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REVIEW ARTICLE

Combining treatment with myo-inositol and D-chiro-inositol (40:1) is effective in restoring ovary function and metabolic balance in PCOS patients

Giovanni Monastra[1], Vittorio Unfer[2], Abdel Halim Harrath[3], and Mariano Bizzarri[4]

[1]Visiting scientist at the Systems Biology Group, Department of Experimental Medicine, University la Sapienza, Rome, Italy, [2]Department of Medical Sciences, IPUS – Institute of Higher Education, Chiasso, Switzerland, [3]Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia, and [4]Department of Experimental Medicine, Systems Biology Group, University la Sapienza, Rome, Italy

Abstract

Polycystic ovary syndrome (PCOS), a relevant cause of infertility, is a heterogeneous, endocrine disorder affecting up to 10–15% of women in reproductive age. Besides hyperandrogenism, insulin resistance (IR) plays a key role in such syndrome. Insulin-sensitizing drugs, such as Metformin, are effective in treating hyper-insulinemic PCOS patients. Recently, inositols – myo-inositol (MI) and D-chiro-inositol (DCI) – have shown to be an efficient and safe alternative in PCOS management, as both inositol isoforms are able to counteract downstream consequences of insulin resistance. Yet, whereas DCI contributes in mediating insulin activity mainly on non-ovarian tissues, MI displays specific effects on ovary, chiefly by modulating glucose metabolism and FSH-signaling. Moreover, MI may also improve ovarian functions by modulating steroid metabolism through non-insulin-dependent pathways. As DCI and MI activity likely involves different biological mechanisms, both inositol isoforms can be synergistically integrated according to a multitargeted design, by combining MI and DCI in a ratio corresponding to their physiological plasma relative amount (40:1). New experimental and clinical evidence with MI plus DCI evidenced the suitability of such integrated approach, and provided promising results. Further studies need to investigate thoroughly the molecular mechanism and confirm such preliminary data.

Keywords

D-chiro-inositol, hyperandrogenism, insulin resistance, metformin, myo-inositol, polycystic ovary syndrome, sialylated FSH

History

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Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder, affecting up to 10–15% of women of reproductive age in western countries where it represents one of the leading causes of infertility [1]. The simultaneous presence of polycystic ovary and anovulation was first recognized as a distinct syndrome, by Irving F. Stein, Sr., and Michael L. Leventhal [1], in 1935. Diagnostic assessment of PCOS includes a constellation of symptoms such as amenorrhea, obesity, hirsutism and multiple follicular cysts [1]. These criteria were quite limiting, as other pathological features were excluded. PCOS is currently deemed a heterogeneous disorder caused by the combined cross-talk among environmental factors and predisposed multifactorial genetic background [1,2]. In addition, PCOS shows various reproductive, metabolic and cardiovascular anomalies, with long-term health concerns during the life span [3,4].

Throughout the recent years, studies on inositols and its phosphate derivatives (myo-inositol-1-phosphate, myo-inositol-biphosphate, inositol pentakiphosphate to mention just a few) have gained momentum [5].

Cell signaling via inositol and inositol phosphates, in particular via the second messenger myo-inositol 1,4,5-trisphosphate, and phosphoinositides comprises a great amount of biological activities. Despite the fact that inositol has been deemed for a while to be an ''inactive'' molecule [6], current evidence suggests that, by itself, inositol plays significant biological roles. Indeed, some inositols isomers have been proved to be medically relevant: scyllo-inositol (neurodegenerative diseases), D-chiro-inositol (DCI) (diabetes) and, by no doubt, myo-inositol (MI) (cancer, metabolic syndrome, PCOS). It is therefore timely to consider exploration of the roles and applications of these ''new'' simple molecules as pharmacological, pleiotropic agents.

PCOS pathogenesis

The up-to-date definition of PCOS (Rotterdam criteria 2004) requires at least two of the following clinical and endocrine features: chronic ovulatory disorder, hyperandrogenism and polycystic ovaries. Four different ''discrete'' phenotypes, or subsets, can be identified in PCOS subjects, on the basis of the following clinical and endocrine anomalies: (1) chronic ovulatory disorder, hyperandrogenism and polycystic ovaries; (2) chronic ovulatory disorder and hyperandrogenism; (3) hyperandrogenism and polycystic ovaries; (4) chronic ovulatory disorder and polycystic ovaries, without hyperandrogenism (Table 1).

There is a wide agreement on the final outcome to which the syndrome unavoidably leads. The follicular maturation arrest, resulting in the accumulation of numerous, small subcortical follicle ''cysts'' and increased ovarian stromal volume, represents

Address for correspondence: Mariano Bizzarri, Department of Experimental Medicine, Systems Biology Group, University la Sapienza, Rome, Italy. E-mail: mariano.bizzarri@uniroma1.it

Table 1. Clinical index, metabolic and endocrine mean values in PCOS subtypes (from Głuszak et al. [92]).

	Phenotypical groups			
Parameters	**1** OD + HA + PO	**2** OD + HA	**3** HA + PO	**4** OD + PO
Insulin after 30 min of OGTT	47.59 ± 24.20	97.43 ± 57.08	64.88 ± 28.19	84.00 ± 19.80
Weight (kg)	71.6 ± 19.3	82.1 ± 22.3	75.2 ± 19.9	80.8 ± 17.7
Trigliceryde (mg/dL)	96.64 ± 56.78	107.13 ± 65.56	80.33 ± 36.73	92.00 ± 62.23
HOMA-IR index	1.77 ± 1.49	2.04 ± 0.93	1.78 ± 0.80	1.73 ± 1.40
HOMA-index	110.89 ± 55.23	167.14 ± 80.71	128.78 ± 34.25	167.01 ± 65.61
Androstenedione (ng/dL)	485.28 ± 146.60	525.17 ± 146.08	395.25 ± 67.7	192.00 ± 103.2
17-ketosteroids (mg/dL)	16.45 ± 4.94	17.28 ± 3.56	20.78 ± 4.2	18.85 ± 8.3
17-hydroxy-corticosteroids (mg/dL)	5.23 ± 1.71	7.04 ± 1.59	5.54 ± 1.6	7.40 ± 1.3

the chief hallmark of PCOS. The impairment of ovary function leads to abnormal menstrual cycles, infertility, eventually worsening clinical hyperandrogenism. Therefore, the question is how many biochemical paths could actually account for the emergence of such disease.

Hyperandrogenism. The pathophysiology of PCOS has been ascribed mainly to primary endocrinological abnormalities, including deregulation of the hypothalamic–pituitary axis and ovarian steroidogenesis. Yet, none of these mechanisms alone can explain the manifold phenotypes of PCOS, and it is currently deemed that endocrinological cues may differentially interact each other and with other factors in shaping a specific phenotype. However, two primary pathophysiological conditions deserve special attention as PCOS may be *experimentally* induced by disrupting the activity of the suprachiasmatic–hypothalamic–pituitary axis or by impairing androgen steroidogenesis. Indeed, ovary cysts, like those observed during PCOS, may be induced by exposing mature female rats to an environment with constant light [7]. Disruption of the normal light–dark cycle may lead to a severe impairment in the circadian-regulated release of melatonin and it is followed by several changes in endocrine and biochemical pattern [8]. Even if still poorly understood, it can be hypothesized that altered melatonin levels may significantly hinder the LH/FSH balance and the gonadal release of androgens, thus triggering the onset of PCOS [9]. Moreover, melatonin is currently acknowledged in exerting a very relevant role in modulating ovary functions and oocyte maturation [10]. On the other hand, PCOS may be experimentally produced by androgenic treatments and neonatal androgenization [11]. Excessive androgen availability can affect hypothalamic endocrine balance, leading thus to an inappropriate LH/FSH stimulation, mostly if the androgen stimulation occurs early during prenatal or post-puberty periods, when organogenesis take place [12]. Furthermore, hyperandrogenism resulting from genetic defects affecting adrenal androgen production, and androgen-producing tumors are associated with the development of polycystic ovaries [13]. Imbalance in LH/FSH and adrenal hormones homeostasis are usually followed by secondary changes in ovarian steroidogenesis. Eventually, hyperandrogenia may result from an intrinsic alteration in the steroidogenic activity of PCOS theca cells that encompass multiple steps in the biosynthetic pathway [14].

The excessive ovarian androgen production would result in many reproductive abnormalities, including amenorrhea or oligomenorrhea, early follicular atresia, anovulation and infertility, along with all the other typical clinical manifestations of hyperandrogenism, including hirsutism and acne. Therefore, it is not surprising that a few endocrine manipulations, aimed at suppressing abnormal LH release by GnRH super-agonists, have provided some useful clinical results, given that LH surge has a permissive role in increasing androgen production from ovarian theca cells [15]. In turn, the fact that the inhibition of androgen production, even if transient, may enable follicle maturation and subsequent ovulation, strongly argues for a pivotal role played by hyperandrogenism in PCOS pathogenesis. Hormone imbalance in PCOS may also affect post-translational regulation of signaling molecules. Namely, FSH modification through sialic acid linkage has been described in a wide percentage of PCOS patients [16]. As a consequence of sialylation, FSH half-life increases due to reduced hepatic catabolism: sialylation impedes the FSH binding to asialo-glycoprotein receptors, thus preventing the first degradation step. Indeed, a predominance of highly sialylated FSH isoform has been recently described in PCOS [17]. However, if FSH-sialylation alone could account for the observed reduced FSH-activity is a debatable question. Indeed, paradoxically results have been provided by older studies [18] highlighting that less sialylated FSH variants can still display higher receptor-binding activity and biological potency [19]. Furthermore, less sialylated isoforms of FSH appear more potent than the more sialylated in the stimulation of granulosa cells proliferation and in preventing follicular atresia [20]. Irrespective of the FSH sialylation degree, modulation of FSH activity seems mainly be exerted by other endocrine influences, as suggested by recent investigations. Namely, FSH pleomorphism in the ovary seems to be tightly linked not only to sialic acid availability but also to IR [21]. In turn, FSH isoforms seem to selectively modulate TGFβ activity on oocyte maturation [22].

Insulin resistance (IR). Although the Rotterdam consensus meeting explicitly excluded IR from the diagnostic criteria, deregulation of insulin sensitivity and/or abnormalities in glucose metabolism, are usually detected in several PCOS women, associated or not with obesity [23]. It is currently acknowledged that IR in PCOS is primarily due to defects in post-binding signaling, affecting mainly the metabolic pathway [24]. The key role of IR and/or compensatory hyperinsulinemia in the origination and development of PCOS has gained growing cogent evidence [25]. Even if some discrepancies have been recorded in frequencies of both IR and diabetes mellitus type II among PCOS patients [26], it is currently deemed that up to 80% of PCOS women, with upper-body obesity (increased waist circumference and waist–hip ratio) shows IR, whereas 30–40% of PCOS lean women suffer from hyperinsulinemia [27]. In PCOS patients, IR seems to be independent from BMI, and physicians are advised to treat PCOS patients by keeping in mind that IR must be counteracted, regardless patient's weight status [28]. Insulin prompts directly the ovary theca cells to enhance the synthesis and release of androgens. Insulin may also indirectly enhance androgen synthesis through modulation of carbohydrate levels. Indeed, high glucose concentrations, inhibit the hepatic synthesis of sex hormone-binding globulin (SHBG), therefore causing a consequent increase of biologically circulating free-active androgens [29,30]. Furthermore, IR in PCOS women increases the risk for glucose intolerance, type 2 diabetes and lipid abnormalities

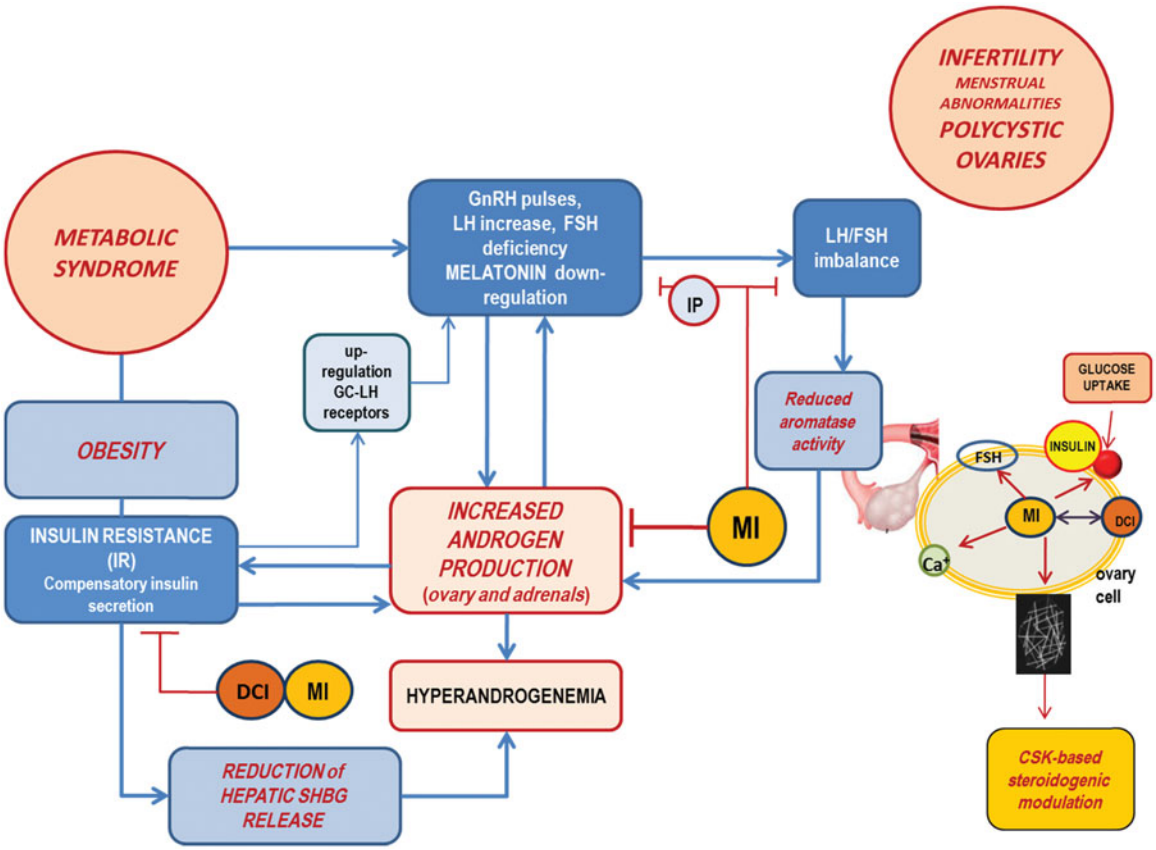

Figure 1. Schematic diagram showing the main factors involved in PCOS pathogenesis. Inhibitory actions triggered by MI and/or DCI are in italic. Inositols counteract hyperinsulinemia effects on glucose metabolism as well as on steroidogenic balance. Additionally, inositol-phosphate participates in modulating LH release. In physiological conditions, MI/DCI ratio in follicular fluid averages 100:1, meanwhile the same ratio in the plasma is 40:1. MI displays several actions within ovary cells: modulates glucose uptake, improving insulin signaling; enhances IP3-dependent calcium release; stabilizes F-actin cytoskeleton (CSK) and probably, through that pathway, MI interfere with ovarian adrenal synthesis; participates in enhancing FSH-mediated effects. DCI: D-chiro-inositol; MI: Myo-Inositol; IP: inositol-phosphate.

[31] (the main factors involved in PCOS pathogenesis are depicted in Figure 1). In turn, the evidence that insulin-sensitizing drugs (ISDs), such as Metformin and Thiazolidinediones (troglitazone, rosiglitazone and pioglitazone) are effective in treating PCOS patients, by lowering their hyperinsulinemia, is an additional proof underpinning the relevance of IR in PCOS [25,32,33].

Current treatments

Given the heterogeneity of clinical presentations, planning of PCOS therapy is usually driven by ''pragmatic'' issues, and mostly depends on the prevailing phenotypic features and reproductive desires. Therefore, tailored strategies include sequential or combined use of both pharmacological drugs and nonpharmacological supports, including weight loss and dietary habits modifications, since life style changes have been proven to significantly improve ovary function and prevent later PCOS-related risks [34].

Hormonal manipulations includes principally the use of oral contraceptives or Metformin, even if a combination of therapeutic options is often required to address specific clinical situations. This subject has recently been reviewed in-depth elsewhere [35].

Combined oral contraceptives (estro–progestin compounds) are used widespread as first-line treatment in PCOS women presenting with moderate or severe hirsutism and/or menstrual abnormalities. Their efficacies rely mostly on progestins and estrogens suppressing the activity on LH release, which in turn leads to decrease in ovarian androgen production. Moreover, estrogens increase the hepatic synthesis of SHBG (thus lowering the levels of circulating free androgens), and promote the peripheral block of androgen receptors [36].

Even if few randomized controlled trials have been so far performed, the available evidences show that both low and high-dose contraceptives are effective in improving hyperandrogenism in 60–100% of patients [37]. However, contraceptives are not an acceptable option if the patient's desire is to restore ovulation for becoming pregnant.

For long time, Metformin has been the first choice in PCOS management for women for whom restoring the ovulatory cycle constitutes the main concern. Metformin exerts several insulin-sensitizing actions by acting on different target tissue, including the ovary, where it facilitates the translocation of glucose transporters from intracellular sites to plasma membrane. Metformin reduces glycemia levels leading consequently to decreased ovarian androgen production, and reduced circulating androgen levels [38]. Indeed Metformin has shown to counteract hyperandrogenism in the short-term [39], but unfortunately no long-term follow-up are available [40]. These effects are mostly evident in obese, insulin-resistant PCOS women, whereas controversial results have been published about Metformin efficacy in non-obese non-insulin-resistant patients [41,42]. Overall, antiandrogenic efficacy of ISDs, including Metformin and Thiazolidinediones, is far lesser than that obtained with contraceptives or hormonal antiandrogens, as stressed by a recent meta-analysis [43]. Furthermore, frequent adverse events are

associated with Metformin assumption, including gastrointestinal symptoms (diarrhea, nausea, vomiting and abdominal bloating) and metabolic complications, and with Thiazolidinediones, such as fluid retention, body weight increase, coronary artery disease, myocardial infarction and bladder cancer. All the above cited side effects may reduce the patients' compliance and limit the use of these drugs. A noteworthy hypothesis is that Metformin-based benefits in PCOS patients are likely to be ascribed to a secondary increase in inositol phosphoglycans availability triggered by Metformin administration [44]. A reliable alternative to conventional medicaments can be obtained by using inositol isomers, MI and/or DCI in PCOS patients.

Inositols

Inositol is a hexahydroxycyclohexane, a 6-carbon ring compound with a hydroxyl group attached to each carbon of the ring. There are nine possible stereoisomeric forms of inositol, related to the epimerization of the six hydroxyl groups. Among these isomeric forms, MI, the mostly represented isoform, stands out for its important biological roles [1], whereas different, integrative functions are displayed by DCI, a stereoisomer produced through the epimerization of the C1 hydroxyl group of MI [1]. In particular, whereas the activation of glucose transporters and glucose utilization take place under the regulation of MI, glycogen synthesis is mainly controlled through DCI [45]. On the other hand, in the ovary, MI regulates glucose uptake and FSH signaling, while DCI modulates insulin-induced androgen synthesis [46]. A significant variability has been noticed in the ratio between MI and DCI in fat, muscle and liver, and this difference reflects the distinct functions that the two isomers are likely to play in those tissues. Moreover, the respective proportions of MI versus DCI are actively maintained as MI is enzymatically transformed into DCI through an NAD–NADH-dependent epimerase, depending on tissue requirement.

When insulin binds to its receptor, two distinct inositolphosphoglycans (IPG), incorporating either MI or DCI (DCI-IPG and MI-IPG), are released by the hydrolysis of glycosylphosphatidylinositol lipids located on the outer leaflet of the cell membrane. IPGs affect intracellular metabolic processes, namely by activating key enzymes controlling the oxidative and non-oxidative metabolism of glucose [47]. The inositol glycans are small oligosaccharides released from insulin sensitive cells upon stimulation by insulin. Isolated IPGs are capable of activating insulin-sensitive cells. Despite some differences have been noticed, both DCI and MI incorporated in IPGs significantly reduce IR and promote an appropriate glucose metabolism [48].

However, while DCI-effects are restricted to insulin signaling transduction, MI has demonstrated to exert other noticeable activities in ensuring oocyte quality and maturation. Given that MI-phosphate is required in GnRH agonist-mediated LH inhibition [49], it can be hypothesized that increased availability of MI-phosphate may be involved in LH modulation. Furthermore, IP3 collaborates in regulating intracellular Ca^{2+} release from mitochondria. In oocytes, this mechanism involves a specific receptor subtype (IP3-R1) [50], deemed to play a pivotal role in oocyte maturation, namely during the final stages of oogenesis, when oocyte sensitivity to calcium fluctuations reaches the maximal value. Indeed, oocyte maturation in rat is triggered by calcium release after IP3 injection [51]. Moreover, oocyte culture supplementation with MI promotes meiotic progression into fertilization-competent eggs, whereas depletion of MI intracellular stores desensitizes inositol-related pathways, reducing IP3 and calcium release [52]. Similarly, an *in vitro* study has evidenced that MI improves also embryo quality and performances [53] (Figure 1).

Inositol and insulin resistance

Women affected by PCOS and IR showed reduced serum levels of DCI and increased urinary loss of DCI-IPG [54]. Indeed, inositols (both MI and DCI) have been shown to participate in the insulin-signaling pathway, given that MI and DCI are constitutive components of inositolglycan, a poor characterized modulator of insulin function. It stimulates pyruvate dehydrogenase phosphatase and allosterically activates protein phosphatase 2C [55]. Additionally, anti-inositolglycan antibodies block the *in vitro* effects of purified insulin mediators as well as the insulin-induced stimulation of pyruvate dehydrogenase in intact BC3H1 myocytes [56].

Once DCI-IPG was ascertained to be a key component of the insulin transduction mechanism in the cells, it was possible to establish a clear mechanistic link between IR and inositol deficiency in PCOS patients [57]. That finding prompted to verify the clinical efficacy of DCI in PCOS therapy. Indeed, earliest studies showed that PCOS women treated with DCI experienced lower free and total cholesterol levels, lower blood pressure, increased insulin sensitivity, decreased serum androgens, and ensure a higher frequency of ovulation, in both lean [58] and obese patients [59]. Furthermore, DCI seems to improve BMI, waist–hip ratio, and both systolic and diastolic blood pressure, hence counteracting the main features of the metabolic syndrome [60]. However, whereas DCI administration improves the systemic consequences of IR, namely by modulating insulin activity in non-ovarian tissues, DCI exerts controversial effects on oocyte function. This paradoxical behavior received a sound confirmation by further studies in which higher DCI doses were administered to PCOS patients [61]. Indeed, encouraging results obtained during the first pilot DCI-based trial [59] prompted Nestler and his team to establish if more beneficial effects could be obtained with even increased doses of DCI. Yet, by treating PCOS women with 2.4 g DCI daily, Nestler was unable to confirm the previous outcomes [46]. As a result, DCI was discontinued from development by the company that conducted Phase I and Phase II studies in both women with PCOS and women with diabetes [46]. Those findings seem to be confirmed by an investigation performed on PCOS patients treated with increasing DCI doses (from 300 to 2400 mg/day), thus indicating that high DCI dosage paradoxically worsens oocyte quality and ovarian response in non-obese and non-insulin resistant PCOS women [62]. Those disappointing data are in some way mirrored by results obtained by treating PCOS patients with Metformin: the antidiabetic drug decreases the follicles number and worsens their quality [63], though Metformin significantly increases the insulin-stimulated release of DCI-phosphoglycans and improves some systemic features of PCOS [44]. It seems as though DCI may exert some beneficial effects at systemic level by properly modulating insulin-based activity, meanwhile hampering ovarian function. Indeed, high release of DCI-phosphoglycans, under insulin stimulation, enhances *de novo* testosterone biosynthesis from ovarian theca cells, thus raising serum androgen levels [64]. In addition, DCI may impair the subtle equilibrium in between MI and DCI within ovary cells. Both DCI and MI are required to ensure a proper glucose metabolism in cooperating with insulin. Yet, MI seems to play a more critical role in oocyte, as suggested by the fact that almost 99% of intracellular inositol pool is constituted by MI [65]. DCI, instead, is produced from MI through an NAD-dependent epimerase whenever it is required. The epimerase conversion of MI to DCI is under insulin control: in type 2 diabetes patients the reduced tissue insulin sensitivity leads to decreased epimerase activity and hence downregulates DCI synthesis [66]. However, ovary retains normal insulin sensitivity even when other tissues display IR [67]. Thus, increased

insulin levels as those recorded in insulin-resistant patients, are likely to paradoxically foster the activity of ovary epimerase, raising in that way the DCI intracellular production and decreasing MI levels. Thereby, in hyperinsulinemic PCOS patients DCI levels are unexpectedly increased in the ovary, and further DCI administration cannot lead to any significant benefit. Moreover, MI depletion will in turn negatively affect the oocyte quality. Such paradox may help in explaining why DCI alone cannot be considered a reliable approach to PCOS management and shed light into the so-called ''DCI paradox in the ovary'' [68]. Indeed, a significant increase in the epimerase activity in the theca cells obtained from ovary of PCOS women has been found to be associated with a dramatic reduction in the MI/DCI ratio [69]. That finding has been indirectly confirmed by the significant decrease in the MI/DCI ratio recorded in follicular fluid from PCOS women [70]. While normal MI/DCI ratio is nearly 100:1, in follicular fluid of PCOS women that value account for only 0.2:1.

Myo-inositol and non-insulin-dependent effects

MI and its phosphate-derivatives play numerous pivotal and unforeseen roles in many cellular functions. Accordingly, either the physiologic and pharmacological function displayed by inositol are currently investigated in deep [71].

MI participates in counteracting physical stresses, namely by modulating osmolarity, and, somehow, it seems to stabilize the structure of cell proteins during environmental challenges [72]; it improves insulin-transduction pathway and takes part in several metabolic regulatory controls [73]. MI and its phosphate derivatives take part also in signaling transduction, membrane dynamics, developmental processes, cytoskeleton rearrangement, just to mention a few [74]. Conversely, an imbalance of MI levels has been suspected to be involved in many diseases, such as cancer, diabetes, PCOS.

Usefulness of MI supplementation in PCOS has been assessed by several reports [75,76]. Morgante et al. have evidenced that MI-treated insulin resistant-PCOS patients show a significant improvement in clinical pregnancy rate (33.3% versus 13.3%) [77]. A randomized, double-blind, placebo-controlled trial with PCOS patients, showed that the frequency of ovulation (40%) was significantly increased in women who received MI, versus the control group [60]. Additional evidence has been provided by Kamenov et al., demonstrating that MI treatment ameliorates IR and body weight, and improves ovarian activity in PCOS patients [78]. Moreover, it is worth noting that MI significantly slowdown the number of FSH treatments needed to trigger ovulation [79].

Additional studies demonstrated that MI treatment lowered lipids, insulin and androgen levels, increased insulin sensitivity, reduced blood diastolic pressure, and was effective in treating acne and hirsutism [80,81]. Taken as a whole, those data evidenced that MI supplementation provides higher efficacy in PCOS management, when compared to conventional therapies or DCI alone. However, a potential bias is represented by the fact that most papers suffer from the lack of a proper randomization, and/or are flawed by a few statistical inconsistencies [82].

Yet, these studies provided the rationale for a different formulation of inositol-based PCOS treatment, as it was recently argued in a thoughtful opinion paper [46]. DCI alone, at low dosage, may restore normal insulin sensitivity in classic insulin target tissues, such as liver and muscle, which would then reduce circulating insulin levels. Then, the enhancement in ovulatory frequency recorded in clinical trial with DCI could be ascribed to the overall improvement in insulin sensitivity and reduction in circulating insulin and androgens. In contrast, higher dose of DCI would likely impair the MI/DCI ratio, leading to a dramatic alteration in ovary functions. As opposed, MI exert its beneficial effects mainly at the ovary level not only by enhancing insulin signaling transduction, but also by directly acting on a number of ovarian functions, including steroidogenesis [80]. This aspect deserves much further attention than previously hypothesized. Indeed, the rationale of the inositol-based treatment of PCOS mostly rely on the insulin-mimicking effect of inositolglycans containing DCI or MI. Yet, these compounds have been suspected to exert additional and even opposite effects on several pathways downstream the insulin signaling, namely, DCI-IPG and MI-IPG influence aromatase activity [83] and 3α-hydroxysteroid dehydrogenase [84] in a subtle diverse fashion. In addition, a synthetic chiro-inositol-containing glycan has been shown to mimic insulin's stimulation of thecal testosterone biosynthesis in a concentration-dependent manner and to a degree at least equal to that of insulin [64]. Furthermore, it is unlikely that physiological benefits of inositols could only be restricted to their insulin-like activity, given that inositols and their phosphate derivatives display a wide range of pleiotropic effects, including among others, modulation of the PI3K/Akt pathway, calcium release, PKA and PKCδ activity [85].

On the other hand, it is worth noting that some reports suggest that MI may also influence cytoskeleton dynamics, while cytoskeleton alterations have been linked to the onset of PCOS [86]. Indeed, remodeling of cytoskeleton is a prerequisite for ovary cells entering into the epithelial-mesenchymal transition (EMT) and EMT is in turn required for appropriate follicle maturation. We may speculate that MI probably modulates microfilaments polymerization, microtubules and intermediate filaments architecture, and by this way it could enable the reversion of the main PCOS features, including abnormal steroid synthesis [82]. Overall those data hint a relevant difference in the biological effects on the ovary triggered by DCI and MI containing IPGs, although both participate in ensuring a proper insulin signaling. Yet, the controversial activity of DCI within the ovary in no way can allow us to forget the positive effects displayed by DCI on non-ovarian tissues. Thereby, a proper treatment should integrate the positive effects exerted by both inositol isoforms, combined according to the physiological MI/DCI plasma ratio (40:1) [87].

A recent trial including 100 PCOS women undergoing IVF-ET, treated with MI combined with DCI according to the 40:1 ratio has provided support to this hypothesis [88]. Significant better results were observed in the MI-DCI (40:1) arm when compared to the control group receiving DCI 500 mg, given that patients treated with this combination required lower dosages of FSH for a shorter period of time, and showed a significant improvement in both oocyte quality and pregnancy rate.

Furthermore, the combined treatment with MI and DCI (40:1) in PCOS patients has ameliorated parameters such as diastolic blood pressure, fasting glucose, fasting insulin and both insulin and glucose AUCs. A similar, positive trend has been detected for HOMA index, triglycerides and both HDL and LDL cholesterol levels. Moreover, the majority of patients achieved again ovulation [89] (for a synthetic comparison among therapeutic treatments for PCOS women, see Table 2).

Yet, further studies are warranted to conclusively determine the exact proportion in which MI and DCI should be combined in order to exert a maximal effect. To address such issue more detailed inositol pharmacokinetics and pharmacodynamics investigations are needed.

Conclusions

Differences in symptoms and clinical presentation of PCOS phenotypes claim for a tailored therapeutic approach, in order to address both reproductive desires and medical issues. In so far as

Table 2. Comparison among therapeutic treatments for PCOS women.

Drug	Route of administration	Patient's desire for pregnancy	Therapeutic target	Chemical structure	Mechanism(s) of action	Side effects
Contraceptive in combined therapy of estrogen and progestin (COCs)	Oral	No	Menstrual disorders, vaginal bleeding, acne, hirsutism, obesity	Among a large number of active molecules, examples are: $C_{20}H_{24}O_2$ (ethinylestradiol) $C_{21}H_{28}O_2$ (levonorgestrel)	Suppressing activity on LH release, SHBG increase, peripheral block of androgen receptors, androgen levels decrease, low-density lipoprotein and total cholesterol reduction, rise of high-density lipoprotein and triglycerides	Dizziness, headache, lightheadedness, stomach upset, bloating, nausea, weight gain, cardio-vascular and thromboembolic events; metabolic disorders may be aggravated or even triggered
Metformin	Oral	Yes	Insulin resistance, acne, hirsutism, ovulation and fertility problems	$C_4H_{11}N_5$	Insulin sensitizer – reduction of hyperinsulinemia and androgen levels, lipid profile improvement, reduced menstrual cycle disorders	Nausea, vomiting, diarrhea, abdominal bloating
Thiazolidinediones (troglitazone, rosiglitazone and pioglitazone)	Oral	Yes	Insulin resistance, acne, hirsutism, ovulation and fertility problems	$C_{24}H_{27}NO_5S$ (troglitazone) $C_{18}H_{19}N_3O_3S$ (rosiglitazone) $C_{19}H_{20}N_2O_3S$ (pioglitazone)	Insulin sensitizers – agonists of peroxisome proliferator–activated receptor γ (PPARγ), modulation of the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism in the lipidic, muscular tissues and in the liver	Fluid retention, body weight increase, coronary artery disease, myocardial infarction, bladder cancer
MI	Oral	Yes	Insulin resistance, acne, hirsutism, ovulation and fertility problems	$C_6H_{12}O_6$ (stereoisomer of DCI)	Insulin sensitizer – enhancer of oocyte and embryo quality – regulation of: glucose transporters activation and glucose utilization (at systemic level); glucose uptake and FSH signaling – modulation of LH and Ca_2+ release from mitochondria (in the ovary) – decrease of lipids and androgen levels, reduction of blood diastolic pressure	None
DCI	Oral	Yes	Insulin resistance	$C_6H_{12}O_6$ (stereoisomer of MI, which is enzymatically transformed into DCI through a NAD–NADH-dependent epimerase, depending on tissue requirement	Insulin sensitizer – regulation of: glycogen synthesis (at systemic level); insulin-induced androgen synthesis (in the ovary)- decrease of free and total cholesterol levels, blood pressure, serum androgens	None
MI + DCI at the 40:1 ratio	Oral	Yes	Insulin resistance, ovulation and fertility problems		Both activities listed for MI and DCI	None

For an updated review on all drugs for PCOS treatment see reference [35].

no conclusive agreement exists upon mechanisms responsible for PCOS pathogenesis, current treatments should be tightly personalized. Hormonal manipulations (estro-progestins, antiandrogens), although effective in counteracting hyperandrogenism effects, negatively affect reproductive outcomes and are thereby reserved to women without immediate desire for pregnancy.

Metformin has long represented a first-line choice for PCOS treatment in women in which restoring ovulation is mandatorily required for becoming pregnant. However, the usefulness of Metformin in non-insulin resistant PCOS women is broadly debatable. Indeed, available data hints that normalizing IR at the systemic level is not enough for restoring a proper ovulatory function, as additionally evidenced by a study in which Metformin administration improved PCOS symptoms only in about 50% of patients [90]. The existing evidence indicates that intra-ovarian androgens deregulation, likely due to the MI/DCI imbalance, may be the main culprit for follicular arrest in PCOS [91]. Hyperandrogenism promotes follicle excess, which in turn increases AMH intra-ovarian levels, and then could exert an inhibiting effect on the FSH-induced aromatase activity, eventually overcoming the ''maturation capacity'' of the ovary.

A reliable clinical alternative to conventional treatment approaches may be offered by inositol-based drugs. Both inositol isoforms – MI and DCI – have been demonstrated to improve insulin signaling. MI is safe and has been proved to interfere with different targets at both ovarian and non-ovarian level. On the contrary, DCI alone is unable to exert any valuable improvement on ovary cell functions, as its beneficial effects are mainly limited to non-ovarian tissue in which DCI may significantly inhibit the hyperinsulinemic consequences.

Though MI and DCI share this property with Metformin, they are devoid of the common side-effects associated with Metformin therapy and could thus be considered safer than conventional ISDs. Modulation of insulin activity, in turn, interferes with steroidogenic pathways leading to reduced androgen synthesis at both ovarian and adrenal level. However, high DCI concentration is detrimental for ovary, where increased activity of epimerase activity leads to enhanced accumulation of DCI and concomitant depletion of MI stores. On the contrary, MI exerts several significant functions within the ovarian tissue, including a direct modulation activity on steroidogenesis through cytoskeleton modification [82]. Moreover, it is worth noting that MI treatment has shown to be effective in PCOS patients with and without IR.

To restore MI content in ovary cells without losing DCI benefits, a proper combination of both isomers should be envisaged. A formulation based on the physiological plasma ratio MI:DCI (40:1) has already proven to induce higher clinical results than MI or DCI alone. It is likely that a safe, natural compound able to counteract both hyperinsulinemia as well as hyperandrogenism in PCOS may significantly change the current prevalent approach in PCOS management. The different responsiveness of hyperinsulinemic and non-hyperinsulinemic patients to inositol treatment should be carefully addressed, and larger, randomized clinical studies are warranted to confirm the promising preliminary clinical results, till now obtained. The possibility in gathering such information in due time must be considered a critical step in expanding the clinician's confidence on this new pharmacological approach.

As final conclusion, let us enumerate just a few critical aspects that merit an in-depth inquiry:

(a) Metabolism of inositol isomers in the ovary cells. To be precise, it must be investigated the quantitative MI uptake and its further processing by the integrated interplay of inositol-kinases and phosphatase. Namely, the reported inhibitory activity of high DCI levels on the MI uptake deserves to be fully clarified, as that mechanism raises a relevant concern on some current clinical practices in which high DCI concentrations are used.
(b) MI has been shown to modulate cytoskeleton architecture. In turn, cytoskeleton may efficiently interfere with steroidogenesis within the ovary. How MI actually participates in modulating this process needs to be deciphered in detail.
(c) The dynamics of MI/DCI interconversion and the regulation of epimerase activity require a novel investigation, possibly carried out in a 3D-model of ovary cells.
(d) Accordingly, the cross-talk in between inositols and endocrine factors (FSH, LH) within ovary should be carefully addressed.

These basic studies are thought to supply more solid foundations for clinical studies in providing new insights into the molecular mechanisms through which MI acts in fostering follicle maturation and ovary functions.

Declaration of interest

Vittorio Unfer is an employee at Lo.Li.Pharma, Rome, Italy. Giovanni Monastra, Abdel Halim Harrath and Mariano Bizzarri declare that there is no conflict of interest regarding the publication of this paper.

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References

1. Porcaro G, Bizzarri M, Monastra G, et al Strategies for the treatment of polycystic ovary syndrome (PCOS) women: the role of myo-inositol (MI) and d-chiro-inositol (DCI) between diet and therapy. In: Cobbs B, ed. Polycystic ovary syndrome (PCOS): clinical aspects, potential complications and dietary management. Hauppauge, NY: Nova science publishers; 2016, in press.
2. Nardo LG, Patchava S, Laing I. Polycystic ovary syndrome: pathophysiology, molecular aspects and clinical implications. Panminerva Med 2008;50:267–78.
3. Orio F, Palomba S. Reproductive endocrinology: new guidelines for the diagnosis and treatment of PCOS. Nat Rev Endocrinol 2014;10: 130–2.
4. Orio F, Vuolo L, Palomba S, et al. Metabolic and cardiovascular consequences of polycystic ovary syndrome. Minerva Ginecol 2008; 60:39–51.
5. Thomas MP, Mills SJ, Potter BV. The ''other'' inositols and their phosphates: synthesis, biology, and medicine (with recent advances in myo- inositol chemistry). Angew Chem Int Ed Engl 2015;54: 2–39.
6. Hoglan FA, Bartow E. Inactive inositol and its derivatives. Ind Eng Chem 1939;31:749–50.
7. Takeo Y. Influence of continuous illumination on estrous cycle of rats: time course of changes in levels of gonadotropins and ovarian steroids until occurrence of persistent estrus. Neuroendocrinology 1984;39:97–104.
8. Lowton IE, Schwartz NB. Pituitary-ovarian function in rats exposed to constant light: a chronological study. Endocrinology 1967;81: 497–508.
9. Jain P, Jain M, Haldar C, et al. Melatonin and its correlation with testosterone in polycystic ovarian syndrome. J Hum Reprod Sci 2013;6:253–8.
10. Tamura H, Nakamura Y, Korkmaz A, et al. Melatonin and the ovary: physiological and pathophysiological implications. Fertil Steril 2009;92:328–43.
11. Anderson E, Lee GY. The polycystic ovarian (PCO) condition: apoptosis and epithelialization of the ovarian antral follicles are aspects of cystogenesis in the dehydroepiandrosterone (DHEA)-treated rat model. Tissue Cell 1997;29:171–89.
12. Abbott DH, Dumesic DA, Franks S. Developmental origin of polycystic ovary syndrome – a hypothesis. J Endocrinol 2002;174: 1–5.
13. Micić D, Zorić S, Popović V, et al. Androgen-producing bilateral large cortical adrenal adenomas associated with polycystic ovaries in a young female. Postgrad Med J 1992;68:219–22.

14. Nelson VL, Legro RS, Strauss III JF, McAllister JM. Augmented androgen production is a stable steroidogenic phenotype of propagated theca cells from polycystic ovaries. Mol Endocrinol 1999;13:946–57.
15. Liu YX, Hsueh AJ. Synergism between granulosa and theca-interstitial cells in estrogen biosynthesis by gonadotropin-treated rat ovaries: studies on the two-cell, two-gonadotropin hypothesis using steroid antisera. Biol Reprod 1986;35:27–36.
16. Wide L, Naessén T, Sundström-Poromaa I, Eriksson K. Sulfonation and sialylation of gonadotropins in women during the menstrual cycle, after menopause, and with polycystic ovarian syndrome and in men. J Clin Endocrinol Metab 2007;92:4410–17.
17. Hernández-Valencia M, Hernández-Quijano T, Sandoval A, et al. Changes in glycosylation of pituitary gonadotropins in polycystic ovary syndrome. Int J Reprod Fertil Sex Health 2014;1:20–4.
18. Ulloa-Aguirre A, Cravioto A, Damián-Matsumura P, et al. Biological characterization of the naturally occurring analogues of intrapituitary human follicle-stimulating hormone. Hum Reprod 1992;7:23–30.
19. Zambrano E, Barrios-de-Tomasi J, Cárdenas M, Ulloa-Aguirre A. Studies on the relative in-vitro biological potency of the naturally-occurring isoforms of intrapituitary follicle stimulating hormone. Mol Hum Reprod 1996;2:563–71.
20. Timossi C, Barrios-de-Tomasi J, Zambrano E, Ulloa-Aguirre A. A naturally occurring basically charged human follicle-stimulating hormone (FSH) variant inhibits FSH-induced androgen aromatization and tissue-type plasminogen activator enzyme activity in vitro. Neuroendocrinology 1998;67:153–63.
21. Salhanick AI, Amatruda JM. Role of sialic acid in insulin action and the insulin resistance of diabetes mellitus. Am J Physiol 1988;255: E173–9.
22. Loreti N, Ambao V, Andreone L, et al. Effect of sialylation and complexity of FSH oligosaccharides on inhibin production by granulosa cells. Reproduction 2013;145:127–35.
23. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev 1997;18: 774–800.
24. De Leo V, la Marca A, Petraglia F. Insulin-lowering agents in the management of polycystic ovary syndrome. Endocr Rev 2003;24: 633–67.
25. Baillargeon JP, Iuorno MJ, Nestler JE. Insulin sensitizers for polycystic ovary syndrome. Clin Obstet Gynecol 2003;46:325–40.
26. Legro RS, Kunselman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. J Clin Endocrinol Metab 1999;4: 165–9.
27. Genazzani AD, Battaglia C, Malavasi B, et al. Metformin administration modulates and restores luteinizing hormone spontaneous episodic secretion and ovarian function in nonobese patients with polycystic ovary syndrome. Fertil Steril 2004;81:114–19.
28. Mayer SB, Evans WS, Nestler JE. Polycystic ovary syndrome and insulin: our understanding in the past, present and future. Womens Health (Lond) 2015;11:137–49.
29. Selva DM, Hogeveen KN, Innis SM, Hammond GL. Monosaccharide-induced lipogenesis regulates the human hepatic sex hormone-binding globulin gene. J Clin Invest 2007;117: 3979–87.
30. Unfer V, Carlomagno G, Dante G, Facchinetti F. Effects of myo-inositol in women with PCOS: a systematic review of randomized controlled trials. Gynecol Endocrinol 2012;28:509–15.
31. Venkatesan AM, Dunaif A, Corbould A. Insulin resistance in polycystic ovary syndrome: progress and paradoxes. Recent Prog Horm Res 2001;56:295–308.
32. Genazzani AD, Lanzoni C, Ricchieri F, et al. Metformin administration is more effective when non-obese patients with polycystic ovary syndrome show both hyperandrogenism and hyperinsulinemia. Gynecol Endocrinol 2007;23:146–52.
33. Stabile G, Borrielli I, Artenisio AC, et al. Effects of the insulin sensitizer pioglitazone on menstrual irregularity, insulin resistance and hyperandrogenism in young women with polycystic ovary syndrome. J Pediatr Adolesc Gynecol 2014;27:177–82.
34. Orio F, Muscogiuri G, Palomba S. Could the Mediterranean diet be effective in women with polycystic ovary syndrome? A proof of concept. Eur J Clin Nutr 2015;69:974.
35. Rocca ML, Venturella R, Mocciaro R, et al. Polycystic ovary syndrome: chemical pharmacotherapy. Expert Opin Pharmacother 2015;16:1369–93.
36. De Leo V, Morgante G, Piomboni P, et al. Evaluation of effects of an oral contraceptive containing ethinylestradiol combined with drospirenone on adrenal steroidogenesis in hyperandrogenic women with polycystic ovary syndrome. Fertil Steril 2007;88: 113–17.
37. Guido M, Romualdi D, Giuliani M, et al. Drospirenone for the treatment of hirsute women with polycystic ovary syndrome: a clinical, endocrinological, metabolic pilot study. J Clin Endocrinol Metab 2004;89:2817–23.
38. Palomba S, Falbo A, Zullo F, Orio Jr F, Evidence-based and potential benefits of metformin in the polycystic ovary syndrome: a comprehensive review. Endocr Rev 2009;30:1–50.
39. Romualdi D, Giuliani M, Cristello F, et al. Metformin effects on ovarian ultrasound appearance and steroidogenic function in normal-weight normoinsulinemic women with polycystic ovary syndrome: a randomized double-blind placebo-controlled clinical trial. Fertil Steril 2010;93:2303–10.
40. Kolodziejczyk B, Duleba AJ, Spaczynski RZ, Pawelczyk L. Metformin therapy decreases hyperandrogenism and hyperinsulinemia in women with polycystic ovary syndrome. Fertil Steril 2000;73: 1149–54.
41. Trolle B, Flyvbjerg A, Kesmodel U, Lauszus FF. Efficacy of metformin in obese and non-obese women with polycystic ovary syndrome: a randomized, double-blinded, placebo-controlled cross-over trial. Hum Reprod 2007;22:2967–73.
42. Baillargeon JP, Jakubowicz DJ, Iuorno MJ, et al. Effects of metformin and rosiglitazone, alone and in combination, in nonobese women with polycystic ovary syndrome and normal indices of insulin sensitivity . Fertil Steril 2004;82:893–902.
43. Cosma M, Swiglo BA, Flynn DN, et al. Clinical review: insulin sensitizers for the treatment of hirsutism: a systematic review and metaanalyses of randomized controlled trials. J Clin Endocrinol Metab 2008;93:1135–42.
44. Baillargeon JP, Iuorno MJ, Jakubowicz DJ, et al. Metformin therapy increases insulin-stimulated release of D-chiro-inositol-containing inositolphosphoglycan mediator in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2004;89:242–9.
45. Sun TH, Heimark DB, Nguygen T, et al. Both myo-inositol to chiro-inositol epimerase activities and chiro-inositol to myo-inositol ratios are decreased in tissues of GK type 2 diabetic rats compared to Wistar controls. Biochem Biophys Res Commun 2002;293:1092–8.
46. Nestler JE, Unfer V. Reflections on inositol(s) for PCOS therapy: steps toward success. Gynecol Endocrinol 2015;31:501–5.
47. Larner J. D-chiro-inositol-its functional role in insulin action and its deficit in insulin resistance . Int J Exp Diabetes Res 2002;3:47–60.
48. Ortmeyer HK. Dietary myoinositol results in lower urine glucose and in lower postprandial plasma glucose in obese insulin resistant rhesus monkeys. Obes Res 1996;4:569–75.
49. Janovick JA, Conn PM. Gonadotropin-releasing hormone (GnRH)-receptor coupling to inositol phosphate and prolactin production in GH3 cells stably transfected with rat GnRH receptor complementary deoxyribonucleic acid. Endocrinology 1994;135:2214–19.
50. Goud PT, Goud AP, Van Oostveldt P, Dhont M. Presence and dynamic redistribution of type I inositol 1,4,5-trisphosphatereceptors in human oocytes and embryos during in-vitro maturation, fertilization and early cleavage divisions. Mol Hum Reprod 1999;5: 441–51.
51. Lowther KM, Weitzman VN, Maier D, Mehlmann LM. Maturation, fertilization, and the structure and function of the endoplasmic reticulum in cryopreserved mouse oocytes. Biol Reprod 2009;81: 147–54.
52. Chiu TT, Rogers MS, Briton-Jones C, Haines C. Effects of myo-inositol on the in-vitro maturation and subsequent development of mouse oocytes . Hum Reprod 2003;18:408–16.
53. Colazingari S, Fiorenza MT, Carlomagno G, et al. Improvement of mouse embryo quality by myo-inositol supplementation of IVF media. J Assist Reprod Genet 2014;31:463–9.
54. Baillargeon JP, Nestler JE, Ostlund RE, et al. Greek hyperinsulinemic women, with or without polycystic ovary syndrome, display altered inositols metabolism. Hum Reprod 2008;23:1439–46.
55. Larner J, Brautigan DL, Thorner MO. D-chiro-inositol glycans in insulin signaling and insulin resistance. Mol Med 2010;16:543–52.

56. Romero G, Gámez G, Huang LC, et al. Anti-inositolglycan antibodies selectively block some of the actions of insulin in intact BC3H1 cells. Proc Natl Acad Sci USA 1990;87:1476–80.
57. Baillargeon JP, Iuorno MJ, Apridonidze T, Nestler JE. Uncoupling between insulin and release of a D-chiro-inositol-containing inositolphosphoglycan mediator of insulin action in obese women with polycystic ovary syndrome. Metab Syndr Relat Disord 2010;8: 127–36.
58. Iuorno MJ, Jakubowicz DJ, Baillargeon JP, et al. Effects of D-chiro-inositol in lean women with the polycystic ovary syndrome. Endocr Pract 2002;8:417–23.
59. Nestler JE, Jakubowicz DJ, Reamer P, et al. Ovulatory and metabolic effects of D-chiro-inositol in the polycystic ovary syndrome. N Engl J Med 1999;340:1314–20.
60. Gerli S, Mignosa M, Di Renzo GC. Effects of inositol on ovarian function and metabolic factors in women with PCOS: a randomized double blind placebo-controlled trial. Eur Rev Med Pharmacol Sci 2003;7:151–9.
61. Galazis N, Galazi M, Atiomo W. D-Chiro-inositol and its significance in polycystic ovary syndrome: a systematic review. Gynecol Endocrinol 2011;27:256–62.
62. Isabella R, Raffone E. Does ovary need D-chiro-inositol? J Ovarian Res 2012;5:14.
63. Palomba S, Falbo A, Di Cello A, et al. Does metformin affect the ovarian response to gonadotropins for in vitro fertilization treatment in patients with polycystic ovary syndrome and reduced ovarian reserve? A randomized controlled trial. Fertil Steril 2011;96: 1128–33.
64. Nestler JE, Jakubowicz DJ, de Vargas AF, et al. Insulin stimulates testosterone biosynthesis by human thecal cells from women with polycystic ovary syndrome by activating its own receptor and using inositolglycan mediators as the signal transduction system. J Clin Endocrinol Metab 1998;83:2001–5.
65. Unfer V, Carlomagno G, Papaleo E, et al. Hyperinsulinemia alters myoinositol to d-chiroinositol ratio in the follicular fluid of patients with PCOS. Reprod Sci 2014;21:854–8.
66. Asplin I, Galasko G, Larner J. Chiro-inositol deficiency and insulin resistance: a comparison of the chiro-inositol- and the myoinositol-containing insulin mediators isolated from urine, hemodialysate, and muscle of control and type II diabetic subjects. Proc Natl Acad Sci USA 1993;90:5924–8.
67. Rice S, Christoforidis N, Gadd C, et al. Impaired insulin-dependent glucose metabolism in granulosa-lutein cells from anovulatory women with polycystic ovaries. Hum Reprod 2005;20:373–81.
68. Carlomagno G, Unfer V, Roseff S. The D-chiro-inositol paradox in the ovary. Fertil Steril 2011;95:2515–16.
69. Heimark D, McAllister J, Larner J. Decreased myo-inositol to chiro-inositol (m/c) ratios and increased m/c epimerase activity in pcos theca cells demonstrate increased insulin sensitivity compared to controls. Endocr J 2013;61:111–17.
70. Chiu TT, Rogers MS, Law EL, et al. Follicular fluid and serum concentrations of myo-inositol in patients undergoing IVF: relationship with oocyte quality. Hum Reprod 2002;17:1591–6.
71. Shears SB, Ganapathi SB, Gokhale NA, et al. Defining signal transduction by inositol phosphates. Subcell Biochem 2012;59: 389–412.
72. Michell RH. Inositol derivatives: evolution and functions. Nat Rev Mol Cell Biol 2008;9:151–61.
73. Holub BJ. Metabolism and function of myo-inositol and inositol phospholipids. Annu Rev Nutr 1986;6:563–97.
74. Lahiri A, Majumder, Biswas BB, eds. Biology of inositols and phosphoinositides. subcellular biochemistry Vol. *39*. Berlin: Springer; 2006.
75. Genazzani AD, Lanzoni C, Ricchieri F, Jasonni VM. Myo-inositol administration positively affects hyperinsulinemia and hormonal parameters in overweight patients with polycystic ovary syndrome. Gynecol Endocrinol 2008;24:139–44.
76. Dinicola S, Chiu TTY, Unfer V, Carlomagno G, Bizzarri M. The rationale of the myo-inositol and D-chiro-inositol combined treatment for polycystic ovary syndrome. J Clin Pharmacol 2014;54: 1079–92.
77. Morgante G, Orvieto R, Di Sabatino A, et al. The role of inositol supplementation in patients with polycystic ovary syndrome, with insulin resistance, undergoing the low-dose gonadotropin ovulation induction regimen. Fertil Steril 2011;95:2642–4.
78. Kamenov Z, Kolarov G, Gateva A, et al. Ovulation induction with myo-inositol alone and in combination with clomiphene citrate in polycystic ovarian syndrome patients with insulin resistance. Gynecol Endocrinol 2015;31:131–5.
79. Lisi F, Carfagna P, Oliva MM, et al. Pretreatment with myo-inositol in non-polycystic ovary syndrome patients undergoing multiple follicular stimulation for IVF: a pilot study. Reprod Biol Endocrinol 2012;23:52.
80. Minozzi M, Costantino D, Guaraldi C, Unfer V. The effect of a combination therapy with myo-inositol and a combined oral contraceptive pill versus a combined oral contraceptive pill alone on metabolic, endocrine, and clinical parameters in polycystic ovary syndrome. Gynecol Endocrinol 2011;27:920–4.
81. Zacchè MM, Caputo L, Filippis S, et al. Efficacy of myo-inositol in the treatment of cutaneous disorders in young women with polycystic ovary syndrome. Gynecol Endocrinol 2009;25:508–13.
82. For a review see: Bizzarri M, Cucina A, Harrath AH, et al. Does myo-inositol effect on PCOS follicles involve cytoskeleton regulation? Med Hypotheses 2016;91:1–5.
83. Nestler JE. Modulation of aromatase and P450 cholesterol side-chain cleavage enzyme activities of human placental cytotrophoblasts by insulin and insulin-like growth factor I. Endocrinology 1987;121:1845–52.
84. Nestler JE. Insulin and insulin-like growth factor-I stimulate the 3 beta-hydroxysteroid dehydrogenase activity of human placental cytotrophoblasts. Endocrinology 1989;125:2127–33.
85. Vucenik I, Ramakrishna G, Tantivejkul K, et al. Inositol hexaphosphate (IP6) blocks proliferation of human breast cancer cells through a PKCdelta-dependent increase in p27Kip1 and decrease in retinoblastoma protein (pRb) phosphorylation. Breast Cancer Res Treat 2005;91:35–45.
86. Ortega HH, Salvetti NR, Müller LA, et al. Characterization of cytoskeletal proteins in follicular structures of cows with cystic ovarian disease. J Comp Pathol 2007;136:222–30.
87. Facchinetti F, Dante G, Neri I. The ratio of MI to DCI and its impact in the treatment of polycystic ovary syndrome: experimental and literature evidences. Front Gynecol Endocrinol 2015;3:103–9.
88. Colazingari S, Treglia M, Najjar R, Bevilacqua A. The combined therapy myo-inositol plus D-chiro-inositol, rather than D-chiro-inositol, is able to improve IVF outcomes: results from a randomized controlled trialArch. Arch Gynecol Obstet 2013;288:1405–11.
89. Nordio M, Proietti E. The combined therapy with myo-inositol and D-chiro-inositol reduces the risk of metabolic disease in PCOS overweight patients compared to myo-inositol supplementation alone. Eur Rev Med Pharmacol Sci 2012;16:575–81.
90. Raffone E, Rizzo P, Benedetto V. Insulin sensitiser agents alone and in co-treatment with r-FSH for ovulation induction in PCOS women. Gynecol Endocrinol 2010;26:275–80.
91. Jonard S, Dewailly D. The follicular excess in polycystic ovaries, due to intra-ovarian hyperandrogenism, may be the main culprit for the follicular arrest. Hum Reprod Update 2004;10:107–17.
92. Głuszak O, Stopińska-Głuszak U, Glinicki P, et al. Phenotype and Metabolic Disorders in Polycystic Ovary Syndrome, ISRN Endocrinol 2012;2012:569862. doi:10.5402/2012/569862, modified.