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REVIEW

Inositol as putative integrative treatment for PCOS

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Abstract Studies over the last decade have demonstrated that some polycystic ovary syndrome (PCOS) patients have abnormal insulin sensitivity (insulin resistance), independently from being overweight or obese. This induces the risk of developing type 2 diabetes in such PCOS patients. The use of insulin sensitizers (i.e. metformin), reduces such metabolic, and most hormonal, impairments. As metformin often induces side effects, new integrative strategies have been proposed to treat insulin resistance, such as the use of inositols. Such compounds are mainly represented in humans by two inositol stereoisomers: myo-inositol (MYO) and D-chiro-inositol (DCI). MYO is the precursor of inositol triphosphate, a second messenger that regulates thyroid-stimulating hormone (TSH) and FSH as well as insulin. DCI derives from the conversion of myo-inositol via an insulin-dependent pathway. Several preliminary studies have indicated possible benefits of inositol therapy in PCOS patients, but to date no meta-analysis has been performed. This review aims to give clinical insights for the clinical use of inositol in PCOS.

KEYWORDS: anovulation, d-chiro-inositol, diabetes, hyperandrogenism, insulin resistance, myo-inositol

Introduction

Polycystic ovary syndrome (PCOS) is a common disease that affects 5 – 21% of women during their reproductive life (Azziz et al., 2004). Both the aetiology and diagnosis of the syndrome are controversial. In fact, the Consensus Meeting in Rotterdam (Fauser et al., 2012; The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004) was organized to better define and reach a consensus in the scientific community of diagnostic criteria of the syndrome. To state the presence of PCOS, at least two of the following criteria need to be present: (i) chronic anovulation

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disorder (oligo- or anovulation up to amenorrhoea); (ii) clinical (acne, hirsutism) or biochemical signs of hyperandrogenism; and (iii) presence of micro polycystic ovaries at ultrasound or presence of 12 or more follicles with a diameter of 2 ± 9 mm in each ovary, and/or increased ovarian volume (>10 ml) (Genazzani et al., 2014a).

Although the Rotterdam criteria have been widely accepted, it has recently become clear that a new clinical aspect needs to be taken into account – the dismetabolic feature of insulin resistance. Indeed, an extensive literature search has demonstrated that insulin resistance is a frequent finding in PCOS patients, regardless of body mass index (BMI). Insulin resistance is a specific biological adaptation that induces a compensatory hyperinsulinaemia in approximately 70 – 80% of women with PCOS and central obesity, as well as in 15 – 30% of lean women diagnosed with PCOS (Ciampelli et al., 1999; Fauser et al., 2012; Genazzani et al., 2010).

In light of the relevant metabolic characteristics described, it has recently been proposed that PCOS patients should be reconsidered according to these metabolic features. Two types of PCOS have been suggested: the classic reproductive phenotype of PCOS and a new one, with high metabolic risk, whose proposed name is 'metabolic reproductive syndrome' (MRS) (Dunaif and Fauser, 2013).

Endocrine profile of PCOS patients

PCOS typically shows: higher plasma concentrations of both ovarian and adrenal androgens; LH concentrations above 8 – 12 mIU/ml; elevated oestrogen concentrations (mainly oestrone) due to extra glandular conversion from androgens; reduced sex hormone-binding globulin (SHBG); and, not rarely, the elevation of prolactin and insulin, the latter in presence of overweight or obesity but also in normal weight or lean PCOS patients (Genazzani et al., 2010; Unfer et al., 2014).

Although the pathogenesis of PCOS is still controversial (Doi, 2008; Genazzani et al., 2010), the presence of abnormal LH and relatively low FSH secretion changes the LH:FSH ratio (>2.5) (Doi, 2008; Genazzani et al., 2010; Hirschberg, 2009). Such gonadotrophin impairment is at the basis of the elevated androgen secretion as well as of the abnormal follicular development, both due to the higher stimulation on theca cells (Nelson et al., 2001). The elevation of androgen plasma concentrations (with or without clinical signs) is a classic feature of PCOS, although not constant (Hirschberg, 2009), and it is mainly of ovarian origin with an adrenal contribution. Indeed, some PCOS patients might show a mild steroidogenic defect in adrenal glands (such as for 21-hydroxylase) and/or some others show a higher stress-induced adrenal hyperactivation (Genazzani et al., 1993). Among the androgens, androstenedione and testosterone have a mainly ovarian origin, whereas adrenal contribution is demonstrated by dehydroepiandrosterone sulphate (DHEAS) elevation. An excess of circulating androgens induces a higher peripheral conversion towards the potent androgen dihydrotestosterone (DHT), and depending on the amount of such conversion and/or on the sensitivity of skin to androgens, hirsutism may easily occur.

It is well known that gonadal steroids classically bind to SHBG, being biologically inactive, and that less than 3% of testosterone circulates as unbound in the serum. However, the excess of androgens in PCOS induces a lower hepatic synthesis of SHBG or other binding proteins, which causes a relative excess of free circulating androgens, facilitating the genesis of hirsutism (Genazzani et al., 2010).

An additional feature is that insulin sensitivity is affected directly and indirectly by androgens, because they may directly inhibit peripheral and hepatic insulin action. In fact, it has been demonstrated that testosterone modulates post-binding signal, reducing the number and efficiency of glucose transport proteins, such as the type 4 glucose transporter (GLUT-4), thus inducing insulin resistance in women with PCOS, especially in the most metabolically active tissues such as muscle and fat (Ciaraldi et al., 2002). This situation is more severe in obese patients, due to abdominal fat, as they show a free androgen and insulin plasma concentration as well as insulin resistance higher than weight-matched controls (Kirschner et al., 1990). In addition, obese subjects show that hepatic insulin excretion and insulin-stimulated glucose uptake in skeletal muscle is improved by free fatty acids and androgens, therefore increasing both insulin resistance and compensatory hyperinsulinaemia (Pasquali et al., 1986; Peiris et al., 1987; Rebuffe-Scrive et al., 1991). This explains, at least in part, how in overweight or obese patients any excess of weight can further induce a reduction of peripheral tissue sensitivity to insulin, thus inducing hyperinsulinaemia (Genazzani et al., 2010).

This abnormal insulin concentration might also be triggered by abnormal plasma concentrations of adiponectin and leptin. Adiponectin, an adipocyte-derived collagen-like protein, is synthesized by adipose tissue and released into the circulation (Stefan and Stumvoll, 2002; Trujillo and Scherer, 2005). Leptin is another adipocyte hormone encoded by the human obese (*ob*) gene and transmits metabolic signals to the neuronal networks in the brain so as to modulate the hypothalamic activity affecting the pituitary–ovarian axis (Chakrabarti, 2013). As PCOS patients – mainly if obese – show reduced adiponectin and elevated leptin plasma concentrations, it has been demonstrated that there is a positive correlation between the serum leptin concentrations and the clinical and hormonal indices of IR (Chen et al., 2015). In addition, leptin is linked to neuropeptide Y modulation on the reproductive axis, thus being involved in reproductive disturbance (Jacobs and Conway, 1999).

Insulin resistance and compensatory hyperinsulinaemia

The exact cause of the insulin resistance observed in PCOS is relatively clear, but the fact that it also occurs in lean patients suggests the hypothesis of a post-receptor defect that could affect glucose uptake (Baillargeon and Nestler, 2006; Dunaif, 1997) rather than being related to excessive serine phosphorylation of the insulin receptor (IR) (Dunaif et al., 1992; Vrbikova and Hainer, 2009). The presence of overweight or obesity worsens the insulin resistance, and the compensatory hyperinsulinaemia becomes a potent negative modulator of ovarian function, as hyperinsulinaemia can increase ovarian androgen synthesis (Barbieri, 1990; Dunaif et al., 1990; Unfer et al., 2014) as well as abnormal ovarian stimulation due to the abnormal LH secretion (Conway et al., 1989, 1990). These hypotheses have been recently supported by the meta-analysis by Behboudi-Gandevani et al. (2016).

A relevant clinical aspect is the fact that PCOS patients might develop abnormal glucose control and type 2 diabetes, and such evolution is more rapid in PCOS patients than normal controls (Celik et al., 2014; Hudecova et al., 2011). For this reason, screening of women with PCOS for glucose intolerance and hyperinsulinaemia is an important clinical step that needs to be performed at least with a 2-h oral glucose tolerance test (OGTT) (American Association of Clinical Endocrinologists Polycystic Ovary Syndrome Writing Committee, 2005; ACOG Committee on Practice Bulletins – Gynecology, 2009; Wild et al., 2010). It is clear that changes in everyday lifestyle and/or the use of specific compounds (such as metformin) delay or block the risk of type 2 diabetes development as well as cardiovascular risks (Genazzani et al., 2010, Moran et al., 2011; Wild et al., 2010).

As hyperinsulinaemia was also observed in non-obese PCOS patients, it is clear that an excess of insulin secretion is not only dependent on obesity and/or on reduced glucose tolerance (Unfer et al., 2014). The exaggerated beta cell function of the Langerhans cells stresses its function and over time induces abnormal activity, predisposing the patient to diabetes (Unfer et al., 2014). As an additional fact, insulin amplifies the LH-induced production of androgens by the theca cells, thus exacerbating hyperandrogenism and hyperandrogenic symptoms in hyperinsulinaemic subjects with PCOS (Sam and Dunaif, 2003).

The assessment of the hyperinsulinaemic condition is important and cannot rely only on simple basic insulin determination following fasting. A more accurate estimation is usually obtained in a test that stimulates insulin release after glucose load, orally or intravenously. Usually insulin maximal response occurs within 30 – 90 min after the glucose load and is considered normal if it is below 50 μU/ml (Legro et al., 1998). Insulin resistance and insulin sensitivity can also be computed by the easy calculation of the glucose-to-insulin ratio (Genazzani et al., 2004; Legro et al., 1998), the value of which should be higher than 4.5; or the more precise homeostasis model assessment (HOMA) index can be computed as HOMA-insulin resistance as follows: (fasting insulin mU/l) × (fasting glucose mmol/l)/22.5 (Madeira et al., 2008). Hyperinsulinaemia is present when the HOMA value is below 2.71 in adults (Geloneze et al., 2009; Madeira et al., 2008) and 2.5 in children and adolescents (Geloneze et al., 2009). Recently two different groups proposed considering all patients showing fasting insulin plasma concentrations above 12 – 13 μU/ml at risk of compensatory hyperinsulinaemia (Genazzani et al., 2012; Lunger et al., 2013).

Hyperandrogenism and hyperinsulinaemia in PCOS: treatment options

PCOS is characterized by the hyperandrogenic condition in most cases, and as this excess of androgens induces several inaesthetisms, such as acne, seborrhoea and hirsutism, the use of the oestro-progestin pill has been widely used (Goodman et al., 2015). Quite often the use of specific anti-androgenic compounds, such as flutamide and finasteride, in combination with oral contraceptives, permits a great improvement and the restoration of the integrity of skin annexes, greatly reducing insults due to hyperandrogenism (Goodman et al., 2015). However, none of these therapeutical strategies controls the metabolic impairment of PCOS patients.

In fact, insulin-sensitizing agents (metformin, pioglitazone and troglitazone) have been used to treat hyperinsulinaemic PCOS patients (Genazzani et al., 2007, 2010; Pasquali and Gambineri, 2006). Although reducing hyperandrogenic signs and androgen concentration (Goodman et al., 2015; Lord et al., 2003; Nestler, 2008a, 2008b), commonly used insulin sensitizers may induce gastrointestinal side effects, thus reducing compliance (Lord et al., 2003).

The discovery that a defect in the inositol phosphoglycan (IPG) second messenger pathway (Asplin et al., 1993; Kennington et al., 1990) could be at the basis of the hyperinsulinaemia, via an impairment of the post-receptor insulin-induced signal, was the basis for new therapeutical strategies for hyperinsulinaemic PCOS patients. In fact, such IPG are produced at the cellular membrane level by hydrolysis of glycosyl-phosphatidylinositol lipids located on the internal surface of the cell membrane (Unfer et al., 2014). IPG are then internalized and take part in the intracellular processes corresponding to the intracellular second messenger, which activates the endocellular pathway that controls the oxidative and non-oxidative metabolism of glucose as well as the uptake of glucose by GLUT4 from the extracellular environment (Croze and Soulage, 2013). For this reason, inositol became interesting as an integrative treatment to improve cellular response to the metabolic cascades following the binding of insulin with its receptor. However, insulin is not the only hormone to use IPG – other peptide hormones such as TSH and FSH also use such second messengers (Unfer et al., 2014; Wild et al., 2010).

Inositol and its relevance in insulin hormone signalling

In 1850 Johannes Scherer isolated from muscle cells a new compound, which he named 'inositol' from the combination of various Greek words (Buttner, 1978; Kompanje et al., 2007) and which was formally included in the sugar family (Bizzarri and Carlomagno, 2014). This inositol has been characterized chemically as a hexahydroxycyclohexane and has nine stereoisomers. One of these, myo-inositol (MYO), has been identified as the most common in all biological systems, and for this reason it has been thought to be a specific probiotic molecule (Agranoff, 2009). Indeed, inositols are generally found in many plants and various kinds of foods, especially beans and fruits, as derivatives of inositol that is as hexaphosphate and phytic acid or its salts derivatives (phyates) (Bizzarri and Carlomagno, 2014). Chemically speaking, inositol belongs to the vitamin complex; although very similar to the glucose molecule, it cannot be considered a real nutrient as it can be synthetized in the human body (Bizzarri and Carlomagno, 2014) as well as in prokaryotes and eukaryotes. In this way it can be synthesized from glucose-6-phosphate, which is isomerized to MYO-1-phosphate and then dephosphorylated by an inositol monophosphate enzyme to free MYO (Loewus et al., 1983), but most of the biological requirement comes from the diet.

Once MYO enters the cell, it is immediately transformed inside cell membranes in phosphatidyl-myo-inositol, precursor of the inositol-triphosphate that acts as intracellular second

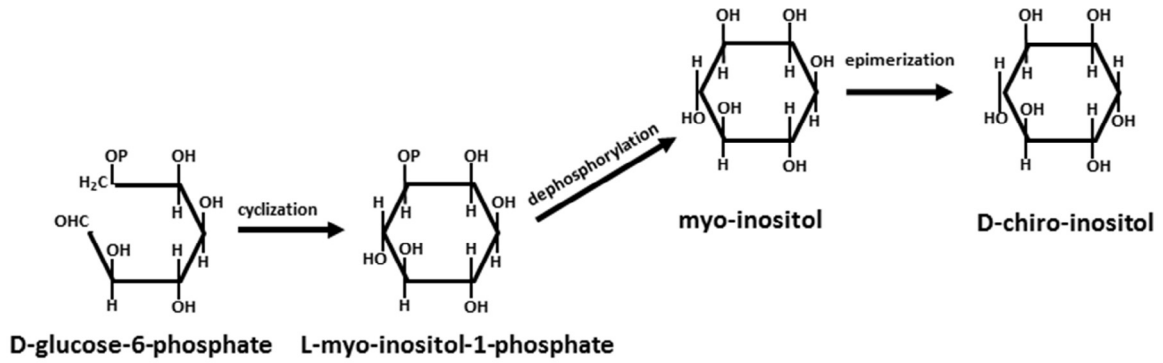

Figure 1 Schematic pathway from D-glucose-6-phosphate to D-chiro-inositol. The limiting enzyme is the epimerase (modified from Larner et al., 2010).

messenger for insulin as well as for FSH and TSH (Buttner, 1978; Thomas et al., 2011; Unfer et al., 2014). MYO is not the only inositol that serves as second messenger. In fact, the inositol family includes nine isomers and Larner reported that two of them, MYO and D-chiro-inositol (DCI), through different mechanisms, are involved in the intracellular transmission of insulin metabolic signal (Larner et al., 1988, 2010). Both MYO and DCI have the same chemical structure but differ in position of a hydroxyl group. *In vivo*, DCI is synthesized through the activity of an epimerase that converts MYO into DCI (**Figure 1**).

After several years, a model was established for these two inositols and for the different ways in which they work in the transmission of the metabolic signal of insulin (Croze and Soulage, 2013; Larner et al., 2010). In brief, binding of insulin on its own receptor activates insulin receptor tyrosine kinase that autophosphorylates, recruits insulin receptor substrates (IRS) proteins and phosphorylates them on Tyr residues to serve as scaffolds (Larner et al., 2010). A principal IR/IRS target is phosphatidylinositol-3-kinase (PI3K), which generates phosphatidylinositol (3,4,5)-trisphosphate (PIP3) to activate the phosphorylation of a protein kinase (PKB)/Akt by the phosphoinositide-dependent kinase (PDK). After some steps, Akt activation leads to the translocation of GLUT-4 vesicles to the plasma membrane to increase glucose transport into the cells (**Figure 2**) (Croze and Soulage, 2013).

In addition to this, Croze and Soulage (2013) described second pathway (**Figure 2**). Insulin binding on the receptor activates a G protein (Gq) that is coupled to a GPI phospholipase (GPI phospholipase D, PLD). Activation of the phospholipase produces an inositol glycan second messenger INS-2 (insulin second messenger with a 4-O-[2-amino-2-deoxy-beta-D-galactopyranosyl]-3-O-methyl-D-chiro-inositol structure) from a GPI lipid precursor in the inner and/or outer surfaces of the cell membrane (Croze and Soulage, 2013). INS-2 is then released inside the cytoplasm as well as outside the cell, where it can be used by neighbouring cells or can re-enter the original cell via an ATP-dependent inositol glycan transporter. Inside the cell, INS-2 activates cytosolic phosphatidylinositol 4,5-bisphosphate (PP2Ca) and mitochondrial pyruvate dehydrogenase phosphatase (PDHP). In the cytosol, activated PP2Ca stimulates glycogen synthase (GS) directly and indirectly via PI3K/PDK/Akt/GSK3 pathway (the pathway previously described). In the mitochondria, PDHP induces PDH, which induces glucose oxidative use. In the cytosol the activation of PKB/Akt leads to the inactivation of glycogen synthase kinase 3, resulting in glycogen storage. The activated Akt induces the activation of mTOR kinase and then GLUT-4 translocation to the plasma membrane (Croze and Soulage, 2013).

It is clear that this rather complicated model supports the idea of the synergistic activity of the two main inositol isomers, MYO and DCI, in the control of insulin signal. In addition, their presence inside the cell is important, as well as an adequate dietary intake and an adequate MYO-to-DCI conversion through the epimerase, for a correct oxidative use of glucose and/or its storage as glycogen.

Inositol-impaired metabolism in diabetes and PCOS

The idea of inositol as a putative integrative treatment for diabetes and PCOS relies on the fact that in both these diseases the insulin metabolic signal does not work correctly. MYO is in balance with the other eight isoforms and when required by metabolic pathways is transformed to DCI by an epimerase, with each tissue having its own typical conversion rate (Larner, 2002). It has been demonstrated (Kennington et al., 1990; Ortmeyer et al., 1993) that the urinary excretion of DCI is reduced, in both humans and experimental animals affected by type 2 diabetes, with an increase in MYO urinary content and that this is not due to the diabetic condition but to an impairment at the basis of the insulin resistance (Kennington et al., 1990; Ortmeyer et al., 1993). In fact, it was demonstrated that the epimerase that determines MYO to DCI conversion, is insulin dependent and that there is a decreased amount of DCI production in insulin-sensitive tissues/organs such as the kidney, liver and muscle of experimental animals with insulin resistance (Pak et al., 1993, 1998). In addition, a marked decrease of epimerase bioactivity was demonstrated in these models (Sun et al., 2002), thus supporting the hypothesis that insulin resistance *per se* is triggered by some kind of abnormal enzymatic expression.

When diabetic patients were evaluated, lower concentrations of DCI and higher concentrations of MYO were observed, not only in tissues but also in urinary excretion in

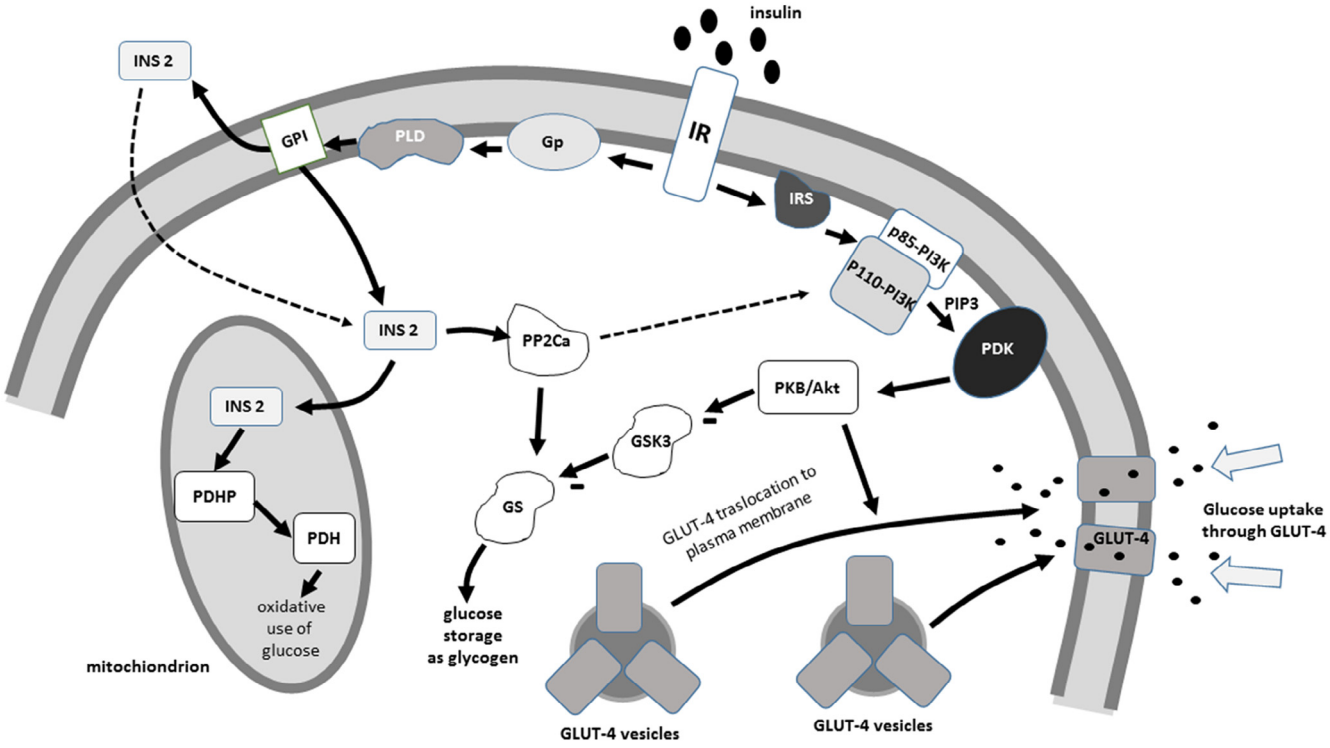

Figure 2 Schematic representation of insulin signalling proposed by Larner et al. (2010). Insulin binding to its receptor (IR) activates a signal via two different and parallel pathways. In the first pathway, the substrates of the insulin receptor (IRS) activate various proteins (PI3K, PDK, PKB/Akt), and PKB/Akt induces glucose transporter 4 (GLUT-4) translocation to the plasma membrane to upload glucose. In the second pathway IR via a G protein (Gp) coupled to a phospholipase D (PLD) causes the hydrolysis of a glycosyl phosphatidylinositol (GPI), which releases an inositol phosphoglycan containing D-chiro-inositol, which acts as second messenger of insulin (INS-2). Then INS-2 enhance glucose storage (GS) as glycogen in the cytosol and also glucose oxidative use in the mitochondria. GSK3 = Glycogen synthase kinase 3; PDH = pyruvate dehydrogenase; PDHP = pyruvate dehydrogenase phosphatase; PDK = phosphoinositide-dependent kinase; PI3K = phosphoinositide 3 kinase; PKB/Akt = protein kinase B/Akt; PP2Ca = phosphoprotein phosphatase 2C alpha (modified from Croze and Soulage, 2013).

baseline conditions (Larner et al., 2010); and MYO concentrations were further increased after insulin administration (Kennington et al., 1990) when compared with non-diabetic controls. Such imbalance in MYO conversion to DCI was expressed as an MYO-to-DCI ratio, which was higher not only in diabetic type 1 or type 2 patients but also in non-diabetic relatives of diabetic patients (**Figure 3**) (Larner and Craig, 1996; Larner et al., 2010). Such data consistently support the hypothesis that the diabetic condition and familial predisposition to diabetes induces an abnormal function/expression of epimerase activity, thus contributing to the systemic occurrence of insulin resistance and to compensatory hyperinsulinaemia. When urine excretion was evaluated in PCOS patients, a lower concentration of DCI was found in the blood than in the blood of control subjects, with no difference in MYO. In addition, PCOS patients undergoing a glucose tolerance test showed a three-fold lower release of DCI than control subjects (Baillargeon and Nestler, 2006; Baillargeon et al., 2006). Moreover, the insulin resistance observed in PCOS patients means that they have a greater chance of developing type 2 diabetes, especially when in conjunction with overweight/obesity and a familial predisposition to diabetes (i.e. diabetic relatives in the family) (Pasquali et al., 2002).

Inositols as putative treatment for PCOS patients

The first data published regarding using inositols to treat PCOS patients were those by Nestler et al. 1999, who administered 1200 mg of DCI to PCOS obese patients for 8 weeks. As DCI has been considered the final and relevant element for the transmission of the post-receptor insulin signal, Nestler was able to demonstrate that these PCOS patients had a consistent improvement in insulin sensitivity as well as free testosterone concentrations. In addition, ovulation was restored in a higher percentage of these patients (86%) than in controls (27%), and similar results were observed in lean PCOS patients, who had ovulatory cycles and the reduction of both insulin and androgen plasma concentrations (Iuorno et al., 2002). Interestingly, when higher doses of DCI were administered no significant improvement was observed in PCOS patients, thus suggesting that it was not a simple problem of nutritional deficiency; only when administered at a dose as high as 2400 mg/day the efficacy was again demonstrated (Cheang et al., 2008). Such results were just the beginning of the inositol story but were indicative that DCI was a possible solution; however, a greater understanding of the

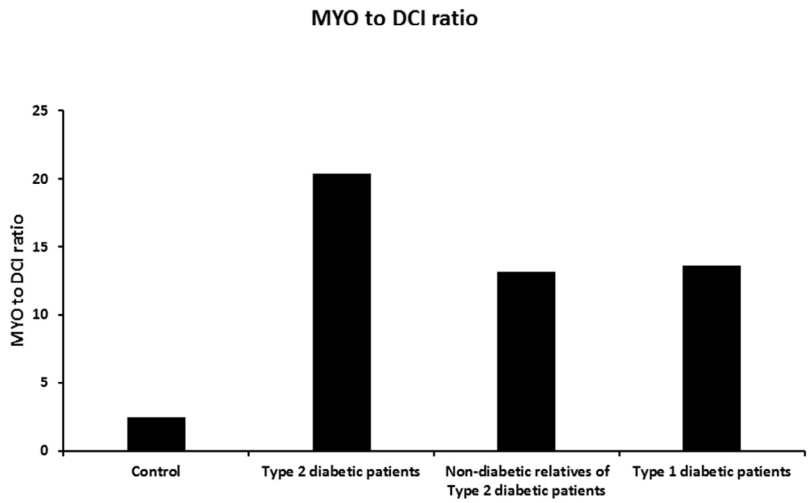

Figure 3 Myo-inositol (MYO) is usually converted to D-chiroinositol (DCI). The MYO to DCI ratio in the urine of controls is completely different from that observed in patients with type 2 diabetes, in non-diabetic relatives, and in patients with type I diabetes (modified from Larner and Craig, 1996 and Croze and Soulage, 2013).

post-receptor mechanism(s) of the insulin signal was needed. In fact, when insulin sensitivity was assessed in different tissues in PCOS patients, various studies reported that although tissues such as muscle, kidney and liver become insulin resistant, ovarian tissue did not (Harwood et al., 2007; Matalliotakis et al., 2006; Rice et al., 2005; Unfer et al., 2012).

Indeed, Chiu et al. (2002), reported that the follicular environment is relevant for oocyte development and maturation and demonstrated that MYO content in human follicular fluid is important and positively correlated with both a better oocyte quality and oestradiol concentrations of the corresponding follicular fluid sample. The same group also provided evidence that adding MYO to the culture medium of mouse oocytes improved meiotic progression of oocytes (Chiu et al., 2003). The fact that recently Unfer et al. (2012) demonstrated that MYO rather than DCI improved both oocyte and embryo quality in FIVET programmes suggests that the ovary has a different environment than muscle or liver in terms of MYO and DCI utilization, that MYO is normally epimerized to DCI in the ovaries of PCOS patients and that higher doses of DCI at the ovarian levels might affect reproductive ability (Carlomagno et al., 2011). These data clearly suggest that ovarian metabolic/endocrine pathways do not require high concentrations of DCI (Rosalbino and Raffone, 2012).

These observations are rather important, as they suggest that what we observe and evaluate in the blood in terms of MYO and DCI concentrations or of insulin resistance/sensitivity represents what is generally occurring in the most metabolically active tissues/organs, i.e. muscles, liver and kidney. These organs represent most (60 – 80%) of our body weight and their metabolic impact is less than minimally influenced by the different ovarian setting and its epimerase activity, as the ovaries are just a few grams in weight. In terms of clinical and practical issues, we have to consider the systemic insulin resistance and the ovarian environment as two distinct metabolically active worlds and that probably our therapeutic choice(s) have to consider which plays the major role.

MYO administration in PCOS patients

As discussed so far, PCOS is an endocrine disease and likely to be a metabolic disease sustained by insulin resistance, always keeping in mind that there are differences when considering the general metabolism of the body or just that of the ovary. Considering metabolic impairment, MYO has been supposed to be a putative integrative strategy for PCOS patients. Recently Unfer et al. (2012) overviewed the clinical outcomes of MYO as a treatment for PCOS patients to improve both metabolic and hormonal parameters. Their review, although not including a formal meta-analysis, considered the recent literature and their analysis was based on 21 studies. Although the study protocols were not homogenous and the daily dose of MYO used varied from 500 to 1500 mg, the authors stated that there were common results after MYO administration (Unfer et al., 2012). Specifically, all PCOS patients showed significant improvement in their hormonal parameters: LH, LH/FSH ratio, testosterone, androstenedione concentrations, insulinaemia and HOMA index. Glucose/insulin ratio was also improved, and when the lipid profile was evaluated total cholesterol concentration decreased and high-density lipoprotein concentration increased (Unfer et al., 2012). BMI and menstrual function improved, as well as fertility. Such an overview of the positive role of MYO administration supported the hypothesis that the reduction of insulinaemia induced by MYO was based on the increased bioavailability of IPG insulin second messenger; once the endocellular metabolic system works better, the whole endocrine function starts to work properly again (Unfer et al., 2012).

Although such considerations are relevant, recent data suggest that all is not as it seems. In fact, although MYO is effective in improving insulin sensitivity and most hormonal parameters in overweight/obese PCOS patients (Genazzani et al., 2008), Genazzani et al. (2012) evaluated a group of obese PCOS patients and showed that although obesity was able to determine a condition of insulin resistance, not all the

obese patients showed significant metabolic improvements under MYO administration. In fact, when subdividing the patients according to baseline insulin plasma concentrations (below or above the cut-off of 12 μU/ml), the efficacy of MYO administration was different. Although MYO administration induced similar endocrine improvement (LH, LH/FSH ratio, androstenedione) and BMI decrease in all obese PCOS, those patients with insulin plasma concentrations below 12 μU/ml did not show any metabolic improvement in terms of insulin response to the oral glucose load.

Such results suggest that obesity is not the only element triggering the hyperinsulinaemia, and a built-in abnormal mechanism(s) might be responsible in most but not all the PCOS patients with insulin resistance. In patients with insulin plasma concentrations below 12 μU/ml, no correlation was observed between BMI and fasting insulin after the treatment, suggesting that some of the obese PCOS patients exhibited hyperinsulinaemia in the presence of low circulating inositol or abnormal DCI–IPG synthesis (Baillargeon and Nestler, 2006; Baillargeon et al., 2006, 2008). The fact that some of the obese patients did not show improvement in both fasting insulin concentrations and insulin response to glucose load, though reducing their BMI, suggests that in these patients the compensatory hyperinsulinaemia is triggered only in part by a change in DCI–IPG synthesis/release, and probably for this reason MYO administration did not induce any visible improvement. Cheang et al. (2008) suggested the presence of a functional defect (e.g. intracellular defect in formation or release of the DCI–IPG mediator) rather than of a simple nutritional deficiency of inositol.

If these are the effects on glucose and insulin-mediated metabolism, interesting data have been collected regarding the ovarian function. Artini et al. pre-treated a group of PCOS patients undergoing IVF with MYO (2 g every day) and observed not only the general improvement in the metabolic and endocrine parameters but also a consistent positive gain in terms of oocyte quality, recruitment, fertility rate and delivery rate. This randomized study showed that MYO was also highly effective in terms of ovarian function in these obese patients with PCOS, permitting the collection of better quality oocytes using less r-FSH than untreated patients. Similarly, Kamenov et al. (2015), demonstrated that a 2 g daily MYO supplementation was able to improve the efficacy of ovulation induction using clomiphene citrate, achieving a higher rate of pregnancy and delivery.

DCI and insulin resistance in PCOS patients

According to previous reports, all hyperinsulinaemic PCOS patients are at risk of diabetes. Interestingly, most of the recent studies that evaluated the efficacy of inositols on PCOS patients and/or on their endocrine and fertility pattern, used mainly MYO. Only recently has the idea that familial predisposition to diabetes might be relevant for the onset of hyperinsulinaemia and/or PCOS been taken seriously. The fact that epimerase activity/expression seems to be abnormal in diabetic patients and abnormal/reduced in those PCOS patients that have familial diabetes (Larner and Craig, 1996; Larner et al., 2010) confirms the importance of the anamnestic evaluation of each single PCOS patient. In fact, recently, we investigated whether any difference was present in terms of clinical results when obese PCOS patients had one or more diabetic first grade relatives and were administered DCI at the daily dose of 500 mg. It was found that DCI administration significantly improved insulin sensitivity in all the patients with insulin resistance (Genazzani et al., 2014b), later confirmed by La Marca et al. (2015). Interestingly, all of the obese PCOS patients with a familial predisposition to diabetes had a greater hyperinsulinaemic response to glucose load before treatment (and thus a higher insulin resistance) than those who had no such familial predisposition (Genazzani et al., 2014b); and DCI administration improved insulin sensitivity similarly to the group with no familial diabetes. Practically speaking, this observation confirms the fact that predisposition to diabetes probably affects epimerase expression/synthesis, thus reducing endogenous MYO conversion to DCI, and DCI administration in these patients significantly restored DCI levels, permitting the positive improvement of insulin sensitivity (Genazzani et al., 2014b). The presence of diabetic relatives predisposes PCOS patients to the abnormal synthesis/formation of DCI and DCI–IPG, thus triggering insulin resistance and predisposing them to diabetes. Again, these data are in agreement with those of Cheang et al. (2008), who sustained that multiple defects in DCI metabolism (low availability of DCI or low endocellular synthesys of DCI and/or DCI–IPG) may have the same end result. Substantially both defects could be overcome by DCI integrative administration, an idea supported by our data (Genazzani et al., 2014b). In addition, DCI administration has been demonstrated to modulate anti-Müllerian hormone (AMH) secretion, probably through a restored insulin modulation, in PCOS patients (La Marca et al., 2015). Indeed, La Marca et al. (2015) demonstrated that 1 – 1.5 g of DCI administered daily decreased AMH plasma concentrations significantly. Such effect on AMH may clearly indicate a reduction in the state of increased functional ovarian reserve typical of PCOS patients (La Marca et al., 2015).

It is well worth saying that when treating hyperinsulinaemic PCOS patients with the classic insulin sensitizer drug, metformin, there is a good and positive effect on ovarian function as well as on the hormonal pattern, mainly the significant reduction of the androgenic milieu (Dinicola et al., 2014). This positive effect of metformin administration is due to a specific action on the release of DCI–IPG, thus showing that insulin sensitizers improve insulin sensitivity by acting on inositol-based signalling (Baillargeon et al., 2004). Such a view is interesting, as it might explain why, if an abnormal DCI–IPG release is present, metformin administration might also be relatively effective in improving the hormonal and clinical signs of PCOS (Genazzani et al., 2007).

Combining MYO and DCI?

Regarding the various aspects discussed so far, the inositol story seems to be interesting and intriguing, as it opens up the possibility of resolving metabolic impairment through the combination of lifestyle change and integrative inositol administration – with the latter probably being crucial. In our view, both MYO and DCI isomers seem to be potentially effective for PCOS patients (**Table 1**), with specific differences in their action mainly according to genetic predisposition to diabetes and whether only the general metabolism is

Table 1 Summary of the clinical studies cited involving the use of MYO or DCI in PCOS patients.

Author	*Inositol used*	*Design*	*Daily dose*	*Number of patients*	*Results obtained*
Artini et al., 2013	MYO	Effects in PCOS undergoing IVF	2 g or folic acid (controls) for 12 weeks	10 controls 10 treated	No endocrine effects. 3 delivered pregnancies. LH, PRL, T, insulin levels and LH/FSH, significantly reduced. HOMA index significantly decreased. Fewer FSH vials used. Lower number and higher quality of oocytes retrieved. 8 delivered pregnancies.
Genazzani et al., 2008	MYO	Effects in overweight/ obese PCOS	2 g or folic acid (controls) for 12 weeks	10 controls 10 treated	No endocrine changes LH, PRL, testosterone, insulin concentrations and LH/FSH, insulin response to glucose load significantly reduced. HOMA index significantly decreased. No changes in BMI. Menstrual cyclicity restored in all PCOS patients.
Genazzani et al., 2012	MYO	Effects in obese PCOS	2 g for 8 weeks	42 patients	LH, PRL, testosterone, insulin concentrations, LH/FSH, BMI, insulin response to glucose load significantly reduced. Insulin:glucose ratio significantly increased.
Genazzani et al., 2014b	DCI	Effects in obese PCOS	500 mg for 12 weeks	22 patients	LH, androstenedione, testosterone, insulin concentrations, LH/FSH, insulin, BMI, LH response to GnRH test and insulin response to glucose load significantly reduced. Insulin:glucose ratio significantly increased. Patients with familial diabetes showed greater changes.
Iuorno et al., 2002	DCI	Effects on lean PCOS	600 mg for 6 – 8 weeks	10 placebo 10 treated	No endocrine changes Insulin, free testosterone, insulin response to glucose load significantly reduced. Systolic and diastolic blood pressure significantly decreased.
Kamenov et al., 2015	MYO	Effects in PCOS with or without clomiphene citrate	2 g for 6 months	47 patients	29 had ovulation: 11 became pregnant. 18 had no ovulation: no pregnancy. Clomiphene citrate was added: 6 became pregnant. BMI and HOMA index decreased.
La Marca et al., 2015	DCI	Effects in PCOS	1 – 1.5 g for 6 – 15 months	47 patients	Significantly more regular menstrual cyclicity. AMH and HOMA index significantly decreased.
Nestler et al., 1999	DCI	Effects in PCOS	1.2 g for 6 – 8 weeks	22 placebo 22 patients	No changes Tryglicerides, insulin, testosterone, free testosterone, DHEA significantly decreased. SHBG significantly increased. 86% of the patients had ovulatory cycles. Diastolic and systolic blood pressure decreased.

AMH = anti-Müllerian hormone; BMI = body mass index; DCI = D-chiro-inositol; DHEA = dehydroepiandrosterone; GnRH = gonadotrophin-releasing hormone; HOMA = homeostasis model assessment; MYO = myo-inositol; PCOS = polycystic ovary syndrome; PRL = prolactin; SHBG = sex hormone-binding globulin.

involved or also ovarian function. Indeed, according to such a view, MYO is converted to DCI and easily used but when such conversion is not optimal, DCI seems to be the solution (Genazzani et al., 2014b; La Marca et al., 2015). The fact that from the metabolic point of view the ovary is a completely different organ in terms of DCI synthesis than the rest of the female body, suggests there is a need for a putative balanced combination dose of MYO and DCI. Such a balanced

combination would offer the chance to modulate both the hyperinsulinaemic condition (i.e. the insulin resistance) with DCI and to improve optimal ovarian function with MYO (ovaries are able to produce DCI).

According to recent reviews (Dinicola et al., 2014), given the importance of inositol in the optimal control of metabolism, hormonal signal transduction and ovarian function, such a combination of MYO and DCI should match the physiological MYO:DCI ratios evaluated in the plasma and the follicular fluid, which range between 40:1 and 100:1 (Dinicola et al., 2014). Indeed, such an MYO:DCI ratio of 40:1 has been proposed as the first-line approach to the integrative treatment with inositols for hyperinsulinaemic PCOS patients (Nordio and Proietti, 2012), although there are not many data available on the efficacy of such a combination. A recent review of MYO and DCI integrative use, through the analysis of the literature available, clearly stated the efficacy of both these compounds on metabolic syndrome, PCOS and the risk of gestational diabetes and, following many observations, stated that 'a treatment based on the association of MYO and DCI in the physiological ratio seems to be the most appropriate' to correct the metabolic aspects of PCOS (Facchinetti et al., 2015).

In conclusion, a large amount of evidence exists of the positive effects of inositols on our biology and on the modulation of female metabolic and reproductive pathways. It is evident that anamnestic data might be relevant to the choice of inositol integration to get specific and significant effects on most of the impaired aspects of PCOS patients, mainly on hormonal and reproductive function. As inositols are natural compounds, they can be considered an easy but effective integrative treatment for PCOS patients.

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