that alpha-lactalbumin (α-LA) can increase *in vivo* the intestinal absorption of MI [87], thus overcoming the issue of inositol-resistance [44,88]. *In-vitro* experiments on human intestinal Caco-2 cells, in the presence of digested α-LA, demonstrated that an increased permeability of the tight junctions may account for the enhanced inositol absorption [87,89].

A clinical trial on 37 anovulatory PCOS women proved that α-LA is effective in boosting the efficacy of MI in inducing ovulation [90]. In a first phase of the study, all participants received oral MI and 23 patients (62%) ovulated after three months. The remaining 14 inositol-resistant participants were further treated with MI combined with α-LA and, by the end of the treatment, 12 (86%) successfully ovulated. These patients also had increased MI plasma concentration and exhibited improved hormone and lipid profile.

6. Conclusion

MI and DCI display paramount activities in the ovaries. MI mediates the FSH signaling in the granulosa cells and is essential for oocyte development in the follicular fluid. Instead, DCI modulates the biosynthesis of steroids in the thecal cells, stimulating androgen production. As insulin second messengers, both isomers contribute to regulate intracellular glucose metabolism. The available data indicate that physiological MI-to-DCI ratios in the various milieux of the ovarian follicles are tightly regulated, and reflect the specific activities of the different types of follicular cells.

Hyperinsulinemia is common in PCOS women and causes pathological alterations of inositol ratios in the ovaries, which do not develop insulin resistance. As a consequence, hyperandrogenism and impaired ovarian functionality arise. Properly tailored inositol supplementation proved to help restoring physiological MI-to-DCI ratios and to counteract the symptoms of PCOS, improving the quality of patients' life. Specifically, MI supplementation reduces systemic insulin levels in PCOS women, improving the ovulatory function, and contributes to enhance the oocyte and the blastocyst quality in ART protocols; the combination of MI and DCI in the 40:1 ratio, instead, yields better results than the sole MI supplementation in terms of improving metabolic functions in overweight or obese PCOS patients. Even though meta-analyses confirmed the beneficial effects of inositol supplementation in women with PCOS [61,70], the results derive from studies with limited number of patients and with a certain degree of variability in the treatment. Randomized, double-blinded and placebo-controlled clinical trials with a larger cohort of patients would be advisable to strengthen the current evidence.

7. Expert opinion

Comprehensive rationalization of the physiological activities of inositols, and the underlying molecular mechanisms, is no trivial task. Both MI and DCI are present in every district of the body, where they have structural role in the cell membranes and participate in a plethora of intracellular signaling pathways. Thus, the results of individual studies represent only a small piece of a larger and extremely intricated puzzle, and should always be analyzed considering the context, avoiding risky and potentially misleading generalizations. Based on the available evidence, MI and DCI exert very distinct and specific functions in the body, with notable regulatory and signaling activities in the ovaries. Both isomers have roles of primary importance in the biosynthesis of steroids from ovarian follicles, and the MI-to-DCI ratio likely determines whether a given follicles produces preferentially estrogens or androgens. Actually, DCI may trigger opposite functions to MI by reinforcing the androgen synthesis in ovarian theca cells [91]. Therefore, disruption of inositol balance induces pathological alterations in the steroidogenesis that lead to gynecological disorders and infertility. Maintaining or restoring the physiological balance between MI and DCI is the goal of inositol treatments, which should be always tailored on the patients' specific needs and condition. In particular, a careful evaluation is strongly advisable before opting for a DCI-based treatment, as excess DCI may induce unwanted side effects that could worsen the patients' steroid status in the long run. On the other hand, the positive effects of MI-based treatments is well established to recover metabolic and hormonal unbalances in women with PCOS, restoring the normal ovulatory function when altered or impaired [61,70]. The anti-oxidant activity of MI, generally observed to reduce the oxidative stress in human semen samples [92–94], recently emerged also in women with PCOS [74], which display systemic inflammation and excess production of free radicals and ROS.

On these premises, previous clinical knowledge about inositol supplementation should be reinterpreted in light of the more recent findings on the molecular activities of MI and DCI. Despite some overlap, they are indeed markedly different, and incorrect supplementation may alter the physiologic MI-to-DCI ratios necessary for the hormonal stimuli in specific tissues and organs. Moreover, women with PCOS may have different and very diverse phenotypes, which normally remain unconsidered in clinical trials. Investigating the effects of inositol therapy on the different PCOS phenotypes would be highly informative in order to better tailor the supplementation.

Considerations on the role of inositols in the ovaries prompted some of the authors of the present Expert Opinion to hypothesize that intrinsic MI-to-DCI ratios drive the fate of follicles during the selection process, determining which is more likely to become dominant [33]. Supporting this idea, anovulatory women with PCOS often feature altered inositol content in the ovaries [34]. Further investigations on the inositol ratio in the granulosa cells, and on the MI-to-DCI ratio in follicles that undergo atresia, are called for to further discuss such theory. Studies on inositol content of mammalian follicles are ongoing with the aim of determining the difference of MI-to-DCI ratios between dominant follicles and those in different stages of atresia.

Declaration of interest

G Forte and V Unfer are employees at Lo.Li. Pharma s.r.l. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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