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REVIEW

Inositols in treating polycystic ovary syndrome and non-insulin dependent diabetes mellitus: now and the future

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ABSTRACT

Introduction: This Expert Opinion covers recent updates in the use of Inositol in polycystic ovary syndrome (PCOS) and type II diabetes and gives support to researchers and clinicians.

Areas covered: This article discusses the role of Myo-Inositol (MI) and D-Chiro-Inositol (DCI) in physiological function, the use of MI in PCOS, the risks of using DCI in reproductive conditions, the 40:1 combination of MI/DCI in PCOS. Furthermore, we discuss the issues of insulin resistance and how α-lactalbumin may increase the intestinal bioavailability of MI. The paper then transitions to talk about the use of inositols in diabetes, including type II diabetes, Gestational Diabetes Mellitus (GDM), and double diabetes. Literature searches were performed with the use of PubMed, Google Scholar, and Web of Science between July and October 2023.

Expert opinion: Inositol therapy has grown in the clinical field of PCOS, with it demonstrating an efficacy like that of metformin. The use of α-lactalbumin has further supported the use of MI, as issues with intestinal bioavailability have been largely overcome. In contrast, the effect of inositol treatment on the different PCOS phenotypes remains an outstanding question. The use of inositols in type II diabetes requires further study despite promising analogous data from GDM.

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Polycystic ovary syndrome; type II diabetes mellitus; gestational diabetes mellitus; myo-inositol; D-Chiro-inositol

1. Introduction

Inositol has been the focus of much attention from the medical community in recent years, with the isomers, Myo-Inositol (MI) and D-Chiro-Inositol (DCI), having been known to play a role in treating various pathologies including polycystic ovary syndrome (PCOS), gestational diabetes mellitus (GDM), and preventing neural tube defects [1–3]. These natural molecules are key players in insulin signaling networks in addition to being involved in steroidogenesis and white adipose tissue (WAT)/brown adipose tissue (BAT) trans-differentiation [4]. MI is synthesized by the liver and kidney in the amount of 4–5 g per day [5], and the rest of the recommended daily intake is consumed through the diet primarily in fresh fruit, vegetable, nuts and seeds [6], while DCI is primarily converted from MI through the use of a tissue-specific epimerase.

MI has shown success in treating insulin dependent-hyperandrogenic PCOS. PCOS is a heterogeneous condition, and the diagnosis and treatment of this condition has caused clinicians difficulty since its initial description by Stein and Leventhal in 1935 [7]. Subsequently, many groups have attempted to standardize the definition of PCOS. This was achieved initially in 1990 by the National Institutes of Health (NIH), whereby PCOS was described as a condition presenting clinical and/or biochemical hyperandrogenism and ovulatory dysfunction in the absence of other secondary causes [8]. This was later updated in 2003 upon a meeting of the European Society for Human Reproduction and Embryology in Rotterdam, which laid out a series of diagnostic criteria, that are still used to classify PCOS to date [9]. The Rotterdam Criteria describe PCOS as a condition presenting two of three of the following: clinical and/or biochemical hyperandrogenism, ovarian dysfunction and polycystic ovary morphology (PCOM), resulting in the formation of the four diverse phenotypes for PCOS, three of which are hyperandrogenic (A, B, and C), and the normoandrogenic phenotype D. The hyperandrogenic phenotypes of PCOS are known to have a metabolic component, with insulin resistance (IR) thought to be inherently linked to the hyperandrogenism observed in these patients. Insulin sensitizers such as metformin or MI have shown success in treating this condition, bringing androgen levels back into tolerable ranges.

Given the link between inositol and PCOS with metabolic irregularities, it is not surprising that MI and DCI have been looked at as potential adjunct treatments for non-insulin

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Article highlights

- Myo-inositol (MI) and D-Chiro-Inositol (DCI) are the two most common stereoisomers of inositol and play a vital role in the regulation of insulin signaling, with MI pathways facilitating the transfer of glucose into the cell, and DCI aiding the process of glycogen synthesis.
- MI has shown demonstrable success as an insulin sensitizer in order to counteract hyperandrogenism, menstrual cycle disruption and polycystic ovarian morphology, all of which are associated with insulin resistance.
- The physiological ratio of MI and DCI is 40:1 and supplementation at the ratio has shown some success in PCOS, partially for overweight or obese patients, whereby this treatment can restore physiological ratios and make use of low doses of DCI to help treat insulin resistance in addition to obesity through its action on white adipose tissue (WAT)/brown adipose tissue (BAT) differentiation.
- The use of 40:1 MI/DCI supplementation in type II diabetes has been studied; however, only one trial has been conducted to date. MI and DCI have shown some moderate success in treating gestation diabetes millets (GDM), 'double diabetes,' in addition to diabetic nephropathy.

dependent diabetes mellitus (or type II diabetes). Type II diabetes has become more prevalent worldwide with increasing obesity numbers, according to the International Diabetes Federation 10.5% of the global population currently live with type II diabetes with this percentage expected to raise to 12.2% by 2045 [10]. While a balanced diet coupled with exercise represents the foremost treatment options, insulin sensitizers such as MI and DCI remain a promising adjunct therapy. For this review, literature searches were performed with the use of PubMed, Google Scholar, and Web of Science between July-October 2023. Search terms included: 'Polycystic ovary syndrome,' 'Inositol,' 'myo-inositol,' 'd-chiro-inositol,' 'Gestational Diabetes Mellitus' and 'type II diabetes mellitus,' in addition to combinations and abbreviations of the aforementioned search terms. This expert opinion gives an overview of MI and DCI role in insulin signaling before giving a review of the state of the art regarding inositol therapy in PCOS and type II diabetes.

2. Inositol signaling in physiological function

Inositol is known to mediate insulin signaling pathways. In detail, insulin binds to the insulin receptor which goes on to phosphorylate the insulin receptor substrate (IRS), activating phosphoinositide 3-kinase (PI3K) which stimulates the conversion of phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP3), causing a signaling cascade via the activation of phosphoinositide-dependent kinase-1 (PDK1) and pyruvate dehydrogenase kinase isoform 2 (PDK2) and ultimately protein kinase B (also known as AKT) activation [11]. AKT then inhibits glycogen synthase kinase 3 (GSK3) signaling resulting in eventual glycogen synthesis. Alternatively, AKT activation promotes Ras-associated binding (RAB) signaling, which promotes the formation of glucose transporter type 4 (GLUT-4) containing vesicles. Both MI and DCI are vital for insulin signaling due to their role as secondary messages in the form of inositolphosphoglycans, which derive from PIP and PIP2. MI is primarily involved in facilitating glucose uptake, and as such high levels of MI can be observed in the tissues, which are particularly glucose-dependent such as the brain, heart, and notably for this review the ovary. Furthermore, MI reduces the release of fatty acids from adipose tissue through adenylate cyclase inhibition [12]. In contrast, DCI is primarily involved in glycogen storage, and as such is vital for tissues involved in glycogen storage such as liver or muscle tissue. The aforementioned inositolphosphoglycans are responsible for the metabolism of glucose, with IPG-A (MI containing glycan) governing glucose catabolism and IPG-P (DCI-containing glycan) responsible for glycogen synthesis [11].

PCOS presents with a higher degree of insulin resistance than in the general population, typically 65–95% [13]. While the underlying cause and effect is not entirely understood, it is thought that insulin resistance plays a major role in the hyperandrogenic phenotypes of PCOS, namely A, B, and C, with insulin sensitizers such at metformin and inositols demonstrating to be efficacious across multiple studies [1,14,15].

3. D-Chiro-inositol in polycystic ovary syndrome?

Inositol use in PCOS was first proposed by Nestler *et al*. who treated obese women with PCOS typified by oligomenorrhea and high serum testosterone and/or hirsutism, of note 22% of the women tested were insulin resistant [16]. After a period of once daily doses of 1200 mg over 6 to 8 weeks a significant increase was observed in ovarian function (86% of the study group ovulated vs 27% for the control group). This was later replicated in lean women, resulting in significantly reduced serum testosterone and overall insulin levels [17]. A further study by Genazzani *et al*., where obese patients were treated with DCI over a period of 12 weeks, resulted in a significant reduction in androsterone and testosterone in addition to metabolic parameters such as Body Mass Index (BMI) [18]. However, DCI's use as a monotherapy is up for much debate: in a work performed by Cheang *et al*., no significant change was observed in testosterone levels following a six-month treatment of 1200 mg of DCI administered twice daily [19]. It was later discovered by Sacchi *et al*., that the treatment of granulosa cells *in vitro* with DCI inhibited the transcription of aromatase, which is a vital enzyme in the transformation of androgens to estrogens, thus inhibiting its transcription may cause increased androgen levels [20]. In agreement with the initial studies cited above, the use of DCI counteracted the insulin-dependent expression of steroidogenic enzymes; however, given the inhibition of aromatase activity, concerns remained regarding its suitability for long-term use. It should be noted that although the study by Sacchi et al. was conducted *in vitro*, it gives an indication as to the mechanism of action of DCI in the absence of results in humans. Recently, the consequences associated with high doses and prolonged DCI treatment have been discovered. It was observed by Bevilaqua *et al*., that the administration of DCI alone inhibited the gene expression of aromatase, significantly increasing testosterone levels and altering histological physiology. At increased doses of DCI (10–20 mg/day), the mice developed ovarian lesions resembling lesions typically seen in aged mice. Furthermore, they demonstrated reduced testosterone levels in the absence of altered aromatase levels, taken

together these experimental evidences suggested the failure of steroidogenic gonadal activity [21]. While not directly applicable to PCOS, further evidence of the potential risk associated with high levels of DCI treatment was observed in healthy women, whereby volunteers were treated for 1 month with 1200 mg of DCI, demonstrating a significant increase in total testosterone levels. Subsequently, the trial was terminated due to ethical concerns, suggesting that in the absence of insulin resistance DCI at this dose is not suitable for use in healthy volunteers and potentially PCOS patients; furthermore, it indicates the role of aromatase transcription inhibition in increasing androgen levels in women [22]. It is thought that a difference in dosing regimens is responsible for the conflicting results seen regarding DCI use in PCOS. Shorter treatments of a reduced dose of DCI can treat the underlying metabolic condition, resulting in an immediate drop in androgens and restored ovarian function. However, aromatase inhibition caused by prolonged DCI treatment eventually may cause an increase in testosterones, which negates the effect of DCI on insulin signaling [23,24].

4. Myo-inositol in polycystic ovary syndrome

MI has been demonstrated to reduce hormonal, metabolic, and oxidative abnormalities in PCOS patients, in addition to ameliorating IR, as has been noted by several robust reviews and meta-analyses [1,25,26]. In a double-blind study, Costantino *et al*. investigated the effects of MI on insulin levels, ovulation, and serum androgens in women with PCOS. The MI-treated patients demonstrated increased insulin sensitivity, improved glucose tolerance, and decreased insulin resistance [14]. Furthermore, a 66% decrease in serum total testosterone and a 73% reduction in serum-free testosterone concentration was observed, accompanied by a decrease in blood pressure, plasma triglyceride, and total cholesterol levels. In a similar study, Artini *et al*. measured the effect on the hormonal profile of PCOS patients following MI supplementation. In this study 50 overweight PCOS patients were split into two groups: the first, a control group, received folic acid and the second, the study group, received folic acid in combination with MI. After a 12-week treatment regimen, a significant reduction of LH, prolactin, testosterone, insulin levels and LH/FSH ratio was observed in the study group [27]. This was coupled with a significant improvement in IR, glucose to insulin ratio, HOMA index, and an improvement in menstrual regularity. MI has demonstrated success both a monotherapy, in addition to in combination with oral contraceptive pills (OCPs), whereby it was observed that the combination of OCPs and MI caused a significant reduction in hyperinsulinemia and hyperandrogenism in comparison to OCPs alone [28]. Subsequently, Phkaladze *et al*. investigated the combination of MI and OCPs in two groups of adolescents over a three-month period, in order to restore the metabolic parameters which are typically worsened through the use of OCPs. The combination group saw significant improvements in metabolic parameters such as weight (change compared to baseline, OCP only 1.00 vs OCP + MI −1.00), BMI (OCP only 0.37 vs OCP + MI −0.38), and HOMA index (OCP only −0.04 vs OCP + MI −0.45) [29]. Given the frequent use of OCPs, MI represents an adjunct therapy for reducing metabolic side effects, for use in contraceptive care and PCOS.

The use of MI as an insulin sensitizer is thought by many in the field to be a natural alternative to pharmaceuticals typically used in the case of insulin resistance, such as metformin. Metformin is prescribed in the treatment of conditions, which rely on insulin signaling and has shown success in treating hyperandrogenism as a consequence of hyperinsulinemia in PCOS. However, side effects are not uncommon in metformin treatment, with gastrointestinal issues reported in a subpopulation of patients [30]. In contrast, MI is very well tolerated, suggesting MI is optimally placed within the therapeutic space as a natural molecule that offers an alternative treatment. The efficacy of both MI and metformin was compared in 2021 by Kutenaei *et al*. via a meta-analysis [15]. In this work, it was found across nine separate studies that no significant difference could be observed in metabolic, endocrine, and reproductive parameters supporting the claim MI may be a valid alternative to metformin therapy.

While MI has demonstrated success in PCOS, this has not been observed across all patient subsets, with a minority of women suffering 'MI resistance' with reduced MI sensitivity as studies have revealed that MI can suffer from poor bioavailability [31,32]. α-Lactalbumin is a protein that is found naturally in mammalian milk, with a high concentration of essential amino acids, for this reason it is routinely added to infant milk formulas to help avoid weight gain and gastric side effects typically associated with regular formula. It has been further demonstrated to increase intestinal absorption of essential molecules consumed through the diet, such as trace elements [33]. Notably, α-lactalbumin can improve the bioavailability of MI in patients that exhibit 'MI resistance.' In an open prospective study by Montanino Oliva *et al*., 37 anovulatory PCOS patients were treated with MI, achieving an ovulation rate of 62%. The other patients were considered 'MI resistant' and subsequently received MI in combination with α-lactalbumin, achieving a rate of ovulation of 86%, coupled with an improvement in their hormone and lipid profiles [34]. Additionally, plasma levels of MI were observed to be higher than at baseline, suggesting the observed effects were due to an increase in MI bioavailability. Recently, Kamenov *et al*. conducted an open-label randomized prospective study, which compared the effect of MI supplementation alone versus MI in combination with α-lactalbumin [35]. In total 50 women with anovulatory PCOS were randomly assigned to receive one of two therapies over a three-month treatment period, with the MI α-lactalbumin group demonstrating an improvement in terms of ovulation rate and menstrual cycle versus MI alone. Furthermore, the combined treatment significantly improved the effect of MI on body weight, hirsutism, and hyperandrogenism.

5. Myo-inositol to D-Chiro-inositol ratio in polycystic ovary syndrome

As mentioned previously, DCI is effective in treating women with PCOS with IR; however, its use has been hamstrung with questions raised over its suitability as a monotherapy in women, due to its inhibition of aromatase transcription. Indeed, at higher concentrations such as a 1.2 g daily dose

for a period in excess of a month, as indicated by Nordio et al., and discussed earlier on in the text, require careful clinical monitoring [22]. However, the use of DCI in small quantities (typically in a 40:1 ratio with MI) to preserve physiological ratios has been posited as a potential therapeutic, with the aim of preserving physiological homeostasis. Furthermore, if used at small doses, DCI can impact IR, in addition to being able to improve obesity by triggering WAT/BAT differentiation.

The combination of MI and DCI aims to use DCI to reduce systemic insulin levels through DCI-mediated glycogen storage. Phosphorylated MI is involved in the signaling of FSH and insulin, whereby it can facilitate glucose uptake by the cell, thus reducing systemic glucose levels. Given its role in FSH signaling, high levels of MI in the follicular fluid have been reported as a marker of high oocyte quality [36]. During systemic insulin resistance, the ovary maintains its sensitivity to insulin. Consequently, increased insulin levels lead to increased epimerase stimulation, which catalyzes the conversion of DCI from MI, increasing DCI levels and causing a deficit of MI within the follicular fluid, impairing FSH signaling. In this context, MI supplementation provides a method to restore the ratio between DCI and MI resulting in the restoration of FSH sensitivity, thus improving oocyte quality [37], while small quantities of DCI help maintain the physiological ratio. Following a 3-month twice daily treatment regimen of 2 g total inositol in several MI/DCI ratios including 5:1, 20:1, 40:1, and 80:1, Bevilaqua *et al*. found that only the latter two were able to restore ovulation in a PCOS mouse model [38]. This effect was less evident in 80:1 than the 40:1 cohort, and while the ratio of theca/granulosa cell layer thickness was restored with both the 40:1 and 80:1 formulation, the 40:1 treatment was the most effective at restoring normal uterine function and structure, in addition to fertility outcomes. The authors of this study do not explicitly mention their rationale for choosing the ratios in this study; however, they were the same ratios used by Nordio *et al*. in a previous paper [39]. It could be hypothesized that this range considered physiological ratios typically seen in blood and within various tissues in the body, which can range from 65:35 in fat tissue to 99.5:0.5 in theca cells, with MI typically in greater amounts than DCI [40]. As such, it is important to consider these ratios when designing treatments consisting of MI to DCI, so to not disturb the homeostasis dramatically between these two stereoisomers.

Subsequently, in the first human trial to consider the different combinations of MI and DCI, Nordio *et al*. discovered that treatment of strongly insulin-resistant PCOS patients (HOMA index > 4) with MI and DCI, with a ratio beyond the physiological norm did not improve ovulation; furthermore, higher concentrations of DCI seemed to hinder MI activity and reduce reproductive function [39]. This work confirmed in humans the prior investigation performed by Bevilaqua in mice, as such the 40:1 ratio was identified as most suitable for the restoration of ovulatory function. It is the authors' opinion that MI supplementation in combination with DCI at a ratio of 40:1 is a promising approach for the treatment patients with IR, making use of the insulin sensitizing characteristics of both MI and DCI and preserving serum physiological ratios, without the associated risks of prolonged high dose DCI treatment regimens.

In a 2020 review article, Roseff *et al*. argued strongly against the seemingly random PCOS inositol treatments, which either show no efficacy or are actively harmful in the treatment of PCOS [37]. The authors posited that the reduction in MI efficacy caused by DCI was due to two separate factors: on the one hand, DCI inhibition of aromatase leads to reduced estrogen levels, which may go on to reduce blastocyst quality [41]; on the other hand, DCI may interfere with MI intestinal absorption when administered at high concentrations. Both MI and DCI use the same transporter, SMIT2 [42]: in intestinal absorption and at physiological concentrations both MI and DCI have a similar K_m value (120–150 μM vs 110–130 μM), suggesting that the amount of DCI does not interfere with MI. However, at higher concentrations DCI is able to compete with MI and thus may cause issues of bioavailability for MI [43]. It is thought that due to the severity of insulin resistance often observed in obese PCOS patients, the 40:1 ratio would be more applicable to obese populations over lean populations where metabolic irregularities are less common.

In addition to the posited combination between MI and DCI, inositols have also been explored as a potential treatment of PCOS in combination with alpha-lipoic acid (ALA) [44,45]. ALA is a natural biologically active molecule involved in various physiological processes, demonstrating anti-inflammatory, immunomodulatory, antioxidant, detoxifying, and notably, insulin sensitizing effects [46]. The inositol/ALA combination has primarily been suggested for use in PCOS in patients with a familial history of diabetes. It has been reported that patients with a familial history of diabetes have impaired epimerase activity leading to reduced DCI conversion, and therefore may become more prone to develop insulin resistance, as such the insulin sensitizing properties of ALA may aid these patients [47]. However, the effectiveness of ALA in combination with inositols has been brought into question, as few studies have been performed which demonstrate a significant increase in inositol activity as a result of the use of ALA, with the association between the two molecules not being well understood. Furthermore, across eight clinical trials ALA did not influence reproductive hormones, with its properties seemingly limited to an insulin sensitizing effect and any hormonal effect being due to action of MI [48]. Therefore, it is apparent further studies are required to properly interrogate this hypothesis; however, the use of ALA seems to be at best a symptomatic treatment of insulin resistance, rather than addressing the underlying hyperandrogenism, it is therefore not recommended for use in PCOS until further efficacy can be proven [48]. A summary of the notable human trials regarding the use of inositols in PCOS is available in Table 1.

6. Myo-inositol to D-Chiro-inositol ratio in type II diabetes

Due to the role Inositol plays in various metabolic diseases and given the link between inositol action and insulin signaling, type II diabetes is an obvious potential application. In the first study of its kind, Pintaudi *et al*. explored the use of 40:1 MI/DCI in type II diabetes, observing improved glycemic para-

Table 1. A summary of the notable human trials regarding the use of inositols in PCOS.

Paper title	Study type	Treatment	Duration	Number of subjects	Inclusion criteria	Exclusion criteria	Summary of findings
Nestler (1999) [16]	Randomized controlled	Treatment group: DCI 1200 mg/d Control group: Placebo	6–8 weeks	Total 44 Treated 22 Control 22	Age 18–40 PCOS according to NIH criteria BMI >28 Normal thyroid function PRL levels, BMI > 26	Diabetes, medications in the last two months	• DCI increased insulin sensitivity and ovulatory function. • DCI reduced serum androgen concentrations, blood pressure, and triglyceride concentrations.
Iuorno (2002) [17]	Randomized double blind	Treatment group: DCI 600 mg/d Control group: Placebo	6–8 weeks	Total 20 Treated 10 Control 10	Age 18–40 PCOS according to NIH criteria BMI>28 Normal thyroid function PRL levels, BMI>26 BMI 20.0–24.4	Diabetes, medications in the last two months	• In lean women, DCI decreased circulating insulin, serum androgens, and improved some metabolic parameters such as blood pressure.
Genazzani (2014) [18]	Non-randomized trial	Treatment group: DCI 500 mg/d	12 weeks	Total 22	PCOS according to Rotterdam, normal PRL levels, BMI>26	Enzymatic adrenal deficiency and/other endocrine disease, hormonal treatment in the 6 months prior to the study.	• DCI increased insulin sensitivity and improved hormonal patterns in obese hyperinsulinism PCOS patients, in particular those with a history of familial diabetes.
Cheang (2008) [19]	Randomized controlled	Treatment group DCI 1200 mg twice daily Control: placebo twice daily	6 weeks terminated early due to a sudden unavailability of drug	Total 11 Treated 6 Control 5	Age 18–40 PCOS according to NIH criteria.	Hyperprolactinemia, thyroid dysfunction, late-onset adrenal hyperplasia, diabetes, no oral contraceptives or insulin mediating medications 3 months prior to treatment	• In all subjects, increased release of DCI-IPG was observed regardless of treatment group. This resulted in an increase in insulin sensitivity and vice versa.
Nordio (2023) [22]	Retrospective study	Treatment group DCI 1200 mg/d	6 months	Total 20	Age 18–50, glycemia ≥100mg/dL, HOMA IR ≥2.5	Pregnancy, delivery in prior 6 months, menopause (natural or iatrogenic); alcohol or drug abuse; overt diabetes, tumor lesions, treatment with drugs, or other supplements containing inositols, steroidal hormone unbalance.	• DCI treatment resulted in a significant decrease of BMI, glycemia, insulinemia, HOMA-IR, serum levels of LH, total testosterone and DHEAS. • Serum estradiol and menstrual abnormalities increased during the treatment
Nordio (2023) [22]	Non-randomized trial	Treatment group DCI 600 mg twice daily	6 months but terminated after 1 month due to safety concerns	Total 10	Age 18–50. Good state of health, regular menstrual cycle.	Pregnancy, breastfeeding, menopause, alcohol or drug abuse, HOMA IR ≥2.5, other medical morbidities such as hypertension or PCOS, oligo/amenorrhea, current treatment with corticosteroids or hormones, use of GnRH or SPRMs within the last 6 months.	• All women exhibited a significant increase in testosterone and asprosin levels after 1 month of treatment, leading to termination of the study.

(*Continued*)

Table 1. (Continued).

Paper title	Study type	Treatment	Duration	Number of subjects	Inclusion criteria	Exclusion criteria	Summary of findings
Artini (2013) [27]	Randomized controlled	Treatment group: MI 2 g + FA 200 mg/d Control group: FA 200 mg/d	12 weeks	Total 50 Treatment 25. Control 25	PCOS according to Rotterdam, overweight, PRL 5–25 ng/ml	Enzymatic adrenal deficiency and/or other endocrine disease, hormonal treatment in the 6 months prior to the study,	• Plasma LH, FSH, PRL, E2, 17OHP, A, T, glucose, insulin, and LH/FSH levels were significantly reduced. HOMA was significantly increased due to MI treatment. • In all amenorrheic and oligomenorrheic patients' menstrual regularity were restored vs no change in the FA only group.
Minozzi (2011) [28]	Prospective open labeled	Treatment group: low dose OCP[1] + 4 g of MI and FA 400 µg. Control group: low dose OCP[1]	12 months, 21 days of assumption followed by 7 days of suspension.	Total 155 Treatment 80 Control 75	PCOS according to Rotterdam	Secondary endocrine disorder, wish to conceive in the next 12 months, oral contraceptive use. No hypertension, diabetes or cardiovascular events, oral contraceptive treatment, or other drugs in the 6 months prior to the study.	• The treatment group demonstrated significantly decreased hyperinsulinemia. • The lipid profile of the treatment group was significantly improved as evidenced by reduced LDL and enhanced HDL levels.
Pkhaladze (2021) [29]	Randomized controlled study	Group A low dose OCP[2] Group B MI 2 g + α-LA 50 mg + FA 200 mcg. Group C low dose OCP[2] + MI 2 g + α-LA 50 mg + FA 200 mcg. Twice daily	3 months	Total118 Group A: (Aged 13–16) 21; Aged 17–19) 19, Group B: (Aged 13–16) 18; Aged 17–19) 20, Group C: (Aged 13–16) 19; Aged 17–19) 21.	Age 13–16 or 17–19, PCOS according to the adolescent diagnostic criteria,	Patients within 2 years of menarche, BMI >25	• Patients aged 13–16 treated with MI exhibited a significant reduction in weight and BMI, in addition to metabolic and hormonal parameters. • Patients aged 17–19 treated with MI in combination with OCP did not undergo increases in weight and BMI associated with OCP treatment and demonstrated improvements in metabolic and hormonal profiles.
Montanino Oliva (2018) [34]	Open and prospective study	Preliminary phase all patients MI 2 g/d. Main phase MI resistant group: MI 2 g + α-LA 50 mg. Twice daily	Preliminary phase 3 months, Main phase 3 months	Total 37 MI resistant group 14	Age 20–35, PCOS according to Rotterdam	Other conditions causing ovulatory dysfunction or androgen excess, in take of other drugs that could influence ovulation, obesity, women with partners with sperm abnormalities.	• During the preliminary phase 14 (38%) of patients were MI resistant. • Subsequent treatment with MI + α-LA induced ovulation in 12 (86%) women in this group, with increase MI levels demonstrating a higher MI bioavailability.

(*Continued*)

Table 1. (Continued).

Paper title	Study type	Treatment	Duration	Number of subjects	Inclusion criteria	Exclusion criteria	Summary of findings
Kamenov (2023) [35]	Open label-randomized prospective study	Treatment group: MI 2 g + α-LA 50 mg. Control group: MI 2 mg	3 months	Total 60 Treatment 30 Control 30	Age 18–45. PCOS according to Rotterdam.	Other conditions causing ovulatory dysfunction or androgen excess, intake of other drugs that could influence ovulation, >5% change in bodyweight in the last 3 months, intake of inositol containing supplements or pharmacological treatment with pioglitazone or metformin in the last three months, diseases related to digestive absorption.	• MI + α-LA improved ovulation rate and menstrual cycle over MI alone. • Body weight improved in the treatment group, while no change was seen in the control. • A greater improvement was observed in hyperandrogenism due to the combination vs MI alone.
Nordio (2019) [39]	Randomized open label	Treatment groups: MI + DCI in 7 different ratios (0:1, 1:3.5, 2.5:1, 5:1, 20:1, 40:1,80:1) amounting to 2 g, taken twice daily	3 months	Total 56 8 patients per treatment group	Age 18–45. PCOS according to Rotterdam	Other pathologies or age-related conditions that cause ovulatory dysfunction, androgen dysfunction or poor ovarian reverse. The intake of drugs that influence ovulation. BMI > 29.9, women with partners with sperm abnormalities,	• The 40:1 ratio significantly restored ovulation compared to other ratios. • At ratios below 40:1 efficacy was significantly reduced across hormonal and metabolic parameters.
Genazzani (2019) [44]	Retrospective study	Group A: MI 1 g/d Group B: ALA 400 mg/day Group C: MI 1 g + 400 mg/day	12 weeks	Total 76 Group A: 24 Group B: 24 Group C: 28	PCOS according to Rotterdam, PRL 5–25 ng/ml, BMI >25.1	Enzymatic adrenal deficiency and/or other endocrine disease including diabetes.	• Group A: MI improved hormonal profiles and insulin response to OGTT in PCOS without familial diabetes. • Group B: ALA improved insulin response to OGTT, and metabolic parameters with no effect on hormonal profile. • Group C: MI + ALA improved insulin response to OGTT, and metabolic and hormonal profiles in all patients.
Genazzani (2018) [45]	Retrospective study	Treatment group: ALA 400 mg/d	3 months	Total 32	PCOS according to Rotterdam, PRL 5–25 ng/ml, BMI >25	Enzymatic adrenal deficiency and/or other endocrine disease including diabetes, hormonal treatment in the last 6 months	• ALA administration decreased insulin and glucose levels, BMI, HOMA, hyperinsulinemia and insulin response to OGTT. • No hormonal response was observed.

ALA (alpha-lipoic acid), BMI (body mass index, kg/m2), DCI (D-chiro-inositol), DCI-IPG (D-chiro-inositol-containing inositolphosphoglycan), FA (folic acid), HOMA-IR (Homeostatic Model Assessment for Insulin Resistance, α-LA (α-lactalbumin), MI (myo-inositol), NIH (National Institutes of Health), OCP (oral contraceptive pills, 1estradiol 30 µg/gestodene 75 µg 2(drospirenone 3 mg/ethinylestradiol 0.03 mg), OGTT (glucose tolerance test), PCOS (polycystic ovarian syndrome, PRL (prolactin).

meters in the study group vs the control after a 3-month treatment of MI/DCI [49]. Furthermore, a notable drop in blood glucose (control 192.6 ± 60.2 vs study group 160.9 ± 36.4; $p = 0.02$) and HbA1c levels (control 8.6 ± 0.9 vs study group 7.7 ± 0.9; $p = 0.02$), was observed, two metabolic parameters commonly associated with type II diabetes. The authors were unable to observe differences in other commonly altered parameters in type II diabetes such as BMI, blood pressure and lipid profile; regardless, no side effects were observed for the combined inositol treatment, suggesting the possible application of MI and DCI in type II diabetes.

In a recent randomized crossover trial in type II diabetes patients, Sanchis *et al*. studied the effect of inositol hexaphosphate (INSP6) supplementation in type II diabetes [50]. INSP6, the phosphorylated form of MI, had a beneficial effect decreasing the levels of HbA1c, a key biomarker in the identification of diabetes mellitus, and adiponectin, a adipokine, which is secreted in adipose tissue and been shown to improve insulin sensitivity [51]. Furthermore, adiponectin levels negatively correlate with obesity levels and the antidiabetic actions of adiponectin, performed by stimulating lipid oxidation and anti-inflammatory responses, are well understood [52]. High adiponectin levels have been found to decrease the risk of developing type II diabetes in Chinese populations [53], with other studies noting low levels in type II diabetic populations [54]. MI and INSP6 are not directly comparable: for example, in this study no significant decrease was observed in HOMA IR, a hallmark of MI activity. However, *in vitro* data has previously suggested MI phosphates restore insulin sensitivity in a similar fashion to MI by boosting lipid storage capacity, increasing glucose absorption, and reducing lipolysis [55]. Therefore, these results indicate that MI could have a promising application in type II diabetes.

Further evidence of the potential of inositols can be observed in the promising work conducted regarding the use of MI in GDM. GDM describes the onset of diabetes-like symptoms during pregnancy, with women typically screened for GDM via a glucose tolerance test between the 24th and the 28th week of pregnancy [56]. Treatment typically can be administered with a change in diet, however in 20% of cases insulin administration is required [57]. GDM can cause numerous adverse pregnancy complications such as high fetal weight, pre-term birth, and gestational hypertension; therefore, presenting risks to both the mother and the fetus. Two similar meta-analyses have investigated the link between MI and GDM. The smaller of the two meta-analyses considered four studies, conducted in Italy and Iran. Within this work, it was demonstrated that MI supplementation significantly lowered the incidence of GDM versus control groups. Further significant reductions were observed between fasting glucose levels and oral glucose tolerance tests after 1 and 2 hours [58].

Wei *et al*. conducted a similar meta-analysis regarding inositol supplementation. The patient population consisted of 1321 women with GDM, of whom 485 received MI and 154 received a 40:1 MI/DCI supplement [2]. In patients who received only MI supplementation the incidence of GDM was reduced, and the requirement for insulin treatment was lowered. A reduction in pregnancy complications such as preterm delivery, lower birth rate and incidence of neonatal hypoglycemia was also observed. In contrast, no difference was witnessed between MI/DCI supplementation and control cohorts across all measured parameters. However, it should be noted that due to the aforementioned aromatase transcription inhibition and the resulting increase in testosterone, DCI may not be suitable in cases of pregnancy.

There is a high degree of correlation between type II diabetes and obesity rates with both increasing throughout the 21st century [59], with obesity causing higher incidence of both insulin resistance and β cell dysfunction. Obesity is characterized by an excessive buildup of adipose tissue leading to a multitude of health problems. Adipose tissue, however, is not uniform and can exist as one of three variants: WAT, BAT, and brite tissue, which has features of both WAT and BAT [60]. Generally, WAT is stored around the body as either subcutaneous or visceral fats and is used for fat storage and energy stockpiling. Subcutaneous WAT around the gluteal-femoral region is thought to be metabolically healthy [61]; however, excessive visceral WAT is considered harmful for human health. BAT in contrast is primarily used to disperse heat and maintain homeostasis and does not appear subcutaneously. BAT is clearly identifiable through its characteristic brown color, which it gains from the high number of mitochondria within the cell. A specific protein, uncoupling protein 1 (UCP-1) is commonly used to identify BAT biochemically as it is a biomarker for WAT to BAT trans-differentiation. In a study conducted by Monastra *et al*., MI and DCI were evaluated for their ability to induce WAT/BAT differentiation, whereby it was discovered that both isomers were effective, with DCI showing activity across multiple cell lines [4]. This was coupled with an increase in mitochondrial copy number and oxygen consumption ratio. As obesity is commonly coupled with hyperinsulinemia, inositol supplementation may offer dual-pronged activity in these patients.

A recent posited potential application of inositols in relation to type II diabetes is the treatment of 'double diabetes,' a term used to define type I diabetic patients who are overweight and with a family history of type II diabetes. This can put such individuals at a higher risk of diabetic complications, and they commonly develop a form of insulin resistance that requires higher doses of insulin. Inositols have not been investigated explicitly for treatment of double diabetes; however, DCI supplementation in combination with folic acid has been explored in obese type I diabetes patients, a group that is at risk for double diabetes. In a first of its kind study, Maurizi *et al*., performed a 3-month treatment with DCI and observed a significant drop in HbA1c levels, which were maintained following a further 3-months of treatment. However, reduction in either BMI or insulin resistance in these patients failed to reach significance.

It has been considered that elevated urinary Inositol levels may serve as potential biomarkers for diabetes, with Hong *et al*. observing a significant increase in urinary MI in type II diabetes patients [62]. This is of particular importance as the current assay of choice, the 2-h oral glucose tolerance test (OGTT) has come into question, with issues of assay sensitivity and a lengthy run time being associated with the diagnostic test [63]. In the pursuit of developing a suitable metric for detecting type II diabetes the authors settled upon urinary DCI

x MI to serve as a functional biomarker (as measured in ng/ml), with a cut off value of 2.14 (sensitivity 81.3%, specificity 70.3%); however, the mechanism causing this correlation between urine inositol concentration and the prevalence of type II diabetes requires further study.

Diabetes affects the homeostasis of multiple organs, with hyperglycemia known to cause damage or improper function at multiple sites within the human body [64]. As such, damage to the kidneys, termed diabetic nephropathy, is currently anticipated to affect 1 in 5 diabetic patients through the course of their lifespans [65]. The pathogenesis of diabetic nephropathy has been intensely studied, with reactive oxygen species (ROS) thought to play a major role [66,67]. ROS are usually generated as a result of disruption of mitochondrial homeostasis or through the NADPH oxidase system [68]. Within this system, numerous pathways exist, one of which is the glucuronate/xylulose (G/X) pathway, in which disruption of the NADPH to NADP+ and NADH to NAD+ causes oxidative stress with the tubular compartment of the kidneys [69]. As part of the G/X pathway MI is converted into glucuronic acid by the oxygenase MIOX. Expressed primarily within the renal proximal tubes, MIOX is upregulated in the incidence of hyperglycemia, and its activity Is known to be modulated by several factors, notably including oxidative stress. The activity of MIOX in catalyzing MI conversion is known to result in a MI deficiency as observed in transgenic mice modified to overexpress MIOX [70]. In the same study by Sharma *et al*. it was demonstrated that MIOX overexpression worsens diabetic nephropathy through elevating the incidence of oxidant and endoplasmic reticulum-stress processes. This then feedback into promoting MIOX activation creating a vicious cycle, as such MIOX has been identified as a therapeutic target [71]. MI supplementation has been observed to ameliorate diabetic nephropathy, in a recent *in vitro* study conducted by Qi *et al*. following cisplatin treatment, MI alleviated proximal tubular cell death typically associated with cisplatin therapy, likely through combatting oxidative stress [72]. In addition, MI had a beneficial effect against renal ferroptosis, a type of cell death characterized by the generation of lipid peroxides as a result of excessive free iron [73]. Whether these beneficial effects are related to MIOX is not well understood and represents an interesting avenue of research for further mechanistic study.

7. Conclusions

This Expert Opinion has discussed at length MI and DCI in PCOS and diabetes, with these molecules gaining much interest in recent years. MI is well established as a secondary messenger of FSH and an insulin sensitizer for the treatment of PCOS symptoms such as hyperandrogenism that are associated with insulin resistance. The use of DCI in contrast has raised concerns regarding its effect on reproductive health and instead has seen success in combination with MI helping counteract insulin resistance and restore homeostatic ratios between the two isomers. The use of inositol in type II diabetes; however, is still preliminary, with only one clinical trial being conducted to date. Despite this, there is room for further investigation with similar work having been performed in GDM, double diabetes, and for counteracting diabetic nephropathy as a result of hyperglycemia. Furthermore, the action of inositols on WAT/BAT differentiation may have application in addressing obesity typically associated with type II diabetes. It is hoped that this expert opinion may trigger further research in the field so to make use of these unique and interesting molecules.

8. Expert opinion

The use of inositols has continued to grow, building upon the measurable success in PCOS toward other applications, with obesity care and diabetes care representing notable examples. MI is proven as a safe approach for treating PCOS, in particular for those patients with hyperandrogenism as a consequence of ongoing metabolic irregularities such as insulin resistance. This expert opinion has highlighted meta-studies which report no significant difference between metformin and MI, further supporting the use of MI in PCOS. The application of DCI is somewhat more complicated, as it is thought to be unsuitable for prolonged treatment in PCOS because of potential adverse effects to ovarian physiology and localized elevated testosterone levels. However, the combination of both isomers at a 40:1 ratio can aid to rebalance physiological ratios, which may become perturbed in the case of PCOS. This 40:1 ratio has been shown to be the optimal because increased DCI concentrations may impair MI entry into the cell through competition for the same cellular transport mechanisms. Accumulating evidence indicates that the 40:1 ratio supplementation of MI and DCI is most effective in obese women who may benefit more from the insulin resistance counteracting characteristics of DCI, and who typically have a more severe version of the syndrome. The story of inositol use in type II diabetes is more complex: while the outlook is promising with the initial clinical trial of MI/DCI 40:1 supplementation showing positive results, it is the only clinical trial performed in this therapeutic space to date. The promising results achieved in GDM should incentivize sorely needed further clinical studies, if the use of inositols is to continue to grow.

Furthermore, comparative studies of inositol effectiveness in type II diabetes versus and/or in combination with current standard of care type II diabetes treatments are required, since these types of meta-studies are obviously reliant on more initial clinical trials; however, such large-scale comparisons would allow inositol treatment to be better positioned within the therapeutic space. In addition, as PCOS is a disease characterized by the existence of the four Rotterdam phenotypes, further work is required to evaluate firstly the etiopathogenesis between these four phenotypes, in addition to how they respond to inositol therapy. An initial first of its kind study was recently conducted by Unfer *et al*. which demonstrated that phenotype D patients did not benefit from MI supplementation, as metabolic and hormonal alterations do not seem to be involved in the pathogenesis of this specific phenotype. However, larger trials are still required.

Within the next five to 10 years, the authors would hope that the role of MI and MIOX will become more well understood, potentially resulting in the use of MI in diabetic patients with diabetic nephropathy. If this could be combined with DCI supplementation, this would offer a dual action as insulin

resistance could be addressed with the same treatment. Furthermore, more mechanistic understanding is required for MI and DCI's role within the ovaries, for example, little is known regarding the effect of inositol supplementation on luteinizing hormone. In the short-term, this basic science would aid guide correct prescription of inositols to benefit a wider range of patients.

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