

Natural molecules for the therapy of hyperandrogenism and metabolic disorders in PCOS

V. CAPPELLI¹, M.C. MUSACCHIO², A. BULFONI³, G. MORGANTE¹, V. DE LEO¹

¹Department of Molecular Medicine and Development, University of Siena, Siena, Italy

²Obstetric and Gynaecology, USL 5, Pisa, Italy

³Humanitas-Pio X, Milan, Italy

Abstract. – OBJECTIVE: Polycystic ovary syndrome (PCOS) is the most common endocrinopathy of women of reproductive age and a complex endocrine condition, due to its heterogeneity and uncertainty about its etiology. However, PCOS is also associated with other metabolic abnormalities such as insulin resistance, impaired glucose tolerance, and diabetes. There are few medications that are approved for the most common symptoms of PCOS, leading to the off-label use of medications that were approved for other indications. One of the most common medications being used off label for PCOS is metformin.

Research of other effective therapeutic options has included the utility of inositol.

PATIENTS AND METHODS: A systematic literature search of PubMed was performed using the following combination of terms: 'PCOS', 'hyperandrogenism', 'inositol', 'natural molecules'. Only papers published between 2000 and 2016 were included in our analysis. The present review analyzes all aspects of the choice of natural molecules in the treatment of hyperandrogenism and metabolic disorders in PCOS women.

RESULTS: The rationale underlying the use of inositols as a therapeutic application in PCOS derives from their activities as insulin mimetic agents and their salutary effects on metabolism and hyperandrogenism without side effects.

CONCLUSIONS: In this review will discuss the role of a number of natural associations between inositol and different substances in the treatment of hyperandrogenic symptoms in PCOS women.

Key Words:

PCOS, Hyperandrogenism, Insulin-resistance, Inositol, Lipoic acid, Monacolin K.

Introduction

PCOS and clinical Hyperandrogenism

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy of women of reproduc-

tive age¹ and a complex endocrine condition, due to its heterogeneity and uncertainty about its etiology.

An international meeting in 1990, held at the U.S. National Institute of Health (NIH), was recommended that the diagnostic criteria for PCOS should comprise the concomitant presence of anovulation and hyperandrogenaemia biochemical, clinical (hirsutism/acne) or both¹. Subsequently, the report of a meeting of experts at a joint ESHRE/ASRM meeting held in Rotterdam in 2003 proposed that the presence of two of the three criteria (chronic anovulation, hyperandrogenism and polycystic ovaries on ultrasonography) would be sufficient for PCOS diagnosis². In 2006, The Androgen Excess PCOS Society (AEPCOS) indicated that PCOS is mainly a hyperandrogenic disorder and that the second criterion essential for the diagnosis could be either chronic anovulation or polycystic ovarian morphology³.

However, PCOS is also associated with other metabolic abnormalities such as insulin resistance, impaired glucose tolerance, and diabetes⁴. In 2011, the Amsterdam ESHRE/ASMR-sponsored 3rd PCOS Consensus Workshop Group identified different phenotype, characterized by hyperandrogenism and chronic anovulation, from those characterized by ovarian dysfunction and polycystic morphology⁵. This Consensus recommended screening for insulin and glucose tolerance by OGTT (75 g, 0 and 2h values) in the presence of phenotype characterized by hyperandrogenism with anovulation, obesity, family history of diabetes⁵.

There are few medications that are approved for the most common symptoms of PCOS, leading to the off-label use of medications that were approved for other indications. One of the most common medications being used off label for

PCOS is metformin. A survey of PubMed reveals > 30 meta-analyses of a randomized trial and > 70 systematic reviews covering the role of metformin therapy in the management of PCOS, including ovulation induction, weight loss, menstrual control, miscarriage, and hirsutism⁶. Also, thiazolidinediones, which are drugs approved for treatment of type 2 diabetes, have been used in the treatment of PCOS.

However, side effects such as nausea and diarrhea (in the case of metformin) and increased body weight (in the case of pioglitazone) may reduce patients compliance and limit the use of these drugs^{7,8}.

Research of other effective therapeutic options has included the utility of inositol. The rationale underlying the use of inositols as a therapeutic application in PCOS derives from their activities as insulin mimetic agents and their salutary effects on metabolism and hyperandrogenism without side effects.

The review is organized by pathogenesis of PCOS, common medications used in the treatment and the efficacy of inositol and other natural molecules used for multiple purposes in PCOS.

Pathogenesis of PCOS: Chronic Anovulation, Hyperandrogenism, and Insulin-Resistance

The PCOS is a multifaceted disease involving genetic/environmental factors but also endocrine factors, such as disordered gonadotropin secretion, uncontrolled ovarian steroidogenesis, aberrant insulin signaling and excessive oxidative stress.

Primary Disordered Gonadotropin Secretion

The first biochemical abnormality that was identified in women with PCOS was disordered gonadotropin secretion, with a preponderance of luteinizing hormone (LH) to follicle stimulating hormone (FSH). Studies of gonadotropin secretion in women with PCOS have established that women have augmented the release of LH in response to a gonadotrophin releasing hormone (GnRH) challenge with appropriate levels of FSH secretion⁹. As the two cell theory of the ovary evolved, i.e., that thecal cells can only produce androgens under stimulation of LH, whereas granulosa cells can only aromatize androgens from the theca cells into estrogens under the influence of FSH, this preponderance of LH was thought to be the primary etiology of the syndrome. Excess LH led

to excess thecal cell development and androgen production, but in the face of inadequate FSH stimulation of granulosa cell development and aromatase production, these androgens were not converted to estrogen leading to multiple abnormalities.

This theory explained the morphology of the ovary, hirsutism, and anovulation. Androgen excess led to an ovarian follicular arrest in the preantral stage, as estrogen is critical to the development and selection of a dominant follicle. The ovary thus contained multiple small preantral follicles due to this ongoing process and also had increased central stroma due to excessive thecal and stromal hyperplasia from the disordered gonadotropin exposure. Secondly this resulted in the spillover of excess androgens into the circulating pool resulting in inappropriate feedback to the hypothalamic pituitary axis and a vicious feedback loop where excess LH leads to excess ovarian androgen production which in turn leads to further LH. Finally, the excess circulating androgen led to stimulation of the pilosebaceous unit increases sebum production, induces terminal hair differentiation, and in rare instances in the scalp lead to pilosebaceous unit atresia and androgenic alopecia.

Primary Ovarian and Adrenal Hyperandrogenism

Ovarian steroidogenesis is perturbed in the syndrome with increased circulating androgen levels frequently noted in women with PCOS. Further intrafollicular androgen levels tend to be elevated in antral follicles supporting a lack of adequate granulosa aromatase activity¹⁰. As noted above, a primary defect in ovarian steroidogenesis could lead through the same feedback loop noted above to disordered gonadotropin secretion and stigmata of peripheral hyperandrogenism such as acne, hirsutism, and alopecia. Thecal cells from PCOS women put into long-term culture exhibit defects in steroidogenesis including hyperproduction of androgens, implying alterations of P450c17 activity that increases the 17OHP/A ratio¹¹. Finally, 20-30% of women with PCOS have evidence of adrenal hyperandrogenism, primarily based on elevated levels of DHEAS an androgen marker of adrenal function¹², suggesting that the defect in steroidogenesis is primary, and affects both androgen secreting glands, i.e. the ovary and the adrenal.

Other ovarian factors than disordered steroidogenesis may contribute to PCOS. For ex-

ample, there appears to be an increased density of small preantral follicles in polycystic ovaries¹³. This could result from increased number of germ cells in the fetal ovary or from decreased loss of oocytes with age, or from decreased rate of loss of oocytes during late gestation, childhood, and puberty. Indeed, there is evidence *in vitro* to support increased survival and diminished atresia in a PCOS follicle¹⁴.

Primary Disorder of Insulin Resistance

Women with PCOS show multiple abnormalities in insulin action. Dynamic studies of insulin action, including hyperinsulinemic euglycemic clamps and frequently sampled intravenous glucose tolerance tests, have shown that women with PCOS are more insulin resistant than weight-matched control women^{15,16}. Early in the ontogeny of the syndrome, as in the ontogeny of type 2 diabetes, this is characterized by increased pancreatic beta cell production of insulin to control ambient glucose levels. Thus, many women with PCOS have fasting and meal challenged hyperinsulinemia¹⁷. However, this compensatory response by the pancreatic beta cell is often inadequate for the degree of peripheral insulin resistance leading to initially post-prandial hyperglycemia in these women and eventually fasting hyperglycemia¹⁶.

At the molecular level, impaired insulin signaling results from mutations or posttranslational modifications of the insulin receptor or any of its downstream effectors molecules. Insulin resistance could be accounted for by a defect in insulin binding to its receptor or to a shortage of insulin receptors; however, there is evidence to suggest that insulin resistance is most often due to a post-binding defect in insulin action¹⁸.

The ovaries of these women have a normal response to insulin, probably because the hyperinsulinemia acts on the ovary through the IGF-1 receptor. Furthermore, the insulin level in ovarian uses as the signal mediator also the inositol-glycan pathway that is different from the activated system in other tissues and which maintains its level in ovarian function also in insulin-resistant subjects¹⁹.

Hyperinsulinemia and/or disordered insulin action may have perturbed the reproductive axis in multiple ways. First insulin may act at the hypothalamic pituitary axis to stimulate gonadotropin production. In animal cell culture models, insulin has been found to enhance pituitary gonadotropin secretion²⁰.

Also, hyperinsulinemia is linked to ovarian and adrenal hyperandrogenism for the ability of insulin in certain conditions to stimulate gonadal and adrenal androgen production. Hyperandrogenism has been further linked to insulin resistance and stigmata of the insulin resistance syndrome in women with PCOS and in PCOS family studies which have found increasing prevalence of the metabolic syndrome in family members with increasing androgen levels²¹. Androgens also induce insulin resistance, best illustrated by the example of female to male transsexuals who have increased insulin resistance after supplementation with androgens²². *In vitro* cultures of PCOS thecal cells have been found to overproduce androgens in response to insulin supplementation²³, by increasing P450 cytochrome expression, LH, and IGF-1 receptors. Therefore, insulin-resistance increases also adrenal steroidogenesis¹⁸. Further, the use of insulin sensitizing agents, including both metformin and troglitazone have been associated with both lowering of circulating insulin levels and levels of both adrenal and ovarian androgens¹⁸.

Finally, increased levels of insulin are associated with the peripheral availability of sex steroids through its impact on circulating sex hormone binding globulin (SHBG). SHBG has been found to be partially regulated by circulating insulin levels with an inverse relationship²⁴. Decreasing levels of SHBG mean increasing levels of free and bioavailable androgens, especially since the preferred substrate of SHBG are androgens (as opposed to estrogens and progestins). Increased free androgens mean increased androgen action in the periphery which can mean the pilosebaceous unit or the hypothalamic pituitary axis (Figure 1).

Altered Oxidative Stress and Chronic Low-Grade Inflammation in PCOS

Oxidative stress (OS) reflects an imbalance between production and scavenging of reactive oxygen/nitrogen species (ROS/RNS)²⁵, and excess ROS accumulated *in vivo* would induce cell²⁶, protein²⁷, and lipid damage²⁸. OS is also intimately involved in PCOS pathogenesis, since PCOS patients show more serious OS compared with the normal²⁹. In addition, OS is involved in the pathological processes of IR, hyperandrogenemia, and obesity as well, which accompany PCOS frequently but not absolutely³⁰. Abdominal obesity is regarded as a common complication of PCOS, and the risk of abdominal obesity in PCOS women ranges from 40%

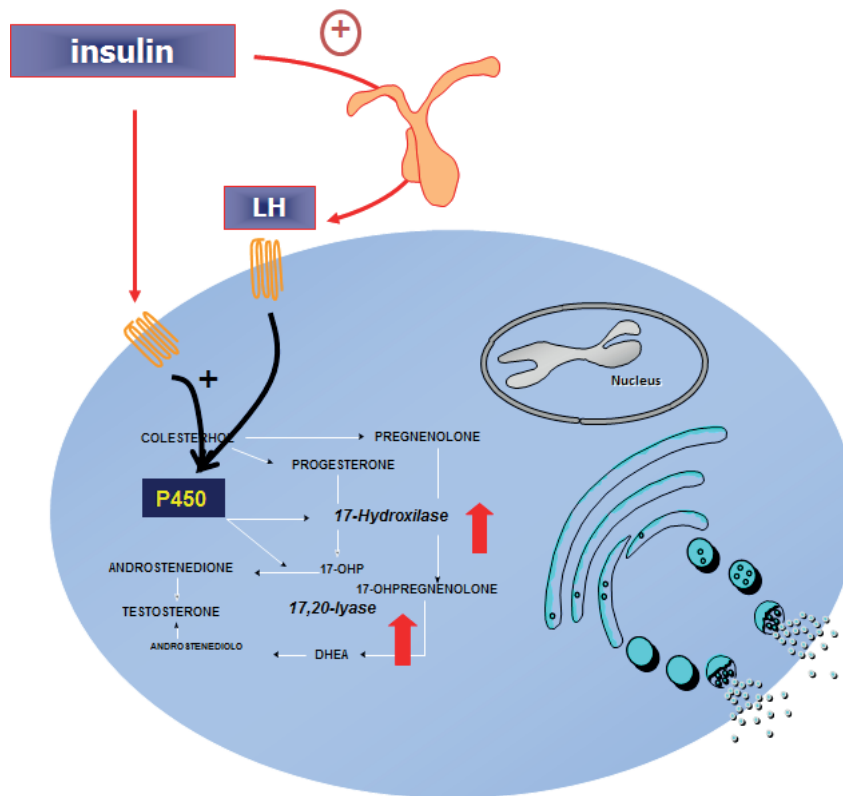


Figure 1. Hyperinsulinemia stimulates directly cytochrome p450 enzymes in the ovary or indirectly through action of LH or IGF-1, causing hyperandrogenism¹¹⁹.

to 80% because of the differences of people and nations³¹. Obese patients are expected to have more serious oxidative stress (OS) levels³², and significant correlations of OS markers with obesity indexes, such as BMI, are discovered³³. Levels of markers that could reflect the degrees of lipid peroxidation and protein peroxidation, such as oxidized low-density lipoprotein (ox-LDL), malondialdehyde (MDA), thiobarbituric reactive substances (TBARS), and advanced oxidation protein products (AOPP), increase significantly in the obese patients compared with the normal, and levels of markers that could reflect the antioxidant ability, such as glutathione peroxidase (GSHPx) and copper- and zinc-containing superoxide dismutase (CuZn-SOD), decreased significantly³⁴. Thus abdominal obesity is directly associated with OS and contributes to the increased OS levels in PCOS³⁶. However, obesity is not the only factor leading to the more serious oxidative status of PCOS, and other factors are considered to have contributions as well, such as insulin-resistance. The IR rate of PCOS patients ranges from 50% to 70%³⁶ and IR markers of women with PCOS, such as HOMA-IR, increase significantly compared with normal women and are usually significantly correlated

with oxidative stress (OS) markers³⁷. IR encourages OS because hyperglycemia and higher levels of free fatty acid lead to reactive oxygen species (ROS) production. In general, insulin receptor substrate (IRS) is the key player of IR pathogenesis³⁸. With the increased OS, various protein kinases are activated to induce serine/threonine phosphorylation of IRS and inhibit normal tyrosine phosphorylation of IRS, reducing the capacity of IRS to combine with insulin receptor, suppressing IRS to activate the downstream phosphatidylinositol 3-kinase (PI3K); and finally insulin signal to the effector via insulin receptor (InsR)/IRS/PI3K pathway is altered³⁹. Therefore, studies with antioxidants such as vitamin E, α -lipoic acid, and N-acetylcysteine indicate a beneficial impact on insulin sensitivity and offer the possibility of new treatment approaches for IR⁴⁰.

Moreover, chronic low-grade inflammation is considered as an important feature of PCOS and has been suggested to participate in the pathogenesis and development of PCOS. Inflammatory markers, such as C-reactive protein (CRP), tumor necrosis factor (TNF), interleukin-6 (IL-6), interleukin-18 (IL-18), monocyte chemoattractant protein-1 (MCP-1), and acute phase serum amyloid A

(APSAA), increased in women with PCOS compared with the normal^{41, 42}. Reactive oxygen species (ROS) could induce releasing inflammatory factors and inflammatory response, via activating the associated signaling pathways⁴³.

Inflammation has also been demonstrated to be associated with IR in PCOS. As well as OS, inflammation could induce insulin resistance (IR) mainly via interfering with post-insulin receptor signaling pathway, insulin receptor substrate 1-phosphatidylinositol 3 kinase-protein kinase B (IRS1-PI3K-PKB/Akt) pathway⁴⁴.

Finally, oxidative stress (OS) and inflammation seem to contribute to hyperandrogenemia in PCOS, but detailed interactions still remain unclear. In several studies, OS and inflammation markers are discovered to be positively correlated with androgen levels in PCOS patients⁴⁵. *In vitro*, OS was reported to enhance the activities of ovarian steroidogenesis enzymes, which could stimulate androgen generation, and antioxidative chemicals, just as statins, inhibit the activities⁴⁶. Tumor necrosis factor- α (TNF- α), an inflammatory marker associated with tissue inflammation, was reported to have the ability to promote the synthesis of androgen in the rat⁴⁷.

Therapeutic Options of PCOS

Treatment of women with PCOS tends to be symptom based, as there are few therapies which address the multitude of complaints with which women with PCOS present. Treatment tends to fall into two categories, either the treatment of anovulatory infertility or the long-term maintenance treatment for PCOS related symptoms (i.e. hirsutism, menstrual disorders, obesity, etc.).

Lifestyle Modification

The gold standard for improving insulin sensitivity in obese PCOS women should be weight loss, diet, and exercise. Unfortunately, there are no effective treatments that result in permanent weight loss, and it is estimated that 90-95% of patients who experience a weight decrease will relapse. Further, in markedly obese individuals, the only treatment that results in sustained and significant weight reduction is bariatric surgery⁴⁸. The current National Institute of Health recommendations are to utilize bariatric surgery in patients with a BMI greater than 40 or with a BMI greater than 35 and serious medical co-morbidities⁴⁹. It is uncertain whether PCOS qualifies as a significant comorbidity. Women

with PCOS appear to experience a dramatic improvement in symptoms after surgery, implying this may be in some subjects a “cure” for the syndrome^{50,51}. However, these studies are primarily case series and need further validation in prospective studies.

Combined Hormonal Contraceptives

Combined hormonal contraceptives are the most commonly used medications for the long-term treatment of women with PCOS and have been recommended by the Task Force and the Endocrine Society⁵², the Australian Alliance⁵³, and the PCOS Consensus Group⁵⁴ as first-line treatment for hyperandrogenism and menstrual cycle irregularities in women with PCOS.

They offer benefit through a variety of mechanisms, including suppression of pituitary LH secretion, suppression of ovarian androgen secretion, and increased circulating SHBG levels.

Individual OC preparations may have different doses and drug combinations and thus have varying risk-benefit ratios. Even though guidelines do not specify the use of one OCP over another^{52,54}, the best choice for symptomatic treatment is considered to be low-dose oral contraceptives that contain anti-androgenic or neutral progestins⁵⁵.

Oral contraceptives may also be associated with a significant elevation in circulating triglycerides as well as in HDL levels, though these do not appear to progress over time⁵⁶. There is no evidence to suggest that women with PCOS experience more cardiovascular events than the general population when they use oral contraceptives, though risk factors for adverse events such as hypertension, obesity, clotting history, and smoking must be considered.

Antiandrogens

A minimum of 6 months of OCP regimen is usually required to obtain satisfactory results against acne and hirsutism⁵⁵. When this treatment it's no useful other therapies may be used.

Spiroglactone

Spiroglactone is primarily used to treat hirsutism and appears effective, though the evidence is weak⁵⁷. It is a diuretic and aldosterone antagonist, also binds to the androgen receptors an antagonist. It has other mechanisms of action, including inhibition of ovarian and adrenal steroidogenesis, competition for androgen receptors in hair follicles, and direct inhibition of 5-reductase activity.

Flutamide

Flutamide, an androgen-receptor agonist, is another nonsteroidal anti-androgen that has been shown to be effective against hirsutism in smaller trials. The most common side effect is dry skin, but its use has been associated with hepatitis in rare cases. The risk of teratogenicity with this compound is significant, and contraception should be used. Flutamide has also been combined with lifestyle and metformin therapy for treatment of PCOS and may have additive effects⁵⁸.

Finasteride

Finasteride inhibits both forms of the enzyme 5-reductase (type I, predominantly found in the skin, and type II, predominantly found in the prostate and reproductive tissues). Finasteride is better tolerated than other anti-androgens, with minimal hepatic and renal toxicity; however, it has a well-documented risk for teratogenicity in male fetuses, and adequate contraception should be used. Overall, randomized trials have found that spironolactone, flutamide, and finasteride to have similar efficacy in improving hirsutism⁵⁷.

Ornithine Decarboxylase Inhibitors

These have been developed for the treatment of female hirsutism. Ornithine decarboxylase is necessary for the production of polyamines, and is also a sensitive and specific marker of androgen action in the prostate. Inhibition of this enzyme limits cell division and function in the pilosebaceous unit. Recently a potent inhibitor of this enzyme, eflornithine, has been found to be effective as a facial cr me for the treatment of unwanted facial hair⁵⁹ (Brand name Vaniqa).

Statins

Another area where there is emerging support in the literature for a cardiovascular and endocrine benefit in women with PCOS, is the use of statins. They have been shown to improve hyperandrogenemia, lipid levels, and reduce inflammation⁶⁰. However, their long-term effects in preventing cardiovascular disease in young women with PCOS is unknown. There are concerns about teratogenicity with the use of this drug in reproductive age women, as it is FDA pregnancy category X. The use of these drugs is still experimental in women with PCOS.

Insulin-Sensitizing Agents

Metformin is the insulin-sensitizing agent most useful in the long-term maintenance of PCOS. Metformin does lower serum androgens, and im-

proves ovulatory and menstrual frequency⁶¹. Metformin tends to be the drug of choice to treat glucose intolerance and elevated diabetes risk in women with PCOS because of its favorable safety profile and the familiarity a wide number of caregivers from varying specialties have with the medication. However, there are no long-term studies of metformin in women with PCOS to show diabetes prevention. Among women with PCOS who use metformin, glucose tolerance improves or stays steady over time⁶². Metformin also may be associated with weight loss in women with PCOS, although the results in other populations are inconsistent⁶³. Metformin is often used in conjunction with lifestyle therapy to treat PCOS.

Gastrointestinal symptoms (diarrhea, nausea, vomiting, abdominal bloating, flatulence, and anorexia) are the most common adverse reactions and may be ameliorated by starting at a small dose and gradually increasing the dose or by using the sustained-release version now available in the United States. The dose is usually 1500-2000 mg/day given in divided doses.

Since metformin and statins administrations are associated with several side effects such as nausea, vomiting, gastric pain causing the end of therapy, combined treatment with natural products: such as monacolin k, inositols, lipoic acid, glucomannan, and bergamot represents a valid alternative, well tolerated and with a similar mode of action⁶⁴.

Inositol

The stereoisomeric family of 9 inositols includes myo-, cis-, allo-, epi-, muco-, neo-, scyllo- and the optical isomers D and L chiro-inositols. Myo-inositol (MI) is the most widely distributed in nature. Biosynthesized from glucose⁶⁵, the cyclase converting the immediate precursor fructose 6-P to myo-inositol has been cloned⁶⁶. L-chiro-inositol is the product of epimerizing hydroxyl #1 of myo-inositol, while D-chiro-inositol (DCI) is the product of epimerizing hydroxyl #3 of myo-inositol⁶⁷ (Figure 2).

In nature inositol (and its derivatives: salts, phosphates and associated lipids) are found in many foods (especially fruits and beans)⁶⁸. In plants, inositol is generally represented in the form of hexaphosphate, and phytic acid or its salts (phytates).

MI was once considered as a member of the vitamin B complex; however, it cannot be considered a 'true' essential nutrient, given that it can be synthesized by the human body. However, it is

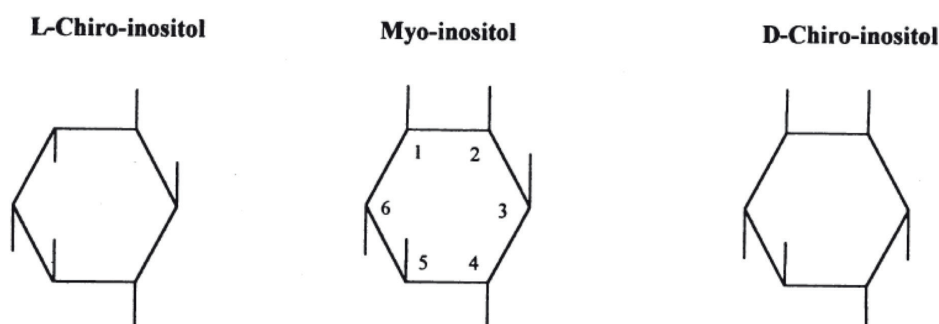


Figure 2. Structures of myo-, D and L chiro-inositols. Myo-inositol is epimerized in position 1 to form L-chiro-inositol and in position 3 to form D-chiro-inositol⁶⁷.

still a matter of controversy if such biosynthesis may provide amounts considered adequate for good health from glucose⁶⁹.

The two inositol stereoisomers, MI and DCI are chemical mediators of insulin, acting through different mechanisms⁷⁰. Both DCI and MI have similar structures, differing in the stereochemistry of only one hydroxyl group⁷¹. Myo-inositol is synthesized from glucose-6-phosphate in two steps. First, glucose-6-phosphate is isomerized to myo-inositol-1-phosphate, which is then dephosphorylated by an inositol monophosphatase enzyme giving free myo-inositol⁷². *In vivo*, DCI is synthesized by an epimerase that converts myo-inositol into DCI. Larner first demonstrated a decreased DCI content in urine as well as tissues of human subjects and animals with type 2 diabetes. Moreover, the decrease of DCI in urine was observed with an increase of myo-inositol^{71,73}. Additional investigations demonstrated that the altered inositol excretion patterns^{71,73,74} were specifically related to the insulin resistance, rather than to diabetes. Larner postulated a defect in the epimerization process that physiologically enacts the conversion of MIDCI.

In addition, MI and DCI function as insulin second messengers and mediate different actions of insulin⁷⁵. MI is converted to an inositolphosphoglycan (IPG) insulin second messenger (MI-IPG) involved in cellular glucose uptake, whereas DCI is converted to an IPG insulin second messenger (DCI-IPG) involved in glycogen synthesis⁷⁶. On the other hand, at the ovarian level, it has been shown that MI-based second messenger is involved in both glucose uptake and FSH signaling whereas DCI-based second messenger is devoted to the insulin-mediated androgen production⁷⁷. In women with PCOS, hyperinsulinemia likely stimulated epimerase activity in

the ovary, resulting in an overproduction of DCI and a concomitant depletion of MI⁷⁵. Also, the resulting deficiency of MI could be responsible for the poor oocyte quality and the impairment of the FSH signaling. Clearly, DCI supplementation would be ineffective in such women as they already have high levels of this molecule in the ovary⁷⁸.

Oral nutritional supplementation with inositol, part of the vitamin B complex (B8) and an intracellular second messenger, was demonstrated to enhance insulin sensitivity and improves the clinical and hormonal characteristics of PCOS patients^{79,80}. Moreover, MI supplementation was shown to restore spontaneous ovulation with the consequent increase in conception, either alone⁸¹ or when combined with gonadotropins⁸².

The 2013 International Consensus Conference on MI and DCI in Obstetrics and Gynecology⁸³ identified opinion leaders in all fields related to MI and DCI and their involvement, as second messengers of insulin, in several insulin-dependent processes, such as metabolic syndrome and PCOS. In conclusion, they postulated that the treatment of PCOS women, as well as the prevention of GDM, seem those clinical conditions which take more advantages from MI supplementation, when used at a dose of 2 g twice/day. The clinical experience with MI is largely superior to the one with DCI. However, the existence of tissue-specific ratios, namely in the ovary, has prompted researchers to recently develop a treatment based on both molecules in the proportion of 40 (MI) to 1 (DCI)⁸³.

In 2015, The International Consensus Conference on MI-and DCI in Obstetrics and Gynecology⁸⁴ postulated that an imbalance between MI and DCI leads to a reduction in insulin and FSH signaling, is observed in PCOS patients^{85,86}.

Indeed, MI depletion induces a defect in glucose uptake. This, in turn, reduces glucose availability in the ovary for both oocytes and follicular cells. Although oocytes are characterized by high glucose consumption, by impairing sugar availability oocyte quality will be compromised. Overall evidence from the literature analyzed by the Conference Scientific Committee points out the beneficial effects of MI treatment in ART, in particular at the level of ovarian response to exogenous gonadotropins as well as oocyte and embryo quality. In this regard, administration of MI, alone or in combination with DCI (in the physiological plasma ratio of 40:1), could be a predictive factor in improving ART outcomes

An important review, published in 2014⁸⁷, analyzed data from the literature about inositols in the treatment of PCOS, in conclusion, Di Nicola et al⁸⁷ postulated that despite the relatively high number of reports, only a few of them fulfill the criteria of the randomized clinical trial. Those studies have been extensively reviewed elsewhere^{81,88}. Among 70 studies focusing on PCOS treatment using different pharmaceutical composition incorporating INS, 21 were considered eligible as they involve MI⁸¹. Yet, only six of them were randomized controlled clinical trials, involving more than 300 PCOS patients. Remarkably, in all the studies analyzed, no side effects were reported. Overall, those studies indicate that MI supplementation improves several of the hormonal disturbances of PCOS. MI mechanisms of action appear to be mainly based on improving insulin sensitivity of target tissues, resulting in a positive effect on the reproductive axis (MI restores ovulation and improves oocyte quality) and hormonal functions (MI reduces clinical and biochemical hyperandrogenism and dyslipidemia) through the reduction of insulin plasma levels⁸⁷.

These data are particularly interesting in PCOS women, in fact, an increased activity of epimerase in theca cells of ovaries of PCOS women is associated with a consistent reduction in the intraovarian ratio of MI and DCI. This is the hypothesis of ovarian paradox postulated by Carmagno et al in 2010⁸⁹; in this way is fundamental to supplement MI and DCI in physiological ratio to restore normally ovary function to improve the oocyte quality⁹⁰.

Because of the absence of side effects with the administration of these products, the scientific interest in recent years has been to discover new natural associations that represent valid alternatives in the treatment of PCOS symptoms. New

valid associations are inositol and monacolin, inositol and lipid acid, inositol and bergamot. These new combinations may oppose the etiopathologies responsible for the onset and deterioration of PCOS-related symptoms. Moreover, these natural choices are more accepted by patients and clinicians who consider metformin only an anti-diabetic drug and then other use are “off-label”⁹¹.

Inositol and Monacolin K

Recently, red yeast rice, a Chinese dietary supplement, has gained popularity due to its properties as a natural statin. It contains varying amounts of natural monacolin K (mevinolin), a metabolite of *Monascus ruber*, which specifically inhibit 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, the rate limiting enzyme in cholesterol synthetic pathway⁹². Monacolin K was suggested to have a similar effect on lipid metabolism, as shown for the mechanism of action of pharmacological statins⁹³, and it was demonstrated to effectively reduce the levels of cholesterol in patients with hypercholesterolemia⁹⁴. Moreover, its ability to inhibit steroid synthesis was claimed to be responsible for the observed decrease in hyperandrogenism, which may further restore ovulation in those PCOS patients, as was already demonstrated following the use of simvastatin alone or in combination with metformin⁹⁵. Furthermore, the antioxidative properties of statins and monacolin K, may further control cellular proliferation and improve ovulatory function⁹⁶.

In 2013, our group published about this natural association⁹⁷; we studied 60 insulin resistant PCOS patients. There were 3 groups were treated for 6 months with either myo-inositol and monacolin k, inositol only or metformin and they compared clinical, metabolic and hormonal assessment at the start, after 3 and 6 months of treatment. In the present study, we demonstrated that all treatments improved patients' clinical, hormonal and metabolic profiles, with a tendency toward better results using the combination of myo-inositol and monacolin k. Regarding the lipid profile, total cholesterol, triglycerides and LDL levels were significantly reduced in all groups, with a more pronounced reduction in LDL following the combined treatment with myo-inositol and monacolin k; the combined treatment with myo-inositol and monacolin k significantly raised in HDL levels.

Since metformin and statins administrations are associated with several side effects such as nausea, vomiting, gastric pain causing the end

of therapy⁹⁸, the combined treatment with the natural products: monacolin k and myo-inositol represent a valid alternative, well tolerated and with a similar mode of action.

Inositol and Lipoic Acid

Alpha lipoic acid (LA) is a potent antioxidant, and controlled release alpha lipoic acid has been reported to improve glucose control in type 2 diabetes patients⁹⁹, and in women with PCOS, to improve insulin sensitivity and reproductive and metabolic disorders¹⁰⁰. Recently, authors showed that inositol combined with alpha lipoic acid can be used as a dietary supplement in insulin-resistant patients in order to increase their insulin sensitiveness^{101,103}.

LA, either as a dietary supplement or a therapeutic agent, modulates redox potential because of its ability to match the redox status between different subcellular compartments as well as extracellularly. Both the oxidized (disulfide) and reduced (di-thiol: dihydro-lipoic acid, DHLA) forms of LA show antioxidant properties. LA exerts antioxidant effects in biological systems through ROS quenching but also via an action on transition metal chelation. Dietary supplementation with LA has been successfully employed in a variety of *in vivo* models of disease associated with an imbalance of redox status: diabetes and cardiovascular diseases¹⁰³.

Recently it's studied the association between myo-inositol, monacolin k and lipoic acid in PCOS patients; the authors recruited 30 women with PCOS and IR, and they studied lipid profile, BMI, and androgens before the treatment and after 6 months of therapy¹⁰². They concluded that this association improves lipid parameters and hyperandrogenism.

These data show that this natural association is not only limited to the reduction of total cholesterol¹⁰³ but that is also stimulated HDL production; the reduction in cholesterol also positively affects the levels of androgenic steroids. The synergy between myo-inositol, monacolin k, and lipoic acid has produced results through a reduction in plasma insulin levels which no longer stimulate the theca cells in androgen production.

Inositol and Glucomannan

Glucomannan is a water-soluble, fermentable dietary fiber extracted from the tuber or root of the elephant yam, also known as konjac (*Amorphophallus konjac* or *Amorphophallus rivieri*). Glucomannan consists of a polysaccharide chain

of beta-D-glucose and beta-D-mannose with attached acetyl groups in a molar ratio of 1: 1.6 with beta 1-4 linkages¹⁰⁶. Because human salivary and pancreatic amylase cannot split beta 1, 4 linkages, glucomannan passes relatively unchanged into the colon. It has a high molecular weight and can absorb up to 50 times its weight in water, making it one of the most viscous dietary fibers known¹⁰⁷. With its low energy density and bulking properties, glucomannan seems to promote weight loss by displacing the energy of other nutrients and producing satiety and satiation as it absorbs water and expands in the gastrointestinal tract. In addition, glucomannan seems to reduce total cholesterol and low-density lipoprotein (LDL) cholesterol levels by stimulating fecal excretion of cholesterol and bile acids and decreasing intestinal absorption of cholesterol¹⁰⁸. Also, glucomannan may improve glycemic parameters by inhibiting appetite and slowing intestinal absorption due to increased viscosity¹⁰⁹. Obesity is present in 50-65% of PCOS women; a lot of studies demonstrated that reduction of body weight and training may improve fertility, and cycle irregularity through reduce the insulin blood levels.

The combination between inositol and glucomannan was studied to reducing glucose levels and improving insulin sensitivity in PCOS overweight patients. In particular, De Leo et al¹¹⁰ in 2014 studied this natural association in 40 PCOS women with insulin resistance. They concluded that this association can improve insulin resistance in PCOS women with significant results, in fact, glucomannan can delay absorption of glucose in the bowel and can extend the action of inositol through the delay of absorption of this substance. This action should improve inositol action to acquire long-acting function.

Inositol and Bergamot

Pharmacological studies have confirmed the activities already known in folk medicine, cholesterol lowering action and lipid lowering action of bergamot juice. These actions are mainly due to the flavonoids. Clinical studies have shown that the activity of individual flavonoid compounds doesn't have the same power of action of the entire plant complex. The lipid-lowering action carried out by the main bioactive compounds (flavonoids) contained in bergamot juice was further confirmed in a major clinical RCT study conducted on 237 patients with hypercholesterolemia either associated to hyperglycemia or not¹¹¹. The results obtained after 30 days have

confirmed that treatment with bergamot extract results in a significant reduction of total and LDL-cholesterol, and a significant increase in HDL-cholesterol values¹¹¹. The plant complex of bergamot has demonstrated in vivo to lower triglyceride levels¹¹²⁻¹¹⁴.

The association between inositol, monacolin k, vitamin K2, methylfolate and bergamot juice has been recently studied for its important action on dyslipidemia¹¹⁵. This association was experimented in 40 perimenopausal women with metabolic syndrome, BMI > 25 kg/m² and insulin resistance: 20 women were treated and the other 20 patients were as the control group.

Regarding the lipid profile, total cholesterol and triglycerides showed a significant decrease while HDL levels have increased. This study demonstrated that the association between d-chiro-inositol, monacolin-K, bergamot extract, methylfolate and vitamin K2 is not limited only to the reduction of total cholesterol, but may increase HDL. The synergic action of nutritional components and plant extracts of this new supplement demonstrated to effectively rebalance the altered functional states of the gluco-lipid metabolism and vascular system.

Conclusions

A growing number of women looking for a mental and physical wellbeing, but at the same time they want to treat their own problems, in particular, those of an aesthetic nature with non-traditional therapies.

In recent years, many studies were performed to clarify the involvement of inositols in PCOS to improve the ovary function and oocyte quality; in fact a large amount of evidence exists to prove the positive effect of inositol in this way; inositol exerts important actions in the glucose homeostasis and when incorporated into phospho glycans has been shown to serve as second messengers involved in the signalling-transduction cascade of insulin¹¹⁶ and in PCOS patients the metabolism of inositol is dysregulated; these data suggest the relationship between insulin resistance and inositol deficiency in PCOS women^{117,118}.

As expressed in this review, we can assert that exist a lot of natural association to help women to resolve primary insulin resistance but enough a lot of related problems. In particular, the use of MI or DCI exerts a reduction of metabolic and endocrine symptoms in PCOS women through

the synergy between insulin sensitizer (inositol) and other product personalized on women request.

Myo-inositol can reduce insulin blood levels to restore physiological menstrual cycle. Restoration of the ovulatory cycle can improve mild acne and hirsutism. The association between myo-inositol and monacolin K (3-10 mg/die) can improve moderate acne and hirsutism after 3, 6 months of therapy, in fact, monacolin interferes with the production of cholesterol and then with the androgen synthesis⁹⁷.

Another relevant association is between myo-inositol and alpha lipoic acid; with this combination, we can restore ovulatory cycles, and we can interfere with inflammation that characterized the onset and maintenance of acne.

In obese PCOS women, the association between inositol and glucomannan; glucomannan is a fibre that can induce a sense of satiety and reduce absorption of lipids and carbohydrates in the bowel with a consequent reduction of circulating insulin, weight reduction and improve hyperandrogenism in these women.

In conclusion, MI and its metabolite DCI have been confirmed as a valid non-pharmacologic alternative to contrast insulin-resistance in PCOS women. Various choices between myo-inositol and other natural substances find specific indications depending on the predominant endocrine and metabolic symptom.

It must be remembered that the first improvements of these symptoms are observed after long treatment (90-120) days. In fact, these therapies should be evaluated in medium and long-term associated with an adequate lifestyle.

It is important to highlight that some studies we have cited are preliminary and conducted on a small population, larger sample sizes will also serve to strengthen future studies.

Conflict of interest

The authors declare no conflicts of interest.

References

- 1) ZAWADSKI JK, DUNAIF A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif A, Givens JR, Haseltine FP, Merriam GR, editors. Polycystic Ovary Syndrome. Boston: Blackwell Scientific Publications, 1992; pp. 377-384.

- 2) ROTTERDAM ESHRE/ASRM-SPONSORED PCOS CONSENSUS WORKSHOP GROUP, AUTHORS. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 2004; 81: 19-25.
- 3) AZZIZ R, CARMINA E, DEWAILLY D, DIAMANTI-KANDARAKIS E, ESCOBAR-MORREALE HF, FUTTERWEIT W, JANSSEN OE, LEGRO RS, NORMAN RJ, TAYLOR AE, WITCHEL SF; Task Force on the Phenotype of the Polycystic Ovary Syndrome of The Androgen Excess and PCOS Society. Task Force on the Phenotype of the Polycystic Ovary Syndrome of The Androgen Excess and PCOS Society. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. *Fertil Steril* 2009; 91: 456-488.
- 4) LEGRO RS, GNATUK CL, KUNSELMAN AR, DUNAIF A. Changes in glucose tolerance over time in women with polycystic ovary syndrome: a controlled study. *Clin Endocrinol Metab* 2005; 90: 3236-3242.
- 5) FAUSER BC, TARLATZIS BC, REBAR RW, LEGRO RS, BALEN AH, LOBO R, CARMINA E, CHANG J, YILDIZ BO, LAVEN JS ET AL. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertility and Sterility* 2012; 97: 28-38.
- 6) VITEK W, ALUR S, HOEGER KM. Off-label drug use in the treatment of polycystic ovary syndrome. *Fertil Steril* 2015; 103: 605-611.
- 7) BAILLARGEON JP, IJURNIO MJ, NESTLER JE. Insulin sensitizers for polycystic ovary syndrome. *Clin Obstet Gynecol* 2003; 46: 325-340.
- 8) PASQUALI R, GAMBINERI A. Insulin-sensitizing agents in polycystic ovary syndrome. *Eur J Endocrinol* 2006; 154: 763-775.
- 9) UNFER V, NESTLER JE, KAMENOV ZA, PRAPAS N, FACCHINETTI F. Effects of Inositol(s) in Women with PCOS: A Systematic Review of Randomized Controlled Trials. *Int J Endocrinol* 2016; 2016: 1849162.
- 10) SCHNEYER AL, FUJIWARA T, FOX J, WELT CK, ADAMS J, MESSERLIAN GM, TAYLOR AE. Dynamic changes in the intrafollicular inhibin/activin/follistatin axis during human follicular development: relationship to circulating hormone concentrations. *J Clin Endocrinol Metab* 2000; 85: 3319-3330.
- 11) DIAMANTI-KANDARAKIS E. Polycystic ovarian syndrome: pathophysiology, molecular aspects and clinical implications. *Expert Rev Mol Med* 2008; 10: e3.
- 12) KUMAR A, WOODS KS, BARTOLUCCI AA, AZZIZ R. Prevalence of adrenal androgen excess in patients with the polycystic ovary syndrome (PCOS). *Clin Endocrinol (Oxf)* 2005; 62: 644-649.
- 13) WEBBER LJ, STUBBS S, STARK J, TREW GH, MARGARA R, HARDY K, FRANKS S. Formation and early development of follicles in the polycystic ovary. *Lancet* 2003; 362: 1017-1021.
- 14) WEBBER LJ, STUBBS SA, STARK J, MARGARA RA, TREW GH, LAVERY SA, HARDY K, FRANKS S. Prolonged survival in culture of preantral follicles from polycystic ovaries. *J Clin Endocrinol Metab* 2007; 92: 1975-1978.
- 15) DUNAIF A, SEGAL KR, FUTTERWEIT W, DOBRJANSKY A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes* 1989; 38: 1165-1174.
- 16) DUNAIF A, FINEGOOD DT. Beta-cell dysfunction independent of obesity and glucose intolerance in the polycystic ovary syndrome. *J Clin Endocrinol Metab* 1996; 81: 942-947.
- 17) CHANG RJ, NAKAMURA RM, JUDD HL, KAPLAN SA. Insulin resistance in nonobese patients with polycystic ovarian disease. *J Clin Endocrinol Metab* 1983; 57: 356-359.
- 18) DE LEO V, LA MARCA A, PETRAGLIA F. Insulin-lowering agents in the management of polycystic ovary syndrome. *Endocr Rev* 2003; 24: 633-667.
- 19) NESTLER JE, JAKUBOWICZ DJ, DE VARGAS AF, BRIK C, QUINTERO N, MEDINA F. 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: 2001-2005.
- 20) ADASHI EY, HSUEH AJ, YEN SS. Insulin enhancement of luteinizing hormone and follicle-stimulating hormone release by cultured pituitary cells. *Endocrinology* 1981; 108: 1441-1449.
- 21) EHRMANN DA, LILJENQUIST DR, KASZA K, AZZIZ R, LEGRO RS, GHAZZI MN. Prevalence and predictors of the metabolic syndrome in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2006; 91: 48-53.
- 22) POLDERMAN KH, GOOREN LJ, ASSCHEMAN H, BAKKER A, HEINE RJ. Induction of insulin resistance by androgens and estrogens. *J Clin Endocrinol Metab* 1994; 79: 265-271.
- 23) WILLIS D, FRANKS S. Insulin action in human granulosa cells from normal and polycystic ovaries is mediated by the insulin receptor and not the type-I insulin-like growth factor receptor. *J Clin Endocrinol Metab* 1995; 80: 3788-3790.
- 24) NESTLER JE, POWERS LP, MATT DW, STEINGOLD KA, PLYMATE SR, RITTMASER RS, CLORE JN, BLACKARD WG. A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. *J Clin Endocrinol Metab* 1991; 72: 83-89.
- 25) PISOSCHI AM, POP A. The role of antioxidants in the chemistry of oxidative stress: a review. *Eur J Med Chem* 2015; 97: 55-74.
- 26) HIRAKU Y, KAWANISHI S. Immunohistochemical analysis of 8-nitroguanine, a nitrative DNA lesion, in relation to inflammation-associated carcinogenesis. *Methods Mol Biol* 2009; 512: 3-13.
- 27) DALLE-DONNE I, ROSSI R, COLOMBO R, GIUSTARINI D, MILZANI A. Biomarkers of oxidative damage in human disease. *Clin Chem* 2006; 52: 601-623.

- 28) TEZIL T, BASAGA H. Modulation of cell death in age-related diseases. *Curr Pharm Des* 2014; 20: 3052-3067.
- 29) MURRI M, LUQUE-RAMÍREZ M, INSENER M, OJEDA-OJEDA M, ESCOBAR-MORREALE HF. Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis. *Hum Reprod Update* 2013; 19: 268-288.
- 30) LEE JY, BAW C, GUPTA S, AZIZ N, AGARWAL A. Role of oxidative stress in polycystic ovary syndrome. *Curr Women's Health Rev* 2010; 6: 96-107.
- 31) KIRCHENGAST S, HUBER J. Body composition characteristics and body fat distribution in lean women with polycystic ovary syndrome. *Hum Reprod* 2001; 16: 1255-1260.
- 32) HOLGUIN F, FITZPATRICK A. Obesity, asthma, and oxidative stress. *J Appl Physiol* 2010; 108: 754-759.
- 33) CHOI HD, KIM JH, CHANG MJ, KYU-YOUN Y, SHIN WG. Effects of astaxanthin on oxidative stress in overweight and obese adults. *Phytother Res* 2011; 25: 1813-1818.
- 34) COUILLARD C, RUEL G, ARCHER WR, POMERLEAU S, BERGERON J, COUTURE P, LAMARCHE B, BERGERON N. Circulating levels of oxidative stress markers and endothelial adhesion molecules in men with abdominal obesity. *J Clin Endocrinol Metab* 2005; 90: 6454-6459.
- 35) KUMANYIKA SK. The obesity epidemic: looking in the mirror. *Am J Epidemiol* 2007; 166: 243-245.
- 36) LEGRO RS, FINEGOOD D, DUNAIF A. A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 1998; 83: 2694-2698.
- 37) MOHAMADIN AM, HABIB FA, ELAHI TF. Serum paraoxonase 1 activity and oxidant/antioxidant status in Saudi women with polycystic ovary syndrome. *Pathophysiology* 2010; 17: 189-196.
- 38) MACLAREN R, CUI W, SIMARD S, CIANFLONE K. Influence of obesity and insulin sensitivity on insulin signaling genes in human omental and subcutaneous adipose tissue. *J Lipid Res* 2008; 49: 308-323.
- 39) GAO D, NONG S, HUANG X, LU Y, ZHAO H, LIN Y, MAN Y, WANG S, YANG J, LI J. The effects of palmitate on hepatic insulin resistance are mediated by NADPH oxidase 3-derived reactive oxygen species through JNK and p38MAPK pathways. *J Biol Chem* 2010; 285: 29965-29973.
- 40) EVANS JL, MADDUX BA, GOLDFINE ID. The molecular basis for oxidative stress-induced insulin resistance. *Antiox Redox Signal* 2005; 7: 1040-1052.
- 41) GONZÁLEZ F. Inflammation in polycystic ovary syndrome: underpinning of insulin resistance and ovarian dysfunction. *Steroids* 2012; 77: 300-305.
- 42) GONZÁLEZ F, NAIR KS, DANIELS JK, BASAL E, SCHIMKE JM, BLAIR HE. Hyperandrogenism sensitizes leukocytes to hyperglycemia to promote oxidative stress in lean reproductive-age women. *J Clin Endocrinol Metab* 2012; 97: 2836-2843.
- 43) TOUYZ RM. Molecular and cellular mechanisms in vascular injury in hypertension: role of angiotensin II. *Curr Opin Nephrol Hypertens* 2005; 14: 125-131.
- 44) KEANE KN, CRUZAT VF, CARLESSI R, DE BITTENCOURT PI, NEWSHOLME P. Molecular events linking oxidative stress and inflammation to insulin resistance and beta-cell dysfunction. *Oxid Med Cell Longev* 2015; 2015: 181643.
- 45) YANG Y, QIAO J, LI R, LI MZ. Is interleukin-18 associated with polycystic ovary syndrome? *Reprod Biol Endocrinol* 2011; 9: 7.
- 46) ZUO T, ZHU M, XU W. Roles of Oxidative Stress in Polycystic Ovary Syndrome and Cancers. *Oxid Med Cell Longev* 2016; 2016: 8589318.
- 47) SPACZYNSKI RZ, ARICI A, DULEBA AJ. Tumor necrosis factor- α stimulates proliferation of rat ovarian theca-interstitial cells. *Biol Reprod* 1999; 61: 993-998.
- 48) SJOSTROM L, LINDROOS AK, PELTONEN M, TORGERTSON J, BOUCHARD C, CARLSSON B, DAHLGREN S, LARSSON B, NARBRO K, SJOSTROM CD, SULLIVAN M, WEDEL H. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *N Engl J Med* 2004; 351: 2683-2693.
- 49) ROBINSON MK. Surgical treatment of obesity-weighting the facts. *N Engl J Med* 2009; 361: 520-521
- 50) ESCOBAR-MORREALE HF, BOTELLA-CARRETERO JI, ALVAREZ-BLASCO F, SANCHO J, SAN MILLAN JL. The polycystic ovary syndrome associated with morbid obesity may resolve after weight loss induced by bariatric surgery. *J Clin Endocrinol Metab* 2005; 90: 6364-6369
- 51) EID GM, COTTAM DR, VELCU LM, MATTAR SG, KORYTKOWSKI MT, GOSMAN G, HINDI P, SCHAUER PR. Effective treatment of polycystic ovarian syndrome with Roux-en-Y gastric bypass. *Surg Obes Relat Dis* 2005; 1: 77-80.
- 52) LEGRO RS, ARSLANIAN SA, EHRLMANN DA, HOEGER KM, MURAD MH, PASQUALI R, WELT CK. Endocrine Society. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2013; 98: 4565-4592.
- 53) MISSO M, BOYLE J, NORMAN R, TEEDE H. Development of evidenced-based guidelines for PCOS and implications for community health. *Semin Reprod Med* 2014; 32: 230-240.
- 54) FAUSER BC, TARLATZIS BC, REBAR RW, LEGRO RS, BALEN AH, LOBO R, CARMINA E, CHANG J, YILDIZ BO, LAVEN JS, BOIVIN J, PETRAGLIA F, WIJEYERATNE CN, NORMAN RJ, DUNAIF A, FRANKS S, WILD RA, DUMESIC D, BARNHART K. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertil Steril* 2012; 97: 28-38.
- 55) YILDIZ BO. Oral contraceptives in polycystic ovary syndrome: risk-benefit assessment. *Semin Reprod Med* 2008; 26: 111-120.
- 56) FALSETTI L, PASINETTI E. Effects of long-term administration of an oral contraceptive containing ethinyl-

- estradiol and cyproterone acetate on lipid metabolism in women with polycystic ovary syndrome. *Acta Obstet Gynecol Scand* 1995; 74: 56-60.
- 57) SWIGLO BA, COSMA M, FLYNN DN, KURTZ DM, LABELLA ML, MULLAN RJ, ERWIN PJ, MONTORI VM. Clinical review: Antiandrogens for the treatment of hirsutism: a systematic review and metaanalyses of randomized controlled trials. *J Clin Endocrinol Metab* 2008; 93: 1153-1160.
 - 58) GAMBINERI A, PATTON L, VACCINA A, CACCIARI M, MORSELLI-LABATE AM, CAVAZZA C, PAGOTTO U, PASQUALI R. Treatment with flutamide, metformin, and their combination added to a hypocaloric diet in overweight-obese women with polycystic ovary syndrome: a randomized, 12-month, placebo-controlled study. *J Clin Endocrinol Metab* 2006; 91: 3970-3980.
 - 59) WOLF JE JR, SHANDER D, HUBER F, JACKSON J, LIN CS, MATHES BM, SCHRODE K; Eflornithine HCl Study Group. Randomized, double-blind clinical evaluation of the efficacy and safety of topical eflornithine HCl 13.9% cream in the treatment of women with facial hair. *Int J Derm* 2007; 207: 94-98.
 - 60) NESTLER JE, JAKUBOWICZ DJ. Decreases in ovarian cytochrome P450C17-alpha activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome. *N Engl J Med* 1996; 335: 617-623.
 - 61) MOGHETTI P, CASTELLO R, NEGRI C, TOSI F, PERRONE F, CAPUTO M, ZANOLIN E, MUGGEO M. Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomized, double-blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation. *J Clin Endocrinol Metab* 2000; 85: 139-146.
 - 62) LORD JM, FLIGHT IH, NORMAN RJ. Metformin in polycystic ovary syndrome: systematic review and meta-analysis. *BMJ* 2003; 327: 951-953.
 - 63) LORD JM, FLIGHT IH, NORMAN RJ. Metformin in polycystic ovary syndrome: systematic review and meta-analysis. *BMJ* 2003; 327: 951-953.
 - 64) DE LEO V, MUSACCHIO MC, CAPPELLI V, DI SABATINO A, TOSTI C, PIOMBONI P. A combined Treatment with Myo-inositol and Monacolin K improve the androgen and lipid profiles of insulin-resistant PCOS patients. *J Metab Syndr* 2013; 2: 127.
 - 65) DAUGHADAY WH, LARNER J, HARTNETT C. The synthesis of inositol in the immature rat and chick embryo. *J Biol Chem* 1955; 212: 869-875.
 - 66) DEAN-JOHNSON M, HENRY SA. Biosynthesis of inositol in yeast. Primary structure of myo-inositol-1-phosphate synthase (EC5.5.1.4) and functional analysis of its structural gene, the INO1 locus. *J Biol Chem* 1989; 264: 1274-1283.
 - 67) 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.
 - 68) CLEMENTS RS JR, DARNELL B. Myo-inositol content of common foods: development of a high-myo-inositol diet. *Am J Clin Nutr* 1980; 33: 1954-1967.
 - 69) BIZZARRI M, CARLOMAGNO G. Inositol: history of an effective therapy for Polycystic Ovary Syndrome. *Eur Rev Med Pharmacol Sci* 2014; 18: 1896-1903.
 - 70) LARNER J, HUANG LC, TANG G, SUZUKI S, SCHWARTZ CF, ROMERO G, ROULIDIS Z, ZELLER K, SHEN TY, OSWALD AS, ET AL. Insulin mediators: structure and formation. *Cold Spring Harb Symp Quant Biol* 1988; 53 pt 2: 965-971.
 - 71) KENNINGTON AS, HILL CR, CRAIG J, BOGARDUS C, RAZ I, ORTMEYER HK, HANSEN BC, ROMERO G, LARNER J. Low urinary chiro-inositol excretion in non-insulin-dependent diabetes mellitus. *N Engl J Med* 1990; 323: 373-378.
 - 72) LOEWUS MW, WRIGHT RW JR, BONDIOLI KR, BEDGAR DL, KARL A. Activity of myo-inositol-1-phosphate synthase in the epididymal spermatozoa of rams. *J Reprod Fertil* 1983; 69: 215-220.
 - 73) ORTMEYER HK, BODKIN NL, LILLEY K, LARNER J, HANSEN BC. Chiroinositol deficiency and insulin resistance. I. Urinary excretion rate of chiroinositol is directly associated with insulin resistance in spontaneously diabetic rhesus monkeys. *Endocrinology* 1993; 132: 640-645.
 - 74) SUZUKI S, KAWASAKI H, SATOH Y, OHTOMO M, HIRAI M, HIRAI A, HIRAI S, ONODA M, MATSUMOTO M, HINOKIO Y ET AL. Urinary chiro-inositol excretion is an index marker of insulin sensitivity in Japanese type II diabetes. *Diabetes Care* 1994; 17: 1465-1468.
 - 75) NESTLER J, UNFER V. Reflections on inositol(s) for PCOS therapy: steps toward success. *Gynecol Endocrinol* 2015; 31: 501-505.
 - 76) LARNER J, HUANG LC, TANG G, SUZUKI S, SCHWARTZ CF, ROMERO G, ROULIDIS Z, ZELLER K, SHEN TY, OSWALD AS, ET AL. Insulin mediators: structure and formation. *Cold Spring Harb Symp Quant Biol* 1988; 53: 965-971.
 - 77) CHEANG KI, BAILLARGEON JP, ESSAH PA, OSTLUND RE JR, APRIDONIZE T, ISLAM L, NESTLER JE. Insulin-stimulated release of D-chiro-inositol-containing inositolphosphoglycan mediator correlates with insulin sensitivity in women with polycystic ovary syndrome. *Metabolism* 2008; 57: 1390-1397.
 - 78) CARLOMAGNO G, UNFER V, ROSEFF S. The D-chiroinositol paradox in the ovary. *Fertil Steril* 2011; 95: 2515-2516.
 - 79) 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: 151-159.
 - 80) ZACCHÈ MM, CAPUTO L, FILIPPIS S, ZACCHÈ G, DINDELLI M, FERRARI A. Efficacy of myo-inositol in the treatment of cutaneous disorders in young women with polycystic ovary syndrome. *Gynecol Endocrinol* 2009; 25: 508-513.
 - 81) PAPALEO E, UNFER V, BAILLARGEON JP, CHIU TT. Contribution of myo-inositol to reproduction. *Eur J Obstet Gynecol Reprod Biol* 2009; 147: 120-123.

- 82) GERLI S, PAPALEO E, FERRARI A, DI RENZO GC. Randomized, double blind placebo-controlled trial: effects of myo-inositol on ovarian function and metabolic factors in women with PCOS. *Eur Rev Med Pharmacol Sci* 2007; 11: 347-354.
- 83) FACCHINETTI F, BIZZARRI M, BENVENGA S, D'ANNA R, LANZONE A, SOULAGE C, DI RENZO GC, HOD M, CAVALLI P, CHIU TT, KAMENOV ZA, BEVILACQUA A, CARLOMAGNO G, GERLI S, OLIVA MM, DEVROEY P. Results from the International Consensus Conference on Myo-inositol and d-chiro-inositol in Obstetrics and Gynecology: the link between metabolic syndrome and PCOS. *Eur J Obstet Gynecol Reprod Biol* 2015; 195: 72-76.
- 84) BEVILACQUA A, CARLOMAGNO G, GERLI S, OLIVA MM, DEVROEY P, LANZONE A, SOULANGE C, FACCHINETTI F, DI RENZO GC, BIZZARRI M, HOD M, CAVALLI P, D'ANNA R, BENVENGA S, CHIU TT, KAMENOV ZA. Results from the International Consensus Conference on myo-inositol and D-chiro-inositol in Obstetrics and Gynecology-assisted reproduction technology. *Gynecol Endocrinol* 2015; 31: 441-446.
- 85) UNFER V, CARLOMAGNO G, PAPALEO E, VAILATI S, CANDIANI M, BAILLARGEON JP. Hyperinsulinemia alters myoinositol to D-chiroinositol ratio in the follicular fluid of patients with PCOS. *Reprod Sci* 2014; 21: 854-858.
- 86) 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* 2014; 61: 111-117.
- 87) DINICOLA S, CHIU TT, 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: 1079-1092.
- 88) UNFER V, CARLOMAGNO G, DANTE G, FACCHINETTI F. Effects of myoinositol in women with PCOS: a systematic review of randomized controlled trials. *Gynecol Endocrinol* 2012; 28: 509-515.
- 89) CARLOMAGNO G, UNFER V, ROSEFF S. The D-chiro-inositol paradox in the ovary. *Fertil Steril* 2011; 95: 2515-2516.
- 90) PAPALEO E, UNFER V, BAILLARGEON JP, FUSI F, OCCHI F, DE SNANTIS L. Myo-inositol may improve oocyte quality in intracytoplasmatic sperm injection cycles. A prospective, controlled, randomized trial. *Fertil Steril* 2009; 91: 1750-1754.
- 91) HSIA Y, DAWOUD D, SUTCLIFFE AG, VINER RM, KINRA S, WONG IC. Unlicensed use of metformin in children and adolescents in the UK. *Br J Clin Pharmacol* 2012; 73: 135-139.
- 92) ZACCHÈ MM, CAPUTO L, FILIPPIS S, ZACCHÈ G, DINDELLI M, FERRARI A. Efficacy of myo-inositol in the treatment of cutaneous disorders in young women with polycystic ovary syndrome. *Gynecol Endocrinol* 2009; 25: 508-513.
- 93) LI Y, JIANG L, JIA Z, XIN W, YANG S, YANG Q, WANG L. A meta-analysis of red yeast rice: an effective and relatively safe alternative approach for dyslipidemia. *PLoS One* 2014; 9: e98611.
- 94) FEUERSTEIN JS, BJERKE WS. Powdered red yeast rice and plant stanols and sterols to lower cholesterol. *J Diet Suppl* 2012; 9: 110-115.
- 95) KAZEROONI T, SHOJAEI-BAGHINI A, DEHBASHI S, ASADI N, GHAFARPASAND F, KAZEROONI Y. Effects of metformin plus simvastatin on polycystic ovary syndrome: a prospective, randomized, double-blind, placebo-controlled study. *Fertil Steril* 2010; 94: 2208-2213.
- 96) DE LEO V, MUSACCHIO MC, PALERMO V, DI SABATINO A, MORGANTE G, PETRAGLIA F. Polycystic ovary syndrome and metabolic comorbidities: therapeutic options. *Drugs Today (Barc)* 2009; 45: 763-775.
- 97) DE LEO V, MUSACCHIO MC, CAPPELLI V, DI SABATINO A, TOSTI C, PIOMBONI P. A Combined Treatment with Myo-Inositol and Monacolin K Improve the Androgen and Lipid Profiles of Insulin-Resistant PCOS Patients. *J Metabolic Syndr* 2013; 2: 2.
- 98) Practice Committee of American Society for Reproductive Medicine. Use of insulin-sensitizing agents in the treatment of polycystic ovary syndrome. *Fertil Steril* 2008; 90(5 Suppl): S69-73.
- 99) EVANS JL, HEYMANN CJ, GOLDFINE ID, GAVIN LA. Pharmacokinetics, tolerability, and fructosamine-lowering effect of a novel, controlled release formulation of alpha-lipoic acid. *Endocr Pract* 2002; 8: 29-35.
- 100) MASHARANI U, GJERDE C, EVANS JL, YOUNGREN JF, GOLDFINE ID. Effects of controlled-release alpha lipoic acid in lean, nondiabetic patients with polycystic ovary syndrome. *J Diabetes Sci Technol* 2010; 4: 359-364.
- 101) CAPASSO I, ESPOSITO E, MAUREA N, MONTELLA M, CRISPO A, DE LAURENTIIS M, D'AIUTO M, FRASCI G, BOTTI G, GRIMALDI M, CAVALCANTI E, ESPOSITO G, FUCITO A, BRILLANTE G, D'AIUTO G, CILIBERTO G. Combination of inositol and alpha lipoic acid in metabolic syndrome-affected women: a randomized placebo-controlled trial. *Trials* 2013; 14: 273.
- 102) CIANCI A, PANELLA M, FICHERA M, FALDUZZI C, BAROLO M, CARUSO S. d-chiro-Inositol and alpha lipoic acid treatment of metabolic and menses disorders in women with PCOS. *Gynecol Endocrinol* 2015; 31: 483-486.
- 103) ROCHETTE L, GHIBU S, RICHARD C, ZELLER M, COTTIN Y, VERGELY C. Direct and indirect antioxidant properties of α -lipoic acid and therapeutic potential. *Mol Nutr Food Res* 2013; 57: 114-120.
- 104) MORGANTE G, CAPPELLI V, DI SABATINO A, MASSARO MG, DE LEO V. Polycystic ovary syndrome (PCOS) and hyperandrogenism: the role of a new natural association. *Minerva Ginecol* 2015; 67: 457-463.
- 105) DE LEO V, DI SABATINO A, MUSACCHIO MC, MORGANTE G, SCOLARO V, CIANCI A, PETRAGLIA F. Effect of oral contraceptives on markers of hyperandrogenism and SHBG in women with polycystic ovary syndrome. *Contraception* 2010; 2: 276-280.

- 106) TYE R. Konjac flour: properties and applications. *Food Technology* 1991; 45: 11-16.
- 107) DOI K. Effect of konjac fibre (glucomannan) on glucose and lipids. *E J Clin Nutr* 1995; 49 Suppl 3: S190-S197.
- 108) CHEN HL, SHEU WH, TAI TS, LIAW Y, CHEN Y. Konjac supplement alleviated hypercholesterolemia and hyperglycemia in type 2 diabetic subjects--a randomized double-blind trial. *J Am Coll Nutr* 2003; 22: 36-42.
- 109) CHEARSKUL S, KRIENGSIYOS W, KOOPTIWUT S, SANGURAI S, ONREABROI S, CHURINTARAPHAN M, SEMPRASERT N, NITIYANANT W. Immediate and long-term effects of glucomannan on total ghrelin and leptin in type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2009; 83: e40-e42.
- 110) DE LEO V, TOSTI C, CAPPELLI V, MORGANTE G, CIANCI A. Valutazione dell'associazione inositolo-glucomannano nel trattamento di donne affette da PCOS. *Minerva Ginecol* 2014; 66: 527-533.
- 111) MOLLACE V, SACCO I, JANDA E, MALARA C, VENTRICE D, COLICA C, VISALLI V, MUSCOLI S, RAGUSA S, MUSCOLI C, ROTIROTI D, ROMEO F. Hypolipemic and hypoglycaemic activity of bergamot polyphenols: from animal models to human studies. *Fitoterapia* 2011; 82: 309-331
- 112) MICELI N, MONDELLO MR, MONFORTE MT, SDRAFKAKIS V, DUGO P, CRUPI ML, TAVIANO MF, DE PASQUALE R, TROVATO A. Hypolipidemic effects of Citrus bergamia Risso et Poiteau juice in rats fed a hypercholesterolemic diet. *J Agric Food Chem* 2007; 5: 10671-10677
- 113) CAPPELLO AR, DOLCE V, IACOPETTA D, MARTELLO M, FIORILLO M, CURCIO R, MUTO L, DHANYALAYAM D. Bergamot (*Citrus bergamia* Risso) flavonoids and their potential benefits in human hyperlipidemia and atherosclerosis: an overview. *Mini Rev Med Chem* 2016; 16: 619-629.
- 114) CHA JY, CHO YS, KIM I, ANNO T, RAHMAN SM, YANAGITA T. Effect of hesperetin, a citrus flavonoid, on the liver triacylglycerol content and phosphatidate phosphohydrolase activity in orotic acid-fed rats. *Plant Foods Hum Nutr* 2001; 56: 349-358
- 115) TOSTI C, CAPPELLI V, DE LEO V. Role of a new oral formulation based on D-chiro-inositol / monocolin K / bergamot extract / methylfolate and Vitamin K2 in perimenopausal women with metabolic syndrome with a BMI >25 kg / m². *J Metab Syndrome* 2016; 5: 3.
- 116) LA MARCA A, GRISENDI V, DONDI G, SIGHINOLFI G, CIANCI A. The menstrual cycle regularization following D-chiroinositol treatment in PCOS women: a retrospective study. *Gynecol Endocrinol* 2015; 31: 52-56.
- 117) GENAZZANI AD, SANTAGNI S, RATTIGHIERI E, CICERCHIA E, DESPINI G, MARINI G, PRATI A, SIMONCINI T. Modulatory role of dichiroinositol (DCI) on LH and insulin secretion in obese PCOS patients. *Gynecol Endocrinol* 2014; 30: 438-443.
- 118) HUANG LC, FONTELES MC, HOUSTON DB, ZHANG C, LARNER J. Chiroinositol deficiency and insulin resistance. III Acute glycolenic and hypoglycemic effects of two phosphoglycan insulin mediator in normal and streptozotocin diabetic rats in vivo. *Endocrinology* 1993; 2: 652-657.
- 119) DE LEO V, MUSACCHIO MC, CAPPELLI V, MASSARO MG, MORGANTE G, PETRAGLIA F. Genetic, hormonal and metabolic aspects of PCOS: an update. *Reprod Biol Endocrinol* 2016; 14: 38.