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Experts' opinion on inositols in treating polycystic ovary syndrome and non-insulin dependent diabetes mellitus: a further help for human reproduction and beyond

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REVIEW

Experts' opinion on inositols in treating polycystic ovary syndrome and non-insulin dependent diabetes mellitus: a further help for human reproduction and beyond

Fabio Facchinetti[a], Marialuisa Appetecchia[b], Cesare Aragona[c], Arturo Bevilacqua [d], Maria Salome Bezerra Espinola[c], Mariano Bizzarri [c], Rosario D'Anna[e], Didier Dewailly[f,g], Evanthia Diamanti-Kandarakis[h], Imelda Hernández Marín[i,j], Zdravko A. Kamenov[k], Eleni Kandaraki[h], Antonio Simone Laganà [l], Giovanni Monastra [c], Mario Montanino Oliva[m], John E. Nestler[n], Francesco Orio[o], Ali Cenk Ozay[p,q], Olga Papalou[h], Lali Pkhaladze[r], Giusy Porcaro[s], Nikos Prapas[t,u], Christophe O. Soulage[v], Annarita Stringaro[w], Artur Wdowiak[x] and Vittorio Unfer[y]

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ABSTRACT

Introduction: This Experts' opinion provides an updated scientific support to gynecologists, obstetricians, endocrinologists, nutritionists, neurologists and general practitioners on the use of Inositols in the therapy of Polycystic Ovary Syndrome (PCOS) and non-insulin dependent (type 2) diabetes mellitus (NIDDM).

Areas covered: This paper summarizes the physiology of Myo-Inositol (MI) and D-Chiro-Inositol (DCI), two important molecules present in human organisms, and their therapeutic role, also for treating infertility. Some deep differences between the physiological functions of MI and DCI, as well as their safety and intestinal absorption are discussed. Updates include new evidence on the efficacy exerted in PCOS by the 40:1 MI/DCI ratio, and the innovative approach based on alpha-lactalbumin to overcome the decreased therapeutic efficacy of Inositols in some patients.

Expert opinion: The evidence suggests that MI, alone or with DCI in the 40:1 ratio, offers a promising treatment for PCOS and NIDDM. However, additional studies need to evaluate some still unresolved issues, such as the best MI/DCI ratio for treating NIDDM, the potential cost-effectiveness of reduced gonadotropins administration in IVF due to MI treatment, or the benefit of MI supplementation in ovulation induction with clomiphene citrate in PCOS patients.

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Myo-inositol (MI); D-chiro-inositol (DCI); alpha-lactalbumin (alpha-LA); insulin resistance; polycystic ovary syndrome (PCOS); non-insulin dependent (type 2) diabetes mellitus (NIDDM); absorption; inositol-resistance

1. Introduction

The significant advancement of experimental and clinical research on inositols in the past few years prompted us to write a paper based on an Experts' Opinion, taking advantage from the expertise of several scientists on pathologies and disorders in which inositol-mediated pathway plays a key role. The promoters (V.U. and F.F.) selected 43 experts on the basis of their experience in the field of inositol. This selection was performed by consulting the database of PubMed/MEDLINE, the Cochrane Library, and ResearchGate. All these experts were asked for their collaboration in this initiative. Among them, 24 (56%) accepted to give their contribution, whereas 19 (44%) declined the invitation. The specific aim of this article is to offer a summary of the most recent findings on both the biochemical and physiological activities of myo-inositol (MI) and D-chiro-inositol (DCI), two essential stereoisomers, and their therapeutic applications. This Experts' Opinion follows the first document delivered by the 'International Consensus Conference on Myo-inositol and D-chiro-inositol in Obstetrics and Gynecology,' held in Florence (Italy) in 2013 and published in two different papers, one edited by Facchinetti, the other by Bevilacqua [1,2].

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Article Highlights

- Myo-inositol (MI) and D-chiro-inositol (DCI) are the most important stereoisomers of inositol: MI can be transformed into DCI by a specific epimerase under insulin control and their respective concentrations (ratio) depends on the specific tissue needs.
- The MI/DCI physiological ratio is 40:1 in plasma and 100:1 in the ovary.
- MI and DCI play several different roles, in some cases also exerting opposite effects (high concentrations of DCI in the follicular fluid are detrimental for blastocyst quality, whereas the MI role is beneficial).
- MI and DCI are insulin sensitizers although using distinct pathways. MI is involved in the cellular uptake of glucose, whereas DCI in glycogen synthesis; furthermore, DCI affects steroidogenesis. DCI reduces in dose–response manner the expression of aromatase gene (CYP19A1) and consequently the conversion of testosterone to estrogen. In addition, MI in the ovary (as InsP3) is one of the second messengers of FSH.
- MI alone or with DCI, in the 40:1 physiological ratio proven to be effective in the treatment of Polycystic Ovary Syndrome and Type 2 diabetes.
- The combination of MI and alpha-lactalbumin (alpha-LA) was found to increase the intestinal absorption and the therapeutic effects of MI.
- Several studies demonstrated that MI and alpha-LA are safe at therapeutic doses. The U.S. Food and Drug Administration included them in the list of generally recognized as safe (GRAS) compounds.

This box summarizes the key points contained in the article.

The current paper, revised and accepted by all the authors, reports the findings of the published studies on the use of inositols in polycystic ovary syndrome (PCOS) and non-insulin dependent (type 2) diabetes mellitus (NIDDM), whose onset can be favored by PCOS-related metabolic alterations [3].

2. Synthesis and activities of myo-inositol and D-chiro-inositol

2.1. The molecules

Inositols have a long evolutionary history and are believed prebiotic 'Ur' compounds [4]. This definition means that such molecules were formed on the Earth before the appearance of the first living beings (prebiotic era). Unlike reducing sugars or acyclic polyols, MI shows per se a strong stability, i.e. without having any defense given by biological structures, and this feature preserves the molecule from degradation due to oxidation or increase of temperature. Obviously, such a condition is mandatory for compounds that had to resist the huge environmental challenges present before the emergence of life. Inositols belong to the group of sugar alcohols (cyclic polyols) and can be found in nine stereoisomeric forms [5–7], among which there are MI and DCI. MI, the most abundant in animals, and DCI are essential for the cell structure and functions. In the human body, MI is actively synthesized in the kidneys, liver, testes, mammary gland, brain (kidneys can produce approximately 4 g MI per day) [8,9]; it derives from the isomerization of glucose-6-phosphate (G6P) to inositol-3-phosphate (Ins3P) by D-3-myo-inositol-phosphate synthase enzyme (inositol synthase, Ino1, or MIPS1) [10]. Then, Ins3P is dephosphorylated to free MI through inositol monophosphatase-1 (IMPA-1 or IMPase) [11]. Free inositol could be obtained also by the dephosphorylation of inositol-1,4,5-trisphosphate (InsP3) and inositol-1,4-bisphosphate (InsP2). In turn, DCI can be obtained from MI by a specific Nicotinamide Adenine Dinucleotide (NAD)–NADH-dependent epimerase, stimulated by insulin [12,13]. The endogenous production of both stereoisomers fluctuates depending on the specific tissue needs [14]: for example, in normal (healthy) women the plasma ratio is 40:1 [15], whereas in ovarian follicular fluid is close to 100:1 [16]. It is still unknown if these endogenous activities allow human beings to be independent of inositol contained in a normal diet.

2.2. MI and DCI activities

MI is one of the most important organic osmolytes [17] and probably this was one of its first functions, then become fundamental for biological structures, such as the brain and the liver. In agreement with this, accumulating evidence suggests that vital organs such, as the brain, need high MI concentrations (10- to15-fold the values detected in blood) [10].

Furthermore, inositols are deeply involved in insulin signaling (Figure 1). Insulin needs the presence of MI and DCI, to exert its activities. The two stereoisomers, in the form of inositolphosphoglycans (MI–IPG and DCI–IPG), are second messengers in the insulin signaling, mediating different effects [7,18–20]. These inositolphosphoglycans derive from glycosyl-phosphatidylinositol lipids (PIP, PIP2), situated in the cell membrane (inner leaflet), after hydrolyzation due to the stimulus of insulin.

Cellular glucose uptake is mainly under MI control, and, in keeping with this physiological role, MI content is significant in tissues with high-glucose utilization, such as the brain, the heart, and the ovary [20–23]. Moreover, MI decreases the free fatty acids release from adipose tissues by means of the adenylate cyclase inhibition [24]. In particular, elevated production of cyclic adenosine monophosphate (cAMP) in adipocytes results in increased activity of protein kinase A (PKA) [25]. Lipolysis is efficiently inhibited by insulin, which under most conditions is mediated by the lowering of cAMP levels, leading to the inhibition of PKA [26,27]. The decreased cAMP is mainly the result of insulin-mediated phosphorylation and activation of phosphodiesterase 3B (PDE3B), the main cAMP-hydrolyzing enzyme in adipocytes [28]. Insulin-induced activation of PDE3B is a process dependent on active phosphatidylinositol 3-kinase (PI3-K): in this mechanism, the inositol phosphoglycan-AMP kinase inhibitor (IPG-A) is able to inhibit both cAMP-dependent protein kinase (PKA) and adenylate cyclase [21], and leads to a reduction of lipolysis mimicking the anti-lipolytic effects of insulin [29]. More recently, inositol hexakisphosphate kinase-1 (IP6 K1), the major inositol pyrophosphate biosynthetic enzyme, has been found to modulate lipolysis via its interaction with the lipolytic regulator protein perilipin1 (PLIN1) at a protein kinase C (PKC)/PKA motif [30]: this may further stress the acknowledged pivotal role of phosphatidylinositol 4-phosphate in activating PKC-mediated pathways [31].

Instead, DCI concentrations are high in tissues that store glycogen (e.g. liver and muscle) and low in those where glucose is actively used [32]. In particular, recent pieces of evidence suggest that DCI is able to reduce free fatty acid uptake by the liver via lipid trafficking inhibition, reduced diacylglycerol deposition, and hepatic Protein kinase C epsilon type (PKCε) translocation, leading to improved insulin sensitivity by

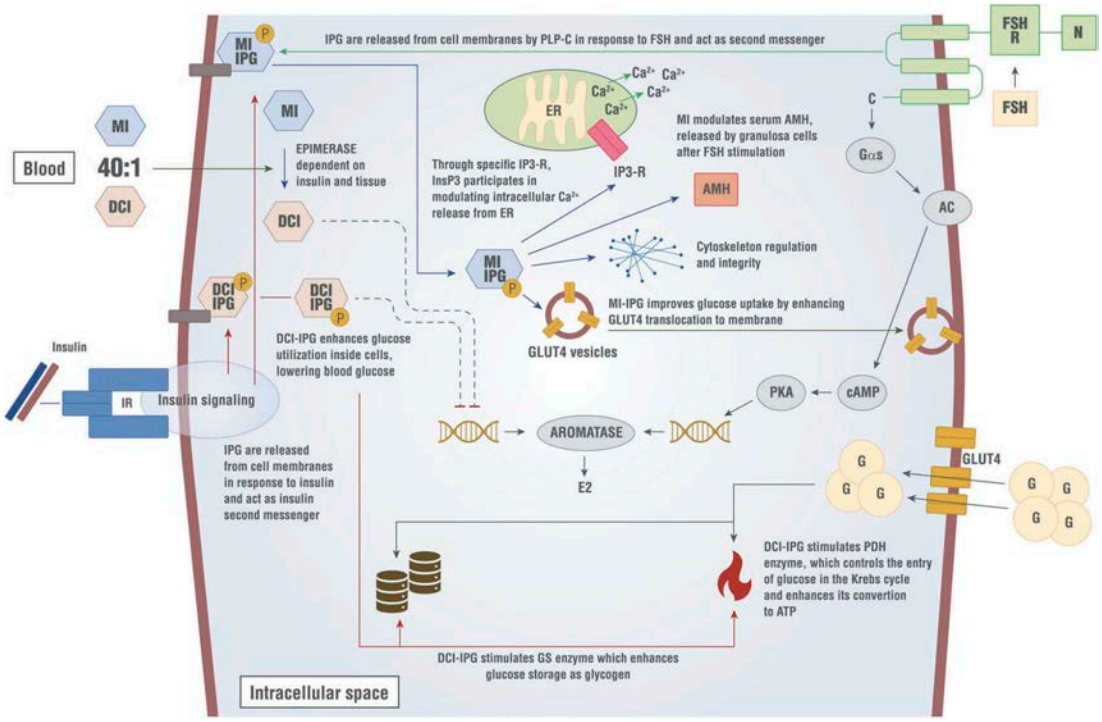

Figure 1. Myo-inositol and D-chiro-inositol mediate insulin-stimulated glucose utilization in the cell; myo-inositol is a second messenger of FSH receptor in follicular cells (granulosa) and oocytes).

AC: adenylate cyclase. AMH: anti-Müllerian hormone. ATP: adenosine triphosphate. cAMP: cyclical adenosine monophosphate. DCI: D-chiro-inositol. E2: Estradiol. ER: endoplasmatic reticulum. FSH: follicle-stimulating hormone. FSHR: FSH receptor. G: glucose. GLUT-4: glucose transporter type-4. GS: Glycogen synthase. Gαs: heterotrimeric G protein. InsP3: inositol-1,4,5-trisphosphate. IP3-R: InsP3 receptors. IPG: inositol phosphoglycan. IR: insulin receptor. MI: myo-inositol. P: phosphate. PDH: pyruvate dehydrogenase. PKA: protein kinase A.

suppressed hepatic gluconeogenesis [33]. On the one hand, DCI has been shown to decrease hepatic glucose output and the expression levels of phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase) in insulin-resistant mice through PKCε- Insulin Receptor Substrate (IRS)/PI3 K/AKT signaling pathway [34]; on the other hand, these actions of DCI were confirmed also in the human liver cancer cell line HepG2 cells with palmitate-induced insulin resistance [35], suggesting a key role to prevent hepatic gluconeogenesis, reduce lipid deposition, and ameliorate insulin resistance via regulation of PKCε-PI3 K/AKT axis.

Glucose metabolism is shifted toward glycogen synthesis by DCI-containing glycan (IPG-P), and toward glucose catabolism by MI-containing glycan (IPG-A). In the latter case, MI enhances pyruvate-dehydrogenase activity (PDH), thus improving glucose utilization. As a general consideration, being the two stereoisomers metabolically linked to each other, a drastic separation of their individual actions of them *in vivo* can be challenging.

MI seems to act at different levels of glucose concentrations in the organism. It significantly prevents glucose absorption at the duodenal level and decreases glucose rise in the blood. This finding can be explained by an interference of MI on glucose intestinal uptake [36]. Furthermore, MI improves insulin sensitivity in adipocytes by increasing lipid storage capacity and glucose uptake, and by inhibiting lipolysis, as demonstrated in an experimental research [37] carried out with 3T3-L1 cells. This *in vitro* study proved that MI exerts its effects also via the expression of peroxisome proliferator-activated receptor gamma (PPAR-gamma), a pathway only recently associated with this stereoisomer. PPAR-gamma, along with the α and β forms, is considered the master regulator of adipogenesis. In humans, PPAR-gamma is most highly expressed in the adipose tissue and, to a lesser extent, in the skeletal muscle, colon, and lungs [38]. Target genes of PPAR-gamma are involved in glucose metabolism [39], adipocyte differentiation and lipid storage [40]. In liver, PPAR-gamma contributes to triglyceride homeostasis and protects other tissues from insulin resistance [41] and triglyceride accumulation [42]. It also downregulates the inflammatory response, particularly in macrophages, probably through the inhibition of pro-inflammatory transcription factors (e.g. STAT, NF-κB, and AP-1) [43].

2.3. Inositols in the ovary and in pregnancy

In addition to the above-cited effects, MI (as InsP3) in the ovary is one of the second messengers of FSH [44]. The concentration of MI in the mammalian female reproductive tract is substantially higher than in blood serum, suggesting that MI plays specific roles at the ovarian level, ensuring correct oocyte maturation [45]. On the other hand, two specific DCI effects should be taken into consideration. In fact, DCI was shown to affect the activity of aromatase enzyme, also called estrogen synthetase, that was detected in fat tissue, ovaries, testicles, placenta, brain, bone, etc. [46]. DCI reduces in dose–response manner the expression of aromatase gene (CYP19A1) and consequently the conversion of testosterone to estrogen [47]. Therefore, the aromatase inhibitors entail a systemic increase in testosterone levels and

a parallel reduction of estrogens. Moreover, it was demonstrated that the IPG-P second messenger (with DCI), acting as insulin mediator, directly stimulates (in dose–response manner) testosterone biosynthesis by human theca cells from healthy and PCOS patients [48]. This effect is more than four times higher in PCOS theca cells than in cells from normal women and contributes to explain the higher amounts of testosterone produced in PCOS patients, in comparison with healthy subjects [48]. In response to FSH and LH, Gαq (a subunit of the heterotrimeric Gq protein) activates PLP-C, which promotes the hydrolysis of phosphatidylinositol bisphosphate (PIP2) from the cell membrane, yielding inositol trisphosphate (MI–InsP3) and diacylglycerol. Another FSH pathway is the cAMP/PKA-mediated induction of aromatase, leading to estrogen biosynthesis from androgens. It was seen in mouse models that MI seems to stimulate the meiotic progression of oocytes into fertilization-competent eggs, as its depletion within the ovary can alter the physiological process of oocyte maturation [49,50]. Moreover, MI is necessary to accelerate the oviduct transport of oocytes [51]. Literature data indicate that MI signaling may also increase anti-Müllerian hormone (AMH) production, induced by FSH, in the granulosa cells of non-PCOS patients with diminished ovarian reserve (DOR) [52]. In this scenario, each organ can regulate the intracellular balance of inositol levels to achieve a tissue-specific intracellular MI/DCI ratio that modulates metabolic processes [16]. In the ovaries, such ratio was found to be 100:1 [16].

The excess of DCI in ovarian follicles is potentially unfavorable in some cases. A recent study successfully related for the first time the concentrations of MI and DCI in the follicular fluid to the quality of blastocysts; according to the results of this study, DCI concentrations above the MI/DCI limit ratio of 70:1 in follicular fluid decrease blastocyst quality [53]. Notably, DCI decreases in dose–response manner the expression of the aromatase gene, named CYP19A1 [47]. Aromatase is an enzyme involved in the transformation of androgens to estrogens; hence, the partial inhibition of its activity may cause an increase of androgen levels [54], which can help to explain the worsening of oocyte and blastocyst quality observed with high DCI levels.

During pregnancy, endogenous MI seems to play a role of paramount importance in the development of the embryo: in particular, at mid-gestation MI concentrations in mixed-umbilical cord serum is five-fold higher than in the mother [8]. At the end of pregnancy, they decrease, although remaining two-to threefold higher than in the mother [8]. In addition, MI seems to prevent oxidative stress damage in the mother and fetus during gestation.

3. Safety of inositols

MI is a natural molecule, used as a nutritional supplement, which proved to be very safe. The United States Food and Drug Administration (FDA) included specifically MI in the list of generally recognized as safe (GRAS) compounds [55]. Such designation means that the substance is judged safe by experts, and is exempted from the usual Federal Food, Drug, and Cosmetic Act (FFDCA) food additive tolerance requirements. Several studies supported this statement [56], also with reference to pregnancy. A recent meta-analysis on 965 pregnant women affected by gestational diabetes mellitus (GDM) randomized to receive inositol or placebo or no treatment found no adverse events. Of note, no congenital malformations were reported either in the fetuses and in the newborns [57]. Moreover, no adverse events associated with antenatal inositol supplementation were reported in studies assessing the relationship between inositol and GDM and included in the Cochrane Review [58].

4. Inadequate intake of inositol

Recent findings strongly support the health benefits of supplementing a normal diet with an extra 1–4 g of inositols per day [7]. Few studies specifically investigated the causes of a critical inositol deficiency and its involvement in the onset of pathological diseases. It is likely that the modern low-fiber diet is one of the reasons. Currently available food, indeed, is processed to eliminate phytates, the main dietary source of inositols. Several lines of evidence suggest the link between diet and inositol deficiency [59]. Such deficiency may be involved also in the pathogenesis of both cancer and metabolic diseases, such as PCOS, diabetes, and metabolic syndrome. Depletion of cellular content of MI (as well as its isomers and phosphorylated derivatives) occurs in both diabetic and cancerous tissues, while deregulation of MI metabolism characterizes a number of conditions (PCOS, metabolic syndrome), mechanistically and epidemiologically associated with high-glucose diet or altered glucose metabolism.

5. Inositol therapy in Polycystic Ovary Syndrome (PCOS)

A crucial effect exerted by MI and DCI in PCOS patients is the insulin-sensitizing action, which improves insulin resistance, mirrored by the homeostatic model assessment (HOMA-IR) index decrease. At the systemic level, DCI improves several components of the metabolic syndrome. In particular, it improves glucose tolerance, decreases systolic and diastolic blood pressure, reduces triglyceride levels, and, up to certain concentrations, lowers serum testosterone [60,61]. The two stereoisomers were found useful in the treatment of the insulin resistance states [62].

5.1. Inositols in an animal model of PCOS

Induction of PCOS in rodents through light cycle manipulation is a well-established model, as LH surges that trigger ovulation is controlled by cyclic light-dark photoperiods [63]. Such a physical mechanism to induce PCOS may have advantages in avoiding the off-target effects of hormone inducers of PCOS models, which may differ from naturally occurring PCOS in women, while the use of hormonal drugs to induce PCOS can dramatically alter other endocrine factors (including downregulation of estrogens) [64]. Exposure of adult rats to continuous light, instead, leads to the gradual development of chronic anovulation [65]. These considerations do not mean that the light-based model is deprived of limitations. Nevertheless, these data may suggest that it is effective in

inducing PCOS while being devoid of some shortfalls showed by other models.

A preclinical study [66] was recently carried out with this animal model of PCOS. Female mice were exposed to continuous light for 10 weeks, developing an androgenic-like phenotype of the ovaries as in PCOS women. The authors provided the first experimental evidence that the efficacy exerted by various MI/DCI molar ratio (5:1; 20:1; 40:1; 80:1) changes, supporting the metabolic link between the two stereoisomers, specifically for PCOS. MI and DCI, being stereoisomers, have the same molecular weight; therefore, the molar ratio is equivalent to mg:mg ratio. The daily treatment of mice with 420 mg/kg MI/DCI in a 40:1 molar ratio allowed to obtain a rapid and almost full recovery from PCOS signs and symptoms. Since the hypertrophy of the theca cell layer is a hallmark of PCOS, strongly associated to an increased production of androgens [67], it is noteworthy that the ovaries from treated mice recovered normal histological features, with reduced ratio between theca and granulosa cell layer thickness. This means that the androgenic phenotype was efficaciously reversed. The other MI/DCI ratios were less effective or even exerted negative effects on the clinical pathological conditions. In particular, the formula with high DCI content demonstrated to be unfavorable, worsening PCOS pathological features. In this regard, a very stimulating hypothesis was formulated few years ago, based on the so-called 'ovarian paradox.' Since the ovaries, unlike the liver and muscle, never become insulin resistant, it was suggested that the overproduction of insulin in PCOS patients might induce an enhanced MI to DCI epimerization into the ovary, which means a DCI increase level and MI deficiency [68]. This hypothesis was proven by two independent laboratories [13,16]. In particular, a study carried out on normal and PCOS patients gave the first empirical evidence of the unbalanced MI-DCI ratio in the ovary of PCOS women [16]. Such ratio drops from 100:1 in healthy women to 0.2:1 in PCOS patients. Since MI is one of the second messengers of follicle-stimulating hormone (FSH) [19], this disorder can impair even FSH signaling.

Therefore, the ovary of PCOS patients suffers from a specific MI depletion and a DCI overload, with impaired FSH signaling and oocytes of poor quality [69]. The restoring of physiological levels of the two stereoisomers in the follicular fluid is crucial for the ovarian functionality [16].

The seminal paper by Heimark [13] demonstrated that 'in PCOS theca cells the inositol imbalance goes in the opposite direction to that observed in insulin-resistant cells, and there is a decreased M/C ratio and an increased myo-inositol to chiro-inositol epimerase activity'. Epimerase has been purified and characterized from rat liver [12], while epimerase activity has been investigated in adipose tissue [70], as well as in ovary (theca) cells [13]. Considering that ovary does not become insulin resistant (as happened in other tissues, like muscle, adipose tissue), upon persistent insulin stimulation, the epimerase activity increases leading hence to sustained conversion of MI into DCI. It is questionable that the impaired MI/DCI ratio in the ovary of PCOS animals could be ascribed to increased renal excretion of MI, as the renal clearance increases for both the inositol isomers in those conditions [71,72]. It is conceivable that deregulation of glucose metabolism (diabetes and insulin resistance) can induce a 'generalized' depletion in inositol content, as previously suggested [59,73]. For example, increased MI and DCI excretion is reported in pathological conditions such as type 1 and type 2 diabetes as well as renal failure [74–76].

In conclusion, it is unlikely that this augmented renal clearance could explain the specific imbalance in the MI to DCI conversion experimentally observed by Haimark et al. [13].

5.2. Inositols in human studies

The results obtained in the animal model were anticipated by human studies in which – besides the limitations due to the insufficient number of patients and the lack of randomization – the treatment with inositols proved to be safe and effective in improving several clinical features of PCOS. Such studies are included in a recent meta-analysis [77] that evaluates the efficacy of treatments with MI, alone or combined with DCI (40:1 ratio between MI and DCI) for 12–24 weeks, in nine randomized controlled trials (RCTs) comprising 247 cases and 249 controls [78–86]. The authors took into consideration fasting insulin concentrations as a primary outcome, and HOMA-IR index, testosterone, androstenedione, and sex hormone binding globulin (SHBG) plasma levels as secondary ones. Significant reductions in fasting insulin (standardized mean difference = −1.021 μU/mL, 95% CI: −1.791 to −0.251, P = 0.009) and HOMA-IR index (standardized mean difference = −0.585, 95% CI: −1.145 to −0.025, P = 0.041) were found after inositol supplementation. The meta-analysis, in particular, suggests a slight trend toward testosterone decrease was observed with respect to controls, whereas androstenedione levels remained unchanged. Finally, MI was able to significantly increase SHBG levels only after at least 24 weeks of administration (standardized mean difference = 0.425 nmol/L, 95% CI: 0.050–0.801, P = 0.026). This evidence strongly suggests that the findings on the primary outcome are conclusive. Concerning the androgenic hormones, the different effects obtained on androstenedione and testosterone levels should be further investigated. The authors of this meta-analysis recommended to avoid exclusive DCI supplementation for three reasons: (a) high doses of DCI/day can be detrimental for ovaries and oocyte maturation; (b) DCI cannot be converted into MI, losing the action of the latter; (c) MI and IPG-A deficiencies are correlated with many conditions of insulin resistance. In conclusion, the meta-analysis gave a new strong support to the supplementation of MI to improve the metabolic profile of PCOS patients.

These results were also supported by another systematic review and meta-analysis [87] of 10 RCTs (a total of 573 patients) [78–81,84,85,88–91]. Total testosterone, estradiol (E2) and HOMA-IR index were the primary outcomes. In comparison with the control group, MI administration exerted significant effects, improving HOMA-IR index (Weighted mean difference −0.65; 95% CI −1.02, −0.28; P = 0. 0005) and rising E2 levels (Weighted mean difference 16.16; 95% CI 2.01, 30.31; P = 0. 03). Only a tendency toward lower total testosterone levels was found. In their conclusions, the authors suggested MI administration for treating PCOS patients with insulin resistance, as well as for improving symptoms caused by decreased estrogen in this syndrome.

A study was carried out in 43 overweight and obese PCOS patients to evaluate the improvements concerning the clinical

and body composition [92]. The patients were randomly divided into three groups and treated for 6 months: group 1 (n = 21) with diet (1200 Kcal) only; group 2 (n = 10) with diet plus MI (2 g MI and 200 µg folic acid in powder, twice daily); group 3 (n = 12) with diet associated to MI and DCI in the 40:1 ratio (2 soft gel capsules, containing 550 mg MI, 13.8 mg DCI, and 200 µg folic acid, per day). Patients were blind to the treatment. The following parameters were recorded: body weight, Body Mass Index (BMI), waist circumference, hip circumference, and hirsutism (Ferriman-Gallwey score). Moreover, a bioimpedentiometry analysis (BIA) was performed to evaluate the body composition using the indices of fat mass (FM), lean mass (LM), total body water (TBW). At the end of the treatment, weight, BMI, waist and hip circumferences decreased significantly in all the patients. The addition of MI plus DCI to the diet seems to accelerate the diminution of weight and FM, with a slight increase of LM. The three groups did not show any significant difference regarding the improvement of the Ferriman-Gallwey score. Instead, the patients significantly differed with regard to the restoration of menstrual regularity. Only 57.2% in group 1, 80% in group 2, and 100% of patients in group 3 reached this goal, respectively. In conclusion, the addition of MI plus DCI in the 40:1 ratio to the diet contributed to improve at most the restoration of regularity of the menstrual cycle.

A recent clinical trial [93] provided additional support to define the best posology with inositols in the treatment of PCOS. This randomized, interventional, open-label study, for the first time, directly compared the efficacy of seven different MI/DCI ratios administered to PCOS patients. Fifty-six women were randomly allocated into seven groups of eight patients each and treated with DCI alone, and in 1:3.5; 2.5:1; 5:1; 20:1; 40:1, 80:1 MI/DCI ratios (in total, 2 g of inositols twice daily) for a period of 3 months. Ovulation was the primary outcome whereas the secondary outcomes included BMI, menses, basal and postprandial insulin levels, HOMA-IR index, FSH, LH, sex hormone binding globulin (SHBG), 17-β-Estradiol (E2), free testosterone. Among the seven tested formulations, the 40:1 ratio achieved the best results, restoring ovulation in 62.5% of women. No ovulation at all was restored with the higher DCI doses, whereas the 5:1, 20:1, and 80:1 ratios were able to induce ovulation however in a lower percentage respect to the 40:1. Furthermore, such ratio more effectively normalized basic parameters such as progesterone, LH, SHBG, estradiol, testosterone, whereas HOMA-IR index similarly improved in all the groups. The results obtained by the 40:1 were significant in comparison to the other formulations, except for HOMA-IR index. Interestingly, these results [93] agree with the study carried out with the PCOS mice [66]. The authors highlighted that the two stereoisomers, even if they share almost the same chemical structure, in some cases demonstrate a different clinical efficacy (for a summary of the most significant studies supporting the inositol use in PCOS patients, see Table 1). In conclusion, the 40:1 MI/DCI ratio is the supplementation approach which could grant the better expectation, as also supported by some clinical experiences (total daily amount of inositols: 4 g subdivided into two doses for at least 3 months or better 6 months). Also, the two Consensus documents on inositols, published few years ago, supported this statement [1,2]. Available pieces of evidence clearly indicate that ovarian epimerase in patients with PCOS works excessively, exposing these women to high concentrations of local DCI. Hence, the better approach at this stage is to use very low doses of DCI as a supplement in PCOS, in consideration that DCI never can be re-converted to MI. Since MI and DCI are in homeostasis in healthy menstruating women (40:1 in circulation; 100:1 in follicular fluid), a rational approach to supplementation should remain within these parameters.

5.3. Treatment of hirsutism

With reference to the phenotypic effects, such as hirsutism due to high testosterone levels (hyperandrogenism), some studies were carried out only with MI. In a clinical trial [94] forty-six women affected by mild or moderate hirsutism were administered with MI (2 g twice daily) for 6 months. At the end of treatment, the authors observed a significant hirsutism decrease ($p < 0.001$) detected by using a modification of the Ferriman–Gallwey score [95] which quantifies the terminal hairs in nine areas of the body. Furthermore, total androgens, FSH, LH, and LDL cholesterol concentrations decreased whereas estradiol and HDL cholesterol concentrations raised. Insulin resistance ($p < 0.01$) significantly decreased. In conclusion, MI administration effectively controlled hyperandrogenism and hirsutism in this study [94], reducing the phenotypic and hormone parameters. Combined oral contraceptives (COCs) are also used to treat PCOS women, when they do not desire to get pregnant. These compounds allow to regularize the menstrual cycles, protect the endometrium, and improve several symptoms, such as those caused by hyperandrogenism (hirsutism, acne, etc.) [96]. COCs are effective; however, they may induce severe side effects (i.e. hepatotoxicity) [97,98]. It was demonstrated that a strategy to overcome these problems can take advantage from the contemporary administration of COCs and MI [99]. The patients (155) were divided into two groups and treated daily for 12 months: 75 women received a monophasic low-dose of COCs (EE 30 mg/gestodene 75 mg) in a cyclic regimen (21 days of assumption followed by 7 days of suspension); the other 80 patients were given the same treatment plus 4 g MI. This combination obtained a better performance, respect to COCs alone, in terms of clinical and biochemical hyperandrogenism and hyperinsulinemia control. Therefore, the addition of MI allows to decrease the active dose of COCs, in such a way reducing the risk of their side effects. Noteworthy, a clinical trial [100] with PCOS women treated with MI (1 g plus 100 µg folic acid twice a day; n = 52) or COC (2 mg cyproterone acetate and 0.035 mg ethinylestradiol; n = 54) was recently performed: MI was administered continuously for 12–16 weeks whereas COC was given for 21 days (the following 7 days without treatment) and the cycle was repeated for 3 months. At the end of the study, HDL increased and LH decreased more significantly only in the COC group when compared with baseline. On the other hand, in the MI group, fasting glucose, LDL, DHEAS, total cholesterol, and prolactin levels decreased significantly. Progesterone and AMH levels, ovarian volume, ovarian antral follicle, and total antral follicle counts decreased significantly in both groups. MI in a greater extent in comparison to COC

Table 1. Studies with the most significant scientific evidences supporting the inositol use in PCOS patients.

	Angik R. (2015) Ref #132	*Artini PG. (2013) Ref #78*	*Benelli E. (2016) Ref #84*	*Bevilacqua A. (2019) Ref #66*	*Costantino D. (2009) Ref#79*	*De Leo V. (2013) Ref #134*
Type of study	RCT	RCT	RCT	Preclinical study	DBR CT	RCT
Patients, treatment and duration of the study	Group 1 (50): 1 g MI twice daily Group 2 (50): 500 mg metformin twice daily 24 weeks	Group 1 (10): 2 g MI + 200 µg f.a.; Group 2 (10): 200 µg f.a. 12 weeks	Group 1 (21): 550 mg MI + 13,8 mg DCI (soft gel cps 40:1 ratio) + 400 µg f.a. Group 2 (25): 400 µg f.a. 24 weeks	Treated (45): 420mg/kg total MI + DCI (at different ratios) in 2ml drinking H2O/day Control (6): plain water 10 days	Group 1 (23): 4 g MI + 400 µg f.a. Group 2 (19): 400 µg f.a. 12-16 weeks	Group 1 (20): 1,5 g MI twice daily Group 2 (20): 850 mg metformin twice daily 24 weeks
Principal outcomes	BMI, WHR, Ferriman-Gallwey score, ovarian volume antral follicle count, fasting insulin and glucose, post meal insulin and glucose, HOMA-IR, testosterone, LH	LH, FSH, prolactin, estradiol, androstenedione, 17-hydroxyprogesterone, testosterone, glucose, insulin, C peptide concentrations, BMI, HOMA-IR, glucose to insulin ratio, menstrual cycles	FSH, LH, 17-β-estradiol, SHBG, androstenedione, DHEA-S, free testosterone, HOMA-IR, fasting glucose and insulin	Macroscopic aspects of uteri/ovaries, reproductive capability	Systolic and diastolic blood pressure, triglycerides, total cholesterol, fasting insulin, fasting glucose, glucose AUC, insulin AUC, index of composite whole body, insulin sensitivity	BMI, FSH, LH, total and free testosterone, SHBG androstenedione, fasting glucose and insulin, total cholesterol, LDL and HDL cholesterol, triglycerides, HOMA-IR
Significant results	In both groups: reduction of Ferriman-Gallwey score, BMI, WHR, fasting insulin and glucose, post meal insulin and glucose, HOMA (significant only in group 1), testosterone, LH, LH/FSH ratio, mean ovarian volume and antral follicle count (AFC)	Group 1: significant reduction of plasma LH, prolactin, testosterone, insulin levels and LH/FSH ratio; improvement of glucose-to-insulin ratio and HOMA index and restoration of menstrual cyclicity	Group 1: significant reduction of LH, free testosterone, fasting insulin, and HOMA index; significant increase of 17-β-estradiol levels	Faster recovery of physiological ovarian phenotype as well as a normal reproductive function when treated with a combination of Mi + DCI in the ratio of 40:1 with respect to water alone or other MI + DCI formulations. Ratio of theca/granulosa cell layer thickness was fully restored in both the 40:1 and 80:1	Group 1: improvement of insulin sensitivity and glucose tolerance. Decrease of glucose-stimulated insulin release, serum total and free testosterone, plasma triglycerides and total cholesterol, systolic and diastolic blood pressure. Ovulation: 69,5% in group 1 *vs* 21% in group 2	In both groups: significant reduction of BMI, modified Ferriman-Gallwey score, LH, total and free testosterone, androstenedione, fasting glucose and insulin, HOMA-IR, total cholesterol, LDL cholesterol, triglycerides. Significant increase of SHBG and HDL only in group 2
Side effects	16% in group 1 and 72% in group 2	No	No	no	No	Yes (mainly in group 2)

	Dona G. (2012) Ref #88	*Emekci Ozay O. (2017) Ref #86*	*Fruzzetti F. (2017) Ref #90*	*Genazzani AD. (2008) Ref #80*	*Gerli S. (2003) Ref #91*	*Gerli S. (2007) Ref #81*
Type of study	RCT	RCT	RCT	RCT	DBR CT	DBR CT
Patients, treatment and duration of the study	Group 1 (18): 1,2 g MI Group 2 (8): placebo daily 12 weeks	Group 1: (52): 4 g MI + 400 µg f.a. Group 2 (54): 400 µg f.a. 12 weeks	Group 1 (25): 1,5 g metformin Group 2 (25): 4 g MI + 400 µg f.a. Control group: no treatment 24 weeks	Group 1 (10): 2 g MI + 200 µg f.a. Group 2 (10): 200 µg f.a. 12 weeks	Group 1 (136): 100 mg MI twice daily Group 2 (147): placebo 16 weeks	Group 1 (45): 4 g MI + 400 µg f.a. Group 2 (47): 400 µg f.a. 16 weeks
Principal outcomes	Testosterone, androstenedione, insulin, glucose, HOMA-IR	Progesterone, fasting insulin and glucose, HOMA-IR, total rFSH used, duration of the rFSH administration, trigger day endometrial thickness, >17mm follicle number, clinical pregnancy rate, abortion rate, cancelled cycle number	BMI, menstrual cycle length, 17-hydroxyprogesterone, testosterone, DHEA-S, androstenedione, HOMA-IR	Plasma LH, FSH, prolactin, E2, 17-hydroxyprogesterone, androstenedione, testosterone, glucose, insulin, C peptide concentration, BMI, HOMA-IR and glucose to insulin ratio, menstrual cycles	BMI, WHR, leptin, fasting insulin and glucose, insulin after OGTT, insulin AUC, total cholesterol, triglycerides, VLDL, LDL and HDL cholesterol	BMI, LH, testosterone, cholesterol (HDL and LDL), SHBG, free androgen index, fasting insulin, insulin AUC (OGTT), fasting glucose, leptin, inhibin B

(*Continued*)

Table 1. (Continued).

	Dona G. (2012) Ref #88	*Emekci Ozay O. (2017) Ref #86*	*Fruzzetti F. (2017) Ref #90*	*Genazzani AD. (2008) Ref #80*	*Gerli S. (2003) Ref #91*	*Gerli S. (2007) Ref #81*
Type of study	RCT	RCT	RCT	RCT	DBR CT	DBR CT
Significant results	Group 1: significant reductions of insulin resistance, androstenedione and testosterone	Group 1: significant increase of progesterone, significant decrease of fasting insulin, glucose and HOMA-IR. Significant decrease of total rFSH dose and cycle duration; higher clinical pregnancy rates	In both groups 1 and 2: significant improvement in BMI, insulin sensitivity and menstrual cycle. No significant difference observed between the two treatments. MI is effective as metformin	Group 1: significant reduction of plasma LH, prolactin, testosterone, insulin levels and LH/FSH. Significant improvement of insulin sensitivity expressed as glucose-to-insulin ratio and HOMA index. Restoration of menstrual cyclicity in all oligomenorrheic and amenorrhoeic subjects	Group 1: significant reduction of BMI, leptin; significant increase of HDL cholesterol. Trend in LDL cholesterol reduction. Significant improvement of the LDL to HDL cholesterol ratio	Group 1: reduction of BMI and leptin; trend in LDL cholesterol decrease; increase of HDL cholesterol, LDL/HDL ratio; significant increase of estradiol and improvement of ovulation. Significant decrease of testosterone and increase of SHBG with lower free androgen index
Side effects	No	No	No for MI	No	Yes	Yes

	Jamilian M. (2018) Ref #140	*Iuorno MJ. (2002) Ref #60*	*Le Donne M. (2019) Ref #92*	*Nehra JK. (2017) Ref #89*	*Nestler JE. (1999) Ref #61*	*Nordio M. (2012) Ref #83*
Type of study	RCT	DBR CT	RCT	RCT	DBR CT	RCT
Patients, treatment and duration of the study	Group 1 (30): 2 g MI + 200 µg f.a. twice daily Group 2 (30): 500 mg metformin thrice daily 24 weeks	Group 1 (10): 600 mg D-chiro-inositol Group 2 (10): placebo orally once daily 6 weeks	Group 1 (21): diet Group 2 (10): diet + 2 g MI + 200 µg f.a. 2 sachets/day Group 3 (12): diet + 550 mg MI + 13,8 mg DCI + 200 µg f.a. 2 softgel caps 40:1 ratio/day 24 weeks	Group 1 (30): 1g MI twice daily Group 2 (30): 500 mg metformin thrice daily 24 weeks	Group 1 (22): 1200 mg of D-chiro-inositol Group 2 (22): placebo once daily 6-8 weeks	Group 1 (26): 550 mg MI + 13,8 mg DCI (soft gel cps 40:1 ratio) Group 2 (24): MI alone same dosage 24 weeks
Principal outcomes	Total testosterone, SHBG, DHEA-S, modified Ferriman-Gallwey score, C-reactive protein, nitric oxide, gene expression of TNF-alpha, IL-1, IL-8	Systolic and diastolic blood pressure, BMI, cholesterol and triglycerides, fasting glucose, fasting insulin, insulin sensitivity index (ISIcomp), testosterone, SHBG, DHEA-S, ovulation	Height, weight, waist circumference, hip Circumference, Ferriman-Gallwey score and body composition. Regularization of the menstrual cycle	Insulin, FSH, LH, LH/FSH ratio, testosterone, HOMA-IR, total cholesterol, triglycerides, VLDL cholesterol, LDL cholesterol, HDL cholesterol	Systolic and diastolic blood pressure, BMI, cholesterol and triglycerides, fasting insulin and glucose, LH, 17-hydroxyprogesterone, testosterone, SHBG, DHEA-S, ovulation	Systolic and diastolic blood pressure, BMI, glucose, insulin, HOMA-IR, total and free testosterone, androstenedione, DHEA-S, SHBG
Significant results	Group 1: significant decrease of total testosterone, modified Ferriman-Gallwey score, C-reactive protein. Significant downregulation of IL-1 gene expression in the PBMCs, in comparison with metformin	Group 1: D-chiro-inositol improved glucose tolerance and decreased glucose-stimulated insulin release, improving the ISIcomp (84%). 66% decrease in serum total testosterone concentrations and 73% decrease in serum free testosterone levels. Decrease in systolic and diastolic blood pressures and in plasma triglyceride concentrations	The weight loss was significant in all groups and highest in group 3 (8.1, 8.5 and 9.8 kg, respectively), as also the fat mass reduction with a slight increase of percent lean mass. Group 3 was the only in which the regularization of the menstrual cycle had a 100% success rate	In both the groups: significant improvement in insulin resistance (glucose/insulin ratio, HOMA-IR); significant improvement of the lipid profile. No significant difference observed between the two treatments. MI is effective as metformin	Group 1: decrease in systolic and diastolic blood pressures, plasma total cholesterol concentration, plasma triglyceride concentration, insulin response to orally administered glucose. Glucose tolerance improved; improvement in ovulatory function (86%). Androgen concentrations also decreased as ovarian androgen production	Group 1: significant reduction of glucose, insulin, HOMA-IR, total and free testosterone, androstenedione, DHEAS and SHBG.
Side effects	No	No	No	No	No	No

	Nordio M. (2019) Ref # 93	*Ozay AC. (2016) Ref #100*	*Pkhaladze L. (2016) Ref #85*	*Pizzo A. (2014) Ref #82*	*Tagliaferri V. (2017) Ref 135*
Type of study	ROL	RCT	RCT	DBR CT	*RCT*
Patients, treatment and duration of the study	Treated (8 pts for each group): 2 g (total MI + DCI at different ratios) twice daily 12 weeks	Group 1 (77): 1 g MI + 100 µg f.a. Group 2 (60): 2 mg cyproterone acetate + 0,035 mg ethinylestradiol daily twice a day continuously 12–16 weeks	Group 1 (20): 4 g MI + 400 µg f.a. Group 2 (20): 3 mg drospirenone + 30 µg ethinylestradiol 12 weeks	Group 1 (25): 4 g MI + 400 µg f.a. Group 2 (25): 1 g DCI + 400 µg f.a. 24 weeks	Group 1 (17): 1 g MI Group 2 (17): 850 mg metformin twice daily 24 weeks
Principal outcomes	Ovulation, progesterone, FSH, LH, SHBG, estradiol, free testosterone, HOMA-IR, fasting and post meal insulin	Fasting plasma glucose and insulin, CRP, AMH, Apo B, Apo A1, HDL and LDL, total cholesterol, triglyceride, FSH, LH, testosterone, DHEAS, SHBG, HOMA-IR	Weight, BMI, glucose, C-peptide, insulin, HOMA-IR, LH, SHBG, total and free testosterone, free androgen index, DHEA-S, AMH	BMI, systolic and diastolic blood pressure, Ferriman-Gallwey score, Cremoncini score, serum LH, LH/FSH ratio, total and free testosterone	BMI, WHR, insulin after OGTT, FSH, LH, estradiol, progesterone, prolactin, testosterone, androstenedione, 17-hydroxyprogesterone, SHBG, DHEA-S, AMH, menstrual cycles
Significant results	The 40:1 MI/DCI ratio was the best to restore ovulation and to normalize important parameters (significant increase of progesterone, estradiol, SHBG; significant decrease of LH and HOMA). The other formulations were less effective or totally ineffective	Group 1: reduction of fasting glucose, LDL, DHEA-S, total cholesterol; total testosterone and fasting glucose were statistically improved. Group 2: increase in HDL and decrease in LH and LH/FSH ratio. AMH showed a statistically significant decrease in both groups	Group 1: significant reduction in weight, BMI, glucose, C-peptide, insulin, HOMA-IR, free testosterone and LH. Group 2: slight increase of weight and BMI. No changes in metabolic parameters	Both stereoisomers were effective, however MI *vs* DCI showed a higher decrease of LH/FSH ratio, total testosterone and HOMA Index, while an increase of SHBG. DCI *vs* MI: decrease of LH and free testosterone, increase of glycemia/IRI ratio. MI and DCI improved the menstrual cycles	In both groups: significant reduction of insulin response to OGTT and improved insulin sensitivity. No clinical and hormonal changes in group 1 In group 2: significant decrease of BMI, LH, estradiol, androgens and AMH, improvement of menstrual pattern and Ferriman-Gallwey score
Side effects	No	No	No	No	No for MI

Abbreviations: AMH: anti-Müllerian hormone; Apo A1: Apoprotein A1; Apo B: apoprotein B; BMI: body mass index; CRP: C-reactive protein; DBR CT: double-blind randomized controlled trial; f.a.: folic acid; pts: patients; RCT: randomized controlled trial; ROL: randomized open label trial.WHR: waist–hip ratio.

significantly decreased total testosterone and AMH levels as well as ovarian volume [100].

5.4. Inositols, PCOS, and cancer

PCOS seems to be associated with an increased risk of developing ovarian and endometrial cancer due, at least in part, to abnormal and unbalanced levels of sex hormones, such as testosterone [101]. In this context, insulin sensitizers might exert an anticancer effect, through the action on different metabolic pathways: they trigger AMPK, which acts as a strong inhibitor of the PI3 K/Akt/mTOR activity; they induce G1 cell cycle arrest and apoptosis; they downregulate the expression of telomerase reverse transcriptase. In addition, an AMPK-independent pathway is speculated to be involved in mTOR repression [102–104]. The reduction of DNA damage and mutation rates, due to insulin-sensitizers may explain the decreased risk of cancer observed in treated PCOS patients [105,106].

5.5. MI and metformin in PCOS patients

5.5.1. A brief profile of metformin

Metformin is one of the longest established oral insulin-sensitizing agents. For decades its use was restricted to the management of NIDDM, although prescribed also for the treatment of prediabetes and PCOS. Metformin limits hepatic glucose production, thus reducing the need for insulin secretion, and decreases intestinal absorption of glucose. Metformin also has an antilipolytic effect that decreases free fatty acid concentrations, thus reducing gluconeogenesis [107–109]. It may also be used as second-line therapy for women with oligomenorrhea due to PCOS, who need endometrial protection (particularly in women with contraindications to COC use) [110]. Indeed, metformin restores ovulatory menses in approximately 50% of women with PCOS [111–113], although some studies report ovulatory rates from 23% to 90% [114–120]. On the one hand, the administration of metformin in women with PCOS before or during IVF cycles does not appear to improve clinical pregnancy and live-birth rates [121,122] or to reduce the number of retrieved oocytes [123]. On the other hand, it seems to decrease the risk of ovarian hyperstimulation syndrome (OHSS) [121,122,124].

The most common side effects of metformin occur at the gastrointestinal level and are diarrhea, nausea, or vomiting, flatulence, indigestion, and abdominal discomfort. Metformin also reduces intestinal absorption of vitamin B12 in up to 30% of patients and decreases serum vitamin B12 concentrations in 5% to 10% of patients but only rarely causes megaloblastic anemia [125]. Lactic acidosis has also been reported, as an extremely rare complication in otherwise healthy individuals. Furthermore, *in utero* exposure to metformin in pregnant PCOS women from the first trimester to birth has been associated with the excessive weight of children at 4 years of age [126].

Even though metformin may reduce serum androgen concentrations, available data do not support its use for the treatment of hirsutism or as first-line treatment for ovulation induction in PCOS patients [127,128]. Data from a number of multicenter trials have lately reported that, while effective for restoring ovulation, metformin appears to be less effective for fertility (in terms of live-birth rate) when compared with clomiphene [129,130]. In addition, the randomized trials performed to date showed no benefit of metformin therapy for reducing the risk of early miscarriage in pregnant PCOS women.

5.5.2. Effects of MI and metformin on hormonal parameters in PCOS patients

A very recent meta-analysis [131], including six trials [89,90,132–135] with a total of 355 patients (178 treated with metformin and 177 with MI), showed that by the end of the treatment (length: 3–6 months) metformin and MI achieve comparable effects on parameters such as fasting insulin, HOMA-IR index, testosterone, androstenedione, SHBG, and BMI. However, the authors found a significant heterogeneity among the analyzed studies for HOMA-IR index, SHBG, BMI changes. It is important to highlight the absence of adverse effects in the subjects receiving MI at the therapeutic dose in comparison with those in the metformin group. A clear evidence of an increased risk of adverse events was found in the metformin group compared to that treated with MI (RR = 5.17, 95% CI: 2.91–9.17, p <.001). As discussed in the section 'Safety of inositols,' MI is safe and has an advantage compared to other compounds such as metformin, ensuring greater patients' compliance to the therapy and avoiding treatment interruptions due to hardly tolerable side effects [136,137] (for a summary of the most significant studies supporting the inositol use in PCOS patients, see Table 1).

MI and metformin share at least one pathway in their mechanism of action, and they could act in an additive or synergistic way. In a recently published RCT [138], PCOS patients, randomly allocated into two groups, received 500 mg of metformin three times a day or 2 g MI two times a day for 12 weeks. MI treatment, compared with metformin, achieved significantly greater effects on glycemic control, triglycerides, and very low-density lipoprotein-cholesterol levels. The authors highlighted that MI exerts its therapeutic activity by means of PPAR-gamma activation, without affecting GLUT-1 and LDLR gene expression. Of note, this study demonstrated that the significant improvement of some lipid parameters seems to be a direct effect of MI by upregulating PPAR-gamma gene expression. MI proved to act with efficacy comparable to metformin, a well-known PPAR-gamma activator. PPAR-gamma activation is consistent with the therapy of PCOS, since decreased PPAR-gamma expression was detected in PCOS rats compared with a control group [139].

5.5.3. Effects of MI and metformin on mental health parameters in PCOS patients

Comparison data on the effect of MI and metformin on mental health parameters in PCOS women are scarce. An interesting randomized controlled study [140] compared such effects to biomarkers of oxidative stress in women with PCOS. The trial included 60 PCOS women, randomly assigned to two groups (30 patients each) to take either 2 g of MI twice a day or 500 mg of metformin three times a day for 12 weeks. All the parameters were recorded at the baseline and after the 12-week intervention. Compared with metformin, MI

supplementation reached significant improvements in the scores of beck depression inventory (BDI), general health questionnaire-28 (GHQ-28), and depression, anxiety, and stress scale (DASS); furthermore, MI significantly increased the plasma total antioxidant capacity (TAC) concentrations but did not change the plasma glutathione and malondialdehyde levels. Overall, these data support that MI supplementation for 12 weeks in patients with PCOS has favorable effects on mental health.

5.6. Treatment with MI plus metformin

A relevant advantage of MI treatment is the combination with reduced doses of metformin in patients intolerant to the normal therapeutic administration of metformin.

A study [141] was planned to evaluate the benefit of the synergistic effect of Metformin plus MI versus Metformin alone in infertile PCOS women undergoing ovulation induction. One hundred and twenty infertile PCOS women were randomized in two groups: Group I (n = 60) received Metformin (500 mg) plus MI (600 mg) three times a day; Group II received Metformin (500 mg) three times a day. Subjects were advised to try for spontaneous conception. Those who did not conceive after 3 months were given three cycles of ovulation induction + intrauterine insemination. Hormonal and biochemical profile parameters were analyzed at the baseline and after 3 months of therapy. The primary outcome measure was live-birth rate. Secondary outcomes were improvement in menstrual cycle pattern; hormonal and biochemical parameters; spontaneous conception rate; abortion rate; multiple pregnancy rate; ovarian hyperstimulation syndrome. Baseline demographic, hormonal, and biochemical parameters were comparable in the two groups. Regarding the results of this study, there was a significant improvement in menstrual cycles (cycle length and bleeding days) in Group I as compared to Group II. Improvement in HOMA-IR index and live-birth rate was significantly higher in Group I as compared to Group II after 3 months [141].

6. Type 2 diabetes (NIDDM)

6.1. Myo-inositol in NIDDM

A preclinical study [36] investigated the effects of MI on muscle glucose uptake and intestinal glucose absorption *ex vivo* as well as in normal and NIDDM model of rats. In the *ex vivo* experiment, both intestinal glucose absorption and muscle glucose uptake were studied in isolated rat jejunum and psoas muscle, respectively, in the presence of increasing concentrations (2.5% to 20%) of MI. In the *in vivo* study, the effect of a single bolus dose (1 g/kg) of oral MI on intestinal glucose absorption, blood glucose, gastric emptying, and intestinal transit was investigated in normal and type 2 diabetic rats after 1 h of co-administration with 2 g/kg glucose. Phenol red was used as a recovery marker. MI significantly reduced the intestinal absorption of glucose, both in normal and diabetic rats, and increased its uptake in the muscle, with or without insulin stimulation. Oral MI not only inhibited duodenal glucose absorption and reduced blood glucose increase but also delayed gastric emptying and accelerated intestinal transit in both normal and diabetic animals.

To date, only a pilot clinical trial [142], relevant for possible clinical implications in the future, evaluated the administration of inositol in 20 NIDDM subjects with suboptimal glycemic control (HbA1 c 7.0–10.0%) already treated with glucose-lowering agents. Patients (five males and fifteen females, mean age of 60.8 ± 11.7 years) were treated orally for 3 months with soft gel capsules (twice a day) containing MI (550 mg) and DCI (13.8 mg) in the 40:1 ratio as add-on supplement to their glucose-lowering drugs. After 3 months of treatment, fasting blood glucose (192.6 ± 60.2 versus 160.9 ± 36.4; $p = 0.02$) and HbA1 c levels (8.6 ± 0.9 versus 7.7 ± 0.9; $p = 0.02$) significantly decreased compared to the baseline. There was no significant difference in blood pressure, lipid profile, and BMI levels. No side effects were detected in inositol-treated patients. The study reached statistical significance in the main parameters detected (i.e. blood glucose and HbA1 c), which define glycemic control. Of note, the metabolic effect of inositol supplementation was tested in a population including also males, whereas almost all the studies published on inositols involved exclusively women with several clinical (i.e. PCOS and GDM) or physiological (i.e. menopause) conditions. Only another randomized, double-blind, placebo-controlled study involved a sample of males with NIDDM, but it focused on investigating the use of MI in the treatment of erectile dysfunction [143], without considering parameters related to glycemic control. The findings reported in Pintaudi's paper suggest extending the target population for inositol use also to males, considering its effect in improving metabolic parameters. As an insulin sensitizer, inositol could represent a possible alternative to metformin or pioglitazone, when their use is not possible (i.e. metformin intolerance, drugs contraindications). This study showed for the first time a direct beneficial effect of the supplementation with MI and DCI on glycemic parameters of subjects with NIDDM. Particularly, a significant reduction in blood glucose and HbA1 c levels was observed.

Finally, it has been shown that diabetic mothers deliver neonates with congenital malformations (namely NTDs) with four to five times higher rate than observed in the healthy population. In this context, MI supplementation proved to significantly reduce the incidence of NTD from 20.4% to 9.5% ($p < 0.01$) in the offspring of diabetic rats. Therefore, it may serve as a useful prophylaxis against diabetes-induced congenital malformations [144].

7. Reproduction in PCOS

7.1. MI treatment in fertility of PCOS women

MI and DCI display their best activity when used in an optimal formulation. This finding was obtained administering MI and DCI in the physiological 40:1 ratio [15]. In a clinical study [84], 46 obese PCOS women (BMI>30) were treated with soft gel capsules containing 550 mg of MI and 13.8 mg of DCI for 6 months. The combined therapy significantly rebalanced the endocrine and metabolic profiles of these patients with respect to the baseline values. Insulin resistance and ovulatory function improved, as demonstrated by HOMA-IR index and ultrasound investigations. LH and free testosterone levels decreased, lowering the LH/FSH ratio and downregulating the hyperandrogenism; HOMA-IR index and fasting insulin were significantly reduced. Moreover,

17-β-Estradiol and SHBG significantly increased, restoring the ovulation. No considerable changes in these sex hormones were reported in the placebo group. BMI, FSH, androstenedione, dehydroepiandrosterone sulfate (DHEAS) and fasting glucose remained unchanged in both treated and control patients. Notably, no side effects were observed during the combined therapy with MI plus DCI [84]. A comprehensive review [145] provided further data to support the inositol treatment in PCOS patients for improving ovarian function, fertility, metabolic and hormonal parameters involved in the reproductive axis function and in ovulation.

High concentrations of MI in human follicular fluid seem to play a role in follicular maturity and have been suggested as potential markers of good oocyte quality. These results were confirmed and extended by an already cited study, which demonstrated that MI improves blastocyst quality, while DCI was found to be harmful above certain levels [53]. Such effect is explainable in the light of the increase of testosterone levels due to DCI, as previously described [47,48].

As previously mentioned, literature data indicate that MI signaling may adjust the level of AMH production induced by FSH in granulosa cells [146]. AMH decreases oocyte sensitivity to FSH and participates in regulating follicle maturation [52]. Therefore, the therapeutic target should be modulated on each pathology considering the available evidence.

Treatment with MI in IVF was associated with decreased administration of rFSH, shortened time of ovulation induction required for follicular development [147,148] and increased clinical pregnancy rates [86].

The paramount importance of preserving the balance between MI and DCI concentrations in follicular fluid was also confirmed in the trials that used combined treatment of MI and DCI. Indeed, the physiologic ratio appears to optimize the improvement of fertility [20].

7.2. Clomiphene citrate (CC) in ovulation dysfunction of PCOS women

'Clomiphene-resistance' refers to a failure to ovulate rather than a failure to become pregnant despite ovulation, which is known as 'clomiphene-failure.' Patients who do not respond to CC are likely to be more obese, insulin resistant and hyperandrogenic than those who respond to the treatment [149].

The combination of various molecules with CC has been extensively evaluated. The majority of data suggests that CC efficacy can be improved when combined with other agents [150]. Metformin in combination with CC was studied in women with PCOS. According to the results of a recent meta-analysis, the clinical pregnancy rate improved when adding metformin to CC in women with CC-resistance, in both obese and nonobese patients. The treatment however failed to increase live-birth rates [150].

Rosiglitazone is another insulin-sensitizing agent investigated in combination with CC. Although limited, the data suggest that the drug has beneficial effects on ovulation rates. The authors of two randomized controlled trials observed that short-term therapy with rosiglitazone enhanced both spontaneous and clomiphene-induced ovulation in overweight and obese women with PCOS [151,152]. When compared with metformin plus CC, the combination of rosiglitazone plus CC was found to be more effective in women with CC-resistant PCOS [153]. Rosiglitazone may cause weight gain and is contraindicated in patients with heart failure and should be administered with caution in patients with osteoporosis.

Inositol was investigated to evaluate possible clinical effects in PCOS women seeking fertility. Regarding its effects in combination with CC, Kamenov et al. [154] examined 50 anovulatory PCOS patients who received MI firstly during three spontaneous cycles. If they remained anovulatory and/or no pregnancy was achieved, a combination of MI and CC was used in the following three cycles. MI improved ovarian activity in PCOS patients, as spontaneous ovulation was observed in 61,7% of women, while 72.2% of MI-resistant patients eventually ovulated after CC treatment. A recent pilot study [155] demonstrated that the combination of MI with CC significantly increases ovulation rates, decreases the rate of resistance to CC rates, and improves pregnancy rates. Even though the differences were not statistically significant for most outcomes, probably due to the small number of patients, this pilot study seems to show a benefit of supplementation with MI during ovulation induction with CC in PCOS patients. Finally, a meta-analysis including 10 RCTs with 601 PCOS patients [156] demonstrated that inositol treatment significantly increased the ovulation rate (relative risk 2.3; 95% CI 1.1–4.7; I^2 = 75%) compared with placebo. In PCOS women with oligomenorrhoea or amenorrhea, inositols increase the frequency of menstrual cycles six-fold (relative risk 6.8; 95% CI 2.8–16.6; I2 = 0%) compared with placebo. In these studies, a significant improvement in the hormonal profile and glycemic parameters was also found. Such outcomes suggest that inositol may represent a potent co-agent in women with PCOS undergoing ovulation induction [156].

However, the available evidence is questionable and further studies are mandatory to reach more definite conclusions [157]. Currently, a double-blinded, randomized controlled trial is recruiting patients with PCOS, who wish to become pregnant and are eligible for simple ovulation induction by CC. Half of them will receive MI + folic acid in addition to CC, whereas the other half will receive a placebo containing only FA in addition to CC. The initial results are expected by April 2020 (https://clinicaltrials.gov/ct2/show/NCT03059173).

8. Fertility in men

Our review aims at evaluating the effects of inositols in PCOS and NIDDM patients, therefore, about fertility, the focus has been on this kind of people (anyway, no data are available on this specific topic for diabetic subjects). However, some information on inositol use both *in vivo* and *in vitro* for the improvement of male fertility can be useful to provide a wider picture. Overall, it was found that MI plays a very beneficial role on many sperm features such as motility, morphology, and concentration. The target of inositol treatment is patients affected by oligoasthenoteratospermia (OAT).

It was found that oxidative stress affects negatively the testicular microenvironment and ends up causing a deficient spermatogenesis, damages to sperm DNA, as well as

reduced motility and altered morphology of spermatozoa [158]. *In vitro* experiments demonstrated that several aspects of sperm deterioration derive from mitochondrial disorders and can be solved by means of MI [159]. Spermatozoa of OAT patients typically show reduced motility and increased levels of the enzyme inositol monophosphatase-1 (IMPA-1), involved in the dephosphorylation of phosphatidylinositol (PI). This data suggests an impaired signal transduction pathway which is fundamental to preserve the motility of male germ cells [160]. The spermatozoa of OAT patients is covered with an amorphous fibrous material that modifies the seminal fluid viscosity, decreasing sperm motility. Furthermore, the mitochondria present damaged cristae. The incubation with MI allowed to drastically reduce the amorphous fibrous material and the damage to the cristae [161]. MI also directly increases the mitochondrial membrane potential (MMP) that is an apoptotic marker in the sperm cells. MI seems to have a beneficial effect on motility, fertilization capacity, and embryo quality, as demonstrated by *in vitro* studies on the sperm of normal and OAT patients undergoing IVF [162].

The most important data for our review come from a clinical trial by Montanino Oliva et al. [163]. This prospective, longitudinal study took into account men with reduced sperm motility and metabolic syndrome, a disorder that shares some features with PCOS and is associated with poor sperm morphology [164]. The authors demonstrated that treating these patients with 1 g of MI, 30 mg of L-carnitine, L-arginine, and vitamin E, 55 µg of selenium, and 200 µg of folic acid (taken twice a day) for 3 months allowed to obtain positive results in the metabolic and reproductive parameters. MI was able to bring back to their normal health conditions. It was found able to increase testosterone levels, improve the insulin sensitivity and semen characteristics (sperm concentration, morphology, and motility) [163]. Other *in vivo* studies confirmed these effects due to MI [165,166].

9. How to overcome some problems in the inositol absorption

An essential field of research concerns the impairment of MI therapy. Solving this problem is a prerequisite for the studies in this area. It may arise through a plethora of mechanisms, including drug-induced decreased biosynthesis and/or transporter activity; inhibition/competition on intestinal and cellular uptake by interfering agents; abnormalities of intestinal absorption due to chronic inflammatory states or other causes; reduced food-dependent intake; increased catabolism and excretion. In particular, two aspects have to be addressed in this regard: reduced absorption of inositol and inositol-resistance.

9.1. Reduced absorption of inositol

Reduced absorption of MI may be caused by the competition with DCI or by the interference of other molecules (e.g. glucose) on the active passage mechanism. When there is competition for the same transporter, MI transport across the intestinal barrier or inside the cells may be insufficient. This condition occurs when a competitor has greater affinity (lower K_m) for the transporter than MI or when a competitor with lower affinity is present in amounts large enough to displace MI. Inositol transporters are divided into two groups with different tissue distribution: sodium/myo-inositol cotransporter 1 and 2 (SMIT1 and SMIT2), coupled with sodium ions, and proton/myo-inositol cotransporter (HMIT), coupled with protons [167]. So far, SMIT2 is the only transporter of MI found in the intestine (duodenum and jejunum). Some *in vitro* experiments identified a slightly lower K_m for DCI than for MI, for this reason, DCI may have a small advantage in the transport. An important confirmation of this came from a very recent pharmacokinetic study in humans [168]. Fasting 18 healthy volunteers received 6 g of MI in a single dose by oral route. After 1 week, they were administered with the same amount of MI plus 1 g of DCI (ratio of 6:1). Such ratio was chosen empirically, in order to obtain well-detectable plasma levels of the two inositol isoforms and to check for the potential inhibitory effect of DCI on MI absorption. The study demonstrated that the combined administration of oral DCI with MI was actually able to inhibit MI *in vivo* absorption, leading to lower plasma concentration respect to the administration of MI alone. The average peak plasma concentration (C_{max}) recorded at 180 min (Tmax) in MI administered alone was about 1.29-fold higher than MI combined with DCI ($p < 0.001$). The area under the time-course curve (AUC) of MI plus DCI plasma concentrations was found reduced of about 19.1% as compared to MI alone ($p = 0.0118$). Furthermore, this inhibitory effect of DCI on intestinal absorption of MI may confirm a potential role of SMIT2 as primary transporter of inositols in intestinal mucosa in humans. It is therefore essential to limit the quantities of DCI to administer, in order to avoid the reduction of MI intestinal absorption in favor of DCI. As far as glucose is concerned, MI and DCI have much greater affinity to the transporter (more than 100 times) [169,170].

9.2. Inositol-resistance

Inositol-resistance refers to the therapeutic inefficacy of inositols in some patients (named 'resistant'), a condition found in several clinical studies [60,81,154,171]. In the 30–40% of PCOS patients, indeed, inositols failed to significantly improve the metabolic and hormonal parameters and restore ovulation. A well-founded hypothesis argues that such problem can derive from the reduced or absent absorption of inositol due to unclear or unpredictable conditions (e.g. obesity, chronic intestinal diseases, dysbiosis). To increase the absorption, MI was combined with alpha-lactalbumin (alpha-LA), a whey protein that is an excellent 'carrier' for metal ions (mainly divalent, such as Ca^{2+} and Fe^{2+}) and for vitamin D [172–175]. In more general terms, alpha-LA can act as a facilitator of passage through biological barriers. This protein has been included by the FDA in the list of GRAS compounds [176].

A recent study [177] was carried out on 18 healthy volunteers: in the first phase, they received a single dose of 6 g of MI and, after a week, 6 g of MI + 150 mg of alpha-LA in single dose. The average peak plasma concentration (C_{max}) and the area under the time-course curve (AUC) of plasma concentration after the combined administration were found significantly higher (+32.4% and +27.5%, respectively) when compared with the administration of 6 g MI alone. Noteworthy, the time of peak plasma concentration was the same for the two formulations. To understand the

mechanism(s) underlying this effect, MI transport alone and together with alpha-LA (as biopeptides, i.e. 'digested' protein) was tested through the human intestinal Caco-2 cell monolayer [177], a commonly used *in vitro* model of gut mucosa [178,179]. The authors found an increased MI passage in the presence of alpha-LA, also in this case, and a concomitant lowering of the Trans-Epithelial Electrical Resistance (TEER), meaning that the tight junctions between the cells were open [177]. Therefore, a 'passive' passage exists in addition to the active one, mediated by transporters. A very important finding is that this opening is transient, i.e. reversible, therefore physiological and not toxic. This indirectly means that MI and alpha-LA, at effective doses, do not damage Caco-2 cells. A subsequent study on PCOS patients treated with MI plus alpha-LA (primary outcome: ovulation restoration) provided a clinical confirmation of the efficacy of the new formulation [180]. This therapy drastically reduced the number of inositol-resistant patients, with a significant progress. In the first phase, 37 anovulatory PCOS women received 2 g of MI, twice a day for 3 months, orally. Following the treatment, 23 subjects (62%) ovulated, whereas 14 (38%) did not and displayed no rise in MI plasma levels, a sign of inositol-resistance. These patients were further treated with the same daily amount of MI with the addition of 50 mg alpha-LA twice a day, for an additional 3 months. Twelve (86%) patients ovulated, displaying significantly higher level of plasmatic MI and a better hormone and lipid profile with respect to the baseline. This clinical trial confirms that a poor intestinal MI absorption represents a relevant cause of inefficacy observed in the treatment of PCOS patients with inositols and, together with the study by Monastra et al. [177], paves a promising way to overcome some current limitations of inositol therapy.

10. Conclusions

Over the past few years, the scientific knowledge on MI and DCI has grown exponentially and most studies were evaluated by several meta-analyses. The present Expert's Opinion summarizes the pivotal role played by the two stereoisomers in the transduction of Insulin signal and highlights the safety of MI administration, as stated by the FDA and the specific clinical trials. Experimental results of human studies suggest convincing evidence about the role and the therapeutic efficacy of inositols in PCOS and compare them with metformin and CC. Moreover, inositols provide very interesting, although still preliminary, outcomes in NIDDM. Several data demonstrate that the most effective treatment in PCOS contains MI and DCI in the 40:1 ratio, similar to that found in normal plasma. It can be thus concluded that the use of DCI has to be carefully considered with reference to the dosage. Increasing DCI concentrations to MI:DCI ratios of 5:1, or even 20:1, may be detrimental to ovarian physiology. To this purpose, it is important to keep in mind that MI and DCI have slightly different affinities for the intestinal inositol transporter. Other new findings, provided in this Experts' Opinion, concern the innovative way to improve MI absorption at the intestinal level, based on the combined administration of alpha-LA.

11. Expert opinion

The current available evidence supports a key role for both MI and DCI in several body microenvironments [5–7]. They are synthesized in the kidneys, liver, testes, mammary gland, brain (kidneys can produce approximately 4 g MI per day) [8,9]. MI can be transformed into DCI by a specific epimerase under insulin control [11,12]. Endogenous production of both stereoisomers varies depending on the specific tissue needs. Their physiological plasma ratio is 40:1 and a scientifically valid treatment should be based on this ratio [14,15]. MI and DCI play several different roles, in some cases also exerting opposite effects. The only variation between them is the steric position of the hydroxyl group; however, this small difference determines a significant change in their functions. Both are insulin sensitizers although using distinct pathways. MI is involved in the cellular uptake of glucose, whereas DCI in glycogen synthesis; furthermore, DCI affects steroidogenesis [7,16,18–22,24]. In addition, MI in the ovary (as InsP3) is one of the second messengers of FSH [44]. As mentioned before, a recent study has highlighted an MI mechanism of action based on PPAR-gamma transcription factor that is crucial for the hypoglycemic effects [138]. All these areas of research are fruitfully enriching and enlarging the promising field of inositol studies related to PCOS and NIDDM. In this scenario, focused clinical studies should be designed and carried out to clarify some aspects with the aim to open new ways of intervention and offer innovative therapeutic approaches.

Future studies should be planned to evaluate the effects on cancer prevention in PCOS patients at epidemiological level, with dedicated meta-analyses that include a wide number of PCOS patients treated with inositols. In addition, a better understanding of the actions of cytokines and growth factors, activated in gynecological tumors and capable of modulating cellular responses to insulin sensitizers in PCOS women, would allow for tailored treatments.

So far only 20 NIDDM patients (five males and fifteen females), with suboptimal glycemic control were successfully administered with MI/DCI in the 40:1 ratio for 3 months, in addition to their glucose-lowering therapy. The improvement was found significant with respect to the baseline for fasting blood glucose and HbA1 c levels; no side effects were noticed [142]. The treatment was based on the results obtained with inositols in PCOS, since MI and DCI exert the most satisfying therapeutic activity in this disorder when administered in the 40:1 ratio, that is the physiological one found in plasma [14,15]. Therefore, carrying out more in-depth clinical studies with a wide number of NIDDM patients is another key topic for future studies with inositols.

Recently, some pieces of evidence support a potential reduction of gonadotropin administered in IVF after MI treatment. However, no assessment was made about the cost-effectiveness coming from such effect. A further point to investigate concerns this key issue, essential in the public and private management of the health policy assessment.

The effects of alpha-LA administration gained also growing attention. Additional researches are required to confirm these findings on larger samples and different PCOS phenotypes. It can be taken also in consideration the specific effects that could be achieved when the alpha-LA treatment continues for months. In fact, such protein is endowed with anti–inflammatory activity, having the capacity to inhibit type 2 cyclooxygenase (COX 2) and to decrease the production of inflammatory cytokines [181]. Moreover, alpha-LA has been found able to reduce glycemia after glucose loading in a rat model of type 2 diabetes [182]. Considering that a chronic inflammation underlays PCOS, as well as hyperglycemia, is a common feature in several women affected by this syndrome, we can reasonably hypothesize that a lengthened alpha-LA treatment *per se* can reduce such inflammatory status and improve glycemia, thus cooperating with the healing activity of MI. However, specific investigations should be carried out.

Overall, all the above studies belong to promising research areas for the identification of new therapeutic opportunities and for the health-care system. In our opinion, by the next 5 years, inositols may gain a much more relevant position among the insulin-sensitizing agents, due to their efficacy and the absence of side effects.

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Declaration of interest

Vittorio Unfer is an employee at Lo.Li. Pharma s.r.l., Rome, Italy. Fabio Facchinetti has been a consultant of the same company. 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. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Obituary

The authors express their most heartfelt condolences for the premature departure of Prof. Francesco Orio, a high skilled researcher and loyal friend, who spent his life with a deep dedication to the patients.

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