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REVIEW

MANAGEMENT OF THE MAIN ENDOCRINE AND DIABETIC DISORDERS IN CHILDREN

# Current treatment for polycystic ovary syndrome: focus on adolescence

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## ABSTRACT

Polycystic ovary syndrome (PCOS) is the most frequent endocrine disorder in women and it is associated with an increased rate of infertility. Its etiology remains largely unknown, although both genetic and environmental factors play a role. PCOS is characterized by insulin resistance, metabolic disorders and low-grade chronic inflammation. To date, the treatment of PCOS is mainly symptomatic and aimed at reducing clinical signs of hyperandrogenism (hirsutism and acne), at improving menstrual cyclicity and at favoring ovulation. Since PCOS pathophysiology is still largely unknown, the therapeutic interventions currently in place are rarely cause-specific. In such cases, the therapy is mainly directed at improving hormonal and metabolic dysregulations typical of this condition. Diet and exercise represent the main environmental factors influencing PCOS. Thus, therapeutic lifestyle changes represent the first line of intervention, which, in combination with oral contraceptives, represent the customary treatment. Insulin resistance is becoming an increasingly studied target for therapy, most evidence stemming from the time-honored metformin use. Relatively novel strategies also include the use of thiazolidinediones and GLP1-receptor agonists. In recent years, a nutraceutical approach has been added to the therapeutic toolkit targeting insulin resistance. Indeed, emerging data support inositol and alpha-lipoic acid as alternative compounds, alone or in combination with the aforementioned strategies, with favorable effects on ovulation, insulin resistance and inflammation. Nevertheless, additional studies are required in adolescents, in order to assess the effectiveness of diet supplements in preventing negative impacts of PCOS on fertility in adult age. This review focuses on the main therapeutic options for PCOS to date.

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KEY WORDS: Polycystic ovary syndrome; Oral contraceptives; Metformin; Inositol; D-chiro-inositol-galactosamine; Insulin resistance.

Polycystic ovary syndrome (PCOS) is a heterogeneous clinical condition, which is considered the most frequent endocrine disorder in women of reproductive age. Its etiology remains unknown, although there is evidence that both genetic and environmental factors may contribute to its origin.[1] According to current diagnostic criteria for adult women, established in a joint consensus workshop of the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM), held in Rotterdam in 2003, PCOS may be diagnosed by the presence of at least two of the following three clinical fea-

tures: clinical and/or biochemical hyperandrogenism, chronic oligo-anovulation, and the micro-polycystic morphology of the ovary (PCOm).[2] Nevertheless, no alterations are specific to PCOS, and other disorders that can potentially cause these abnormalities, such as late-onset congenital adrenal hyperplasia, hyperprolactinemia or androgen-secreting tumors, must be preliminary excluded. Therefore, PCOS is an exclusion diagnosis. It has been estimated that PCOS, as diagnosed by the Rotterdam criteria, may occur in about 10% of women of reproductive age,[3] but this figure could be even higher.[4, 5] Indeed, available epidemiologic data are limited and prevalence estimates may be influenced not only by the diagnostic criteria but also by the methods used for assessing these aspects as well as by the ethnic characteristics of the population investigated. In particular, hirsutism, which is the more frequent and specific clinical feature of hyperandrogenism, is strongly affected by constitutional and ethnic factors. In this regard, the cut-off value for diagnosing excessive body hair growth when using the usually adopted method, *i.e.* the modified Ferriman-Gallwey score, is generally 8 for Caucasian, Middle-Eastern or Afro-American women, but it may be just 3 for Far-East Asian women.[6] Other clinical features, such as acne and scalp hair loss, are less specific and cannot be considered signs of hyperandrogenism, when androgen levels are normal and there is no hirsutism. However, acne can be found in 15-25% and scalp hair loss in 20-30% of adult women with PCOS. Acne is a common, complex, multifactorial process in which androgens play a central role, but other factors are required.[7] Conversely, there is not an obligate link between androgen action and female pattern hair loss, which may be present even in women lacking an androgen receptor. Nevertheless, in many women with hair loss androgen action in the scalp may be enhanced, and assessment of a possible androgen excess is required.[8] Unfortunately, assessment of biochemical hyperandrogenism can also be challenging, being hampered by significant limitations of methods for assaying serum androgens generally used in clinical labs. Indeed, ordinary methods may misclassify, when distinguishing hyperandrogenemic *versus* normoandrogenemic subjects, as many as 30% of women with PCOS.[9] Assessment of ovarian morphology is another problematic issue. In particular, it has been argued that the cut-off value for distinguishing an abnormal follicle count should be adapted according to available probes, from the traditional figure of 12, indicated by the Rotterdam workshop, up to 25 when using modern technology providing greater resolution, with a transducer frequency ≥8 MHz.[10] To overcome these difficulties, measurement of serum Anti-Müllerian hormone (AMH), which is directly associated with follicle count and is increased in many women with PCOS, has been proposed as a potential alternative to assess ovarian morphology on ultrasound.[11] However, this assay still needs adequate standardization. Diagnostic difficulties are even more relevant in adolescents, due to the physiological presence in these subjects of features that mirror some clinical aspects of PCOS. In particular, many girls exhibit a condition of transient anovulation. It has been reported that more than 80% of cycles are anovulatory during the first year after menarche, and 25% remain dysfunctional in the sixth year.[12] PCOm as well may be physiological in adolescents. This morphology was reported in one third of healthy girls 4 years after menarche, without any relationship to the metabolic and endocrine features of PCOS.[13] Nevertheless, while it was hypothesized that isolated PCOm may anticipate ovarian dysfunction in some girls, anovulatory adolescents can show subtle endocrine alterations,[14] suggesting a potential propensity to development of overt PCOS later in life. It has been suggested that age-specific cut-off values for ovarian follicle number should be defined in adolescents.[15] However, transvaginal imaging cannot be performed in many girls, with limitations in ovarian morphology assessment, especially in obese individuals. In this regard, when using transabdominal ultrasound, reporting may be best centered on ovarian volume, maintaining a threshold of ≥10 mL, given the difficulty of consistently measuring follicle number. Finally, hirsutism may require time to develop, whereas acne is a very common feature in young ages, making it often difficult to diagnose clinical hy-

perandrogenism. Therefore, while the definition of PCOS in adolescents should follow the general laws established for adult women, diagnosing the presence of ovarian dysfunction and clinical hyperandrogenism may be a problematic issue in young girls, and no unanimously accepted criteria for diagnosing PCOS in adolescence exist. ESHRE and ASRM, in a consensus workshop held in Amsterdam in 2010, proposed that the presence of all three elements of the Rotterdam criteria is needed in teenagers to diagnose PCOS. In particular, these guidelines suggested that menstrual dysfunction should be present for at least 2 years after menarche; the diagnosis of PCOm on ultrasound should be based on increased ovarian size (>10 $cm^3$); and hyperandrogenemia rather than clinical signs of androgen excess should be documented.[16] Conversely, the Endocrine Society suggested that the diagnosis of PCOS in adolescents should rely on a complete clinical picture, including clinical evidence of androgen excess, increased androgen levels and persistent oligomenorrhea, after exclusion of secondary causes.[17] Notably, according to the International PCOS Network, irregular menstrual cycles cannot be diagnosed in the first year post menarche, whereas they should be defined as <21 or >45 days in the period 1 to <3 years post menarche, and <21 or >35 days thereafter, while primary amenorrhea can be established by age 15 or at least 3 years after thelarche.[18] More recently, an International Consortium of Paediatric Endocrinology update has concluded that appropriate diagnosis of adolescent PCOS requires the presence of both menstrual irregularities, 2 years beyond menarche, and evidence of biochemical and/or clinical hyperandrogenism, in particular progressive hirsutism, after exclusion of other disorders. According to these authors, severe cystic acne and PCOm represent supportive elements, but only in concert with the main features.[19] Finally, other experts recently suggested that PCOS diagnosis should be considered in adolescents with persistence of oligomenorrhea 3-4 years after menarche, in the presence of clinical and/or biochemical hyperandrogenism and after exclusion of other disorders. However, when menstrual dysfunction persists up to 2 years after menarche, girls can be considered at risk for PCOS, suggesting a clinical and possibly ultrasonographic and/or AMH follow up. In these cases, risk of overdiagnosis and unnecessary interventions should be balanced against the individual need of a diagnosis and early therapy.[20] Notably, no current definitions of PCOS include obesity or insulin resistance (IR) among the diagnostic criteria. Nevertheless, these are common features of PCOS women and may play a significant role in the pathophysiology of the syndrome. A meta-analysis of available studies concluded that body fat excess may be found in up to 60% of PCOS subjects, often with a central distribution of adipose tissue.[21] A similar percentage (58%) was reported in the cohort of the Verona 3P Study,[22] which includes a large series of consecutive women with PCOS. However, we must consider that a referral bias could produce an overestimation of these percentages. In actual fact, a comparison of referral women with PCOS with those identified through the screening of an unselected population suggested that frequency of obesity might indeed be similar in PCOS women and in the background population, although contributing to worsening the whole clinical picture of these patients.[23] A body of evidence indicates that IR is a common feature of PCOS independently of obesity, although it is not universal in these patients. In particular, it was estimated that 70-75% of women with this condition have impaired insulin action, as assessed by the gold standard glucose clamp technique.[24, 25] Interestingly, these studies showed that a higher Body Mass Index (BMI) exacerbates the reduction of insulin sensitivity, which is characteristic of PCOS, also suggesting a greater impact of body fat excess in PCOS than in controls.[25] An important point is that IR and the associated hyperinsulinemia may contribute to the origin of PCOS by different mechanisms, including increased androgen production by both the ovary and the adrenal,[26, 27] and possibly by reducing sex hormone binding globulin (SHBG) synthesis in the liver.[28] However, the mechanisms underlying the PCOS-specific impairment of IR remain poorly understood.[29] Another important issue is that the adoption of the Rotterdam diagnostic criteria has generated, un-

der the common label of PCOS, distinct clinical "phenotypes" of the syndrome, based on the different possible combinations of diagnostic features in each individual patient. A recent NIH workshop on PCOS, while confirming the Rotterdam criteria for diagnosis, has recommended distinguishing women with the following phenotypes: androgen excess + ovulatory dysfunction + PCOm (complete phenotype); androgen excess + ovulatory dysfunction (classic phenotype); androgen excess + PCOm (ovulatory phenotype); and ovulatory dysfunction + PCOm (normoandrogenic phenotype).[30] Interestingly, there is a scale of metabolic risk among these different phenotypes. In particular, women with both hyperandrogenism and ovulatory dysfunction, with or without PCOm, and, to a lesser extent, those with the ovulatory phenotype appear to be characterized by IR and the associated abnormalities, whereas those with the normoandrogenic phenotype do not.[31] It is also noteworthy that androgen excess, in turn, may contribute to generating hyperinsulinemia, not only by altering insulin action,[32, 33] but possibly also by impairing the metabolic clearance of insulin.[34, 35] Therefore, there is evidence of a vicious circle between IR and hyperandrogenism in these women. Metabolic syndrome is another alteration that can be found with higher frequency in women with PCOS.[31] Interestingly, this alteration may be found even in many adolescents with this syndrome.[36]

## Goals of therapy in women with PCOS

The main complaints of individuals with PCOS are generally menstrual abnormalities, clinical signs of hyperandrogenism and, in adult women, infertility.[1, 2] Therapy of PCOS is traditionally based on a symptomatic approach, which largely depends on the perceived individual needs of patients. However, while these should be carefully taken into account by physicians, current knowledge of the pathophysiology of the syndrome and its long-term potential sequelae suggests that other aspects, such as body fat excess, metabolic alterations, and risk of endometrial hyperplasia and endometrial cancer and of psychological disturbances should also be considered. Customary treatment of these subjects is generally represented by combined hormonal contraceptives, which can induce regular menses and improve symptoms of hyperandrogenism, in particular of hirsutism and acne, also protecting from endometrial hyperplasia. However, efficacy on hirsutism is limited,[6] and there is no clear evidence of beneficial effects of this therapy on IR and the associated metabolic alterations. In addition, there may be specific contraindications in subjects at risk for thromboembolic and cardiovascular events. These general considerations are also true for adolescents with PCOS,[19, 20] although cardiovascular events are extremely rare in girls. Notably, most knowledge on the long-term effects of OC derives from observational studies in the general population, whereas studies in PCOS women are very limited and short-term and there are no RCTs on these aspects.[37] In order to counteract the mechanisms potentially underlying the origin of PCOS and the associated metabolic abnormalities, and potentially also to prevent some long-term complications of this condition, strategies aimed at improving IR have been assessed, especially with the insulin sensitizer metformin. These studies reported some interesting short-term findings, in terms of effects on reproductive and metabolic alterations.[38] Combined therapy with hormonal contraceptive and metformin has also been proposed, although clinical experience is still limited.[39] Finally, recent findings have suggested that novel strategies, such as incretin mimetic drugs, may be of potential interest for people with PCOS.[40] The following sections will discuss these different therapeutic options for women with PCOS, with specific attention to their use in adolescents.

## Lifestyle intervention modifications

Environmental factors significantly impact on genetic predisposition to PCOS. To date treatment is symptomatic, although lifestyle changes can dramatically affect phenotype, and a first-line intervention is represented by lifestyle changes, in particular, weight loss and prevention of weight gain strategies (Table I).[41-47] Cochrane reviews support lifestyle interventions

TABLE I.—*Main findings relative to the effects of lifestyle intervention modifications in PCOS.*[41-47]

| Study | Main results |
|---|---|
| Domecq *et al*. (2013)[41] | Decreased fasting glucose and fasting insulin concentrations associated with BMI improvement<br>Effect on hirsutism unclear |
| Sedighi *et al*. (2014)[42] | Hypercaloric diet, and scarce physical activity associated with PCOS<br>No association of PCOS with unhealthy behaviors |
| Abazar *et al*. (2015)[43] | Aerobic exercise decreased BMI, WHR, weight, fat mass and triglyceride levels and increased HDL-cholesterol |
| Norman *et al*. (2006)[44] | Psychological Stress: effects not fully understood<br>Caffeine: adverse effects on fertility<br>Alcohol consumption: associated with fertility reduction and weight gain<br>Chemicals and pollutants: may negatively affect fertility<br>Recreational drugs: adverse effects on fertility |
| Patel *et al*. (2018) [45] | Balanced diet and regular and moderate physical exercise are strongly recommended |
| Brennan *et al*. (2017)[46] | Weight management in association with behavioral and psychological strategies especially in reproductive age are recommended |
| Cappelli *et al*. (2017)[47] | Additional interventions consisting in nutritional supplements or drug administration and/or surgery are needed when weight recovery occurs following appropriate diet and physical exercise practice |

BMI: Body Mass Index; WHR: waist-to-hip ratio; HDL: high-density lipoprotein.

for PCOS, nevertheless the behavioral components of these interventions are not yet fully understood and defined.[48] The main environmental factors influencing this syndrome are diet and exercise. The evidence of the influence of lifestyle modification (LSM) interventions on clinical, hormonal and metabolic profiles have been analyzed in several systematic reviews and meta-analyses hereafter reported. Randomized controlled trials (RCTs) have compared PCOS women of ages ranging from 18 to 32 years receiving LSM interventions, to women receiving none or minimal interventions or metformin. LSM significantly decreased fasting blood glucose (FBG) and fasting insulin concentrations, and these changes were associated with BMI improvement. LSM showed better results than metformin in improving glucose or insulin levels whereas the effect on hirsutism measured using the Ferriman-Gallwey score was unclear.[41] A descriptive-comparative study compared 65 women with PCOS with 65 healthy women collecting data by means of questionnaires for diet, physical activity and unhealthy behaviors concluding that a significant association between PCOS and hypercaloric diet, and scarce physical activity was evident, but there was no association between PCOS and unhealthy behavior.[42] Another study compared PCOS women undergoing an aerobic exercise program with a control group: at the end of the exercise program, BMI, waist-to-hip ratio (WHR), weight, fat mass and triglyceride levels decreased significantly and high-density lipoprotein (HDL)-cholesterol increased. However, no change was reported in low-density lipoprotein cholesterol (LDL-C), very low-density lipoproteins (VLDL), and total cholesterol concentrations.[43] Systematic reviews highlight the relevance of quality of life for the health of PCOS women. The effects of LSM interventions, including exercise-only, diet-only, exercise and diet on health-related quality of life or general quality of life were structurally and systematically analyzed.[49] An identified lifestyle factor that adversely affected health and reproductive outcomes in PCOS was smoking since cigarette smoke components had a negative impact on the follicular milieu and affected hormone concentrations in the luteal phase. Psychological stress effects are not fully understood, because it is hard to distinguish whether stress is a contributor or an effect of PCOS. Furthermore, stress is quite difficult to define and measure and common guidelines are still needed. Caffeine may have adverse effects on fertility, affecting ovulation and corpus luteus by changing hormonal levels. Alcohol consumption is associated with fertility reduction and weight gain. Chemicals and pollutants may negatively affect fertility. Recreational drugs are currently taken by some young women and may result into adverse effects on fertility, al-

though data are scarce and uncertain.[44] A holistic review on PCOS as an inflammatory, systemic and endocrine disease suggests that extreme exercise and drastic dieting may have negative effects on human body, which is sensitive to high energy-fluctuations. Therefore, a balanced diet and regular and moderate physical exercise are strongly recommended.[45] Finally, an interesting narrative review highlighted the relevance of supporting in clinical routine practice LSM for weight management in association with behavioral and psychological strategies especially in reproductive age. These authors provided also a list of key recommendations for weight management interventions for PCOS women.[46] Although the gold standard practice for improving insulin sensitivity in obese PCOS women to date consists in weight management by means of appropriate diet and physical exercise, about 90% of these patients experience a weight recovery. Therefore, in particular in these cases, additional interventions consisting in nutritional supplements or drug administration and/or surgery are sometimes needed.[47]

## Oral contraceptives

In patients with diagnosis of PCOS, there are several treatment options depending on the major clinical manifestations. Non-pharmacological treatment (lifestyle interventions such as physical activity, diet and caloric restriction) are considered the first line approaches in overweight and obese patients and in patients with metabolic disturbances. As stated in a separate section of this review, in these patients, treatment with metformin or other insulin sensitizers can be an option. In cases with hirsutism and menstrual irregularity as main clinical issues, pharmacological agents may be used. In these patients, oral contraceptives (OC) are the first line pharmacological approach.[50]

OC reduce hyperandrogenemia manifestations and regulate menstrual cycle. These actions depend on a suppression of pituitary gonadotropin secretion[51] and on an increase in sex hormone binding globulin concentrations with a subsequent reduction in free serum androgen levels.[52] Moreover, progestins contained in some OC (such as dienogest, drospirenone and norgestimate) show anti-androgenic properties (androgen receptor antagonism, inhibition of 5 alpha reductase activity). The progestins contained in third generation contraceptives show a low androgenic activity.[53] Despite medical societies recommend the use of OC in adult women with PCOS,[16, 17, 50] definite conclusions regarding indications, long term risks and safety in adolescents have not been drawn as current evidence is derived from studies with low methodological quality. The only available meta-analysis of RCTs regarding treatment of PCOS in adolescents was published by the group of Al Khalifah[54] where OC were compared with metformin. The authors included four RCTs which enrolled a total of 170 patients. Statistical analysis showed an overall mild improvement in menstrual cycle frequency and in acne scores in patients treated with OC versus metformin. Conversely, the use of metformin was associated with a significant reduction in BMI, total cholesterol, LDL cholesterol and rate of dysglycemia. Interestingly, the impact on hirsutism was similar in patients treated with metformin compared to OC. However, the authors evidenced that the results should be interpreted carefully because of the very low to low quality of evidence of the studies included. A more recent meta-analysis on an adult population including 33 studies and 1521 patients suggested a significant effect in favor of OC in terms of hirsutism scores, when severe hirsute patients were excluded, while it confirmed a superiority of OC in terms of menstrual cycle regularity.[39] When assessing quality of life, OC seemed to have beneficial effect in PCOS patients, at least when associated with lifestyle intervention. A study by the group of Harris-Glocker[55] reported an improvement of all the variables related to quality of life, assessed with a specific questionnaire, in obese adolescent with PCOS enrolled in a lifestyle program and taking OC. With regard to safety, several studies have examined the impact of OC on glucose and lipid metabolism, cardiovascular risk and venous thromboembolism (VTE) risk in PCOS patients. Evidence on glucose and lipid metabolism are conflicting, and some studies suggest differences related to the population enrolled and the type of pill used. A meta-analysis

published by the group of Halpering[56] evaluating prospective cohorts and RCTs that enrolled women, aged 13–44 years with PCOS on OC for at least three months, showed no changes in FBG, insulin and homeostatic model assessment of IR (HOMA-IR) related to these medications. Conversely, the authors showed that OC were associated with an increase in triglycerides and HDL cholesterol levels. Similarly, a more recent meta-analysis by Amiri *et al*.[57] on 27 studies, including adults and adolescents with PCOS, reported that the use of OC was not associated with significant change in BMI, FBG or HOMA-IR but long term use of these medications was found to worsen the lipid profile (increase in total, HDL and LDL cholesterol and increase in triglycerides). It is important to underline that both studies are limited by the heterogeneity of results and by lifestyle interventions that patients with PCOS received during the follow-up period. Moreover, most of participants were not obese and it is therefore difficult to generalize these results to different subgroups of PCOS patients. Overall, the results of these studies suggested no significant change in glucose metabolism and modest worsening of the lipid profile. It is therefore reasonable to check periodically the metabolic profile in PCOS patients on OC and to use low estrogen formulations in patients with known dyslipidemia.[37] The overall impact of OC on cardiovascular health is debated as well, with some authors suggesting that, for PCOS patients, lowering serum androgens with improvement in endothelial and adipocyte function may counteract the mild increase in triglycerides and cholesterol levels.[58] A major known side effect of OC is increased risk of thromboembolism.[59] PCOS patients present impaired fibrinolysis and abnormal endothelial function,[60] with higher risk of VTE compared to the general population. The group of Bird showed how women with PCOS not taking OC have a 1.5-fold increased risk of VTE. The relative risk rises to 2-fold increase in PCOS patients on OC.[61] For this reason, a thorough personal and family history of thromboembolic events and a careful assessment of all other cardiometabolic risk factors needs to be addressed when considering starting the treatment with OC in PCOS patients. Discussing the contraindications for OC goes beyond the purpose of this review. On this topic, we recommend referring to World Health Organization medical eligibility criteria for contraceptive use.[62] Despite the limited number of high-quality studies regarding risk of gynecological cancer in PCOS, data from the literature suggest an increased risk of endometrial cancer in these patients, while data regarding ovarian cancer are contradictory. In addition, epidemiological data indicate no relationship between PCOS and breast cancer.[63] Considering the lack of specific evidence regarding the impact of OC use in PCOS patients and cancer risk, it is reasonable to report data on general population to comment on safety of these medications in PCOS patients. Endometrial cancer risk is reduced by OC use, as strongly suggested by the literature.[64, 65] OC use is protective also for ovarian cancer with persistence in risk reduction for more than 30 years after OC discontinuation.[66, 67] Data on breast cancer risk in OC users indicates a mild increased risk for this tumor, with an attributable risk of +3%. The increased risk appears to be limited to women who are currently using or have recently used these medications, with progressive reduction with time of excessive risk and a rate comparable to the general population after at least 10 years of discontinuation.[68] Overall, whereas OC use is associated with an increased risk of breast cancer, total cancer risk appears to be reduced in most relevant studies, due to the protective effect on endometrial, ovarian and colorectal cancer.[64, 66, 69] OC have different dosages and types of oestrogens which should be associated with different kinds of progestins. In adolescents with PCOS the choice of OC must be individualized for each patient following different considerations. SHBG levels are influenced differently by different types of oestrogens. In turn, oestrogens can decrease serum concentrations of free testosterone (T).[52] Several studies show that ethinyl estradiol (EE) has a greater action in increasing SHBG levels than natural estradiol.[70] Furthermore, progestins have different binding affinity for progesterone receptors suppressing gonadotropin release and subsequently decreasing ovarian androgen synthesis.[71] In PCOS patient with acne and hirsutism progestins, as nogestimate, drospirenone, desogestrel

and cyproterone acetate, are first choice since they have an antiandrogenic action.[72] In particular, cyproterone acetate seems to be more effective than drospirenone in increasing SHBG levels.[73] However, OCs containing cyproterone should be avoided in the adolescent subjects owing to undesirable effects on lipid profile and glucose metabolism.[74-76] In one study, 20 μg EE combined with 3 mg drospirenone were effective as 30 μg EE in improving clinical and hormonal parameters of lean PCOS women, while they showed a moderate impact on the lipid profiles. Nevertheless, both doses improved hirsutism, T, dehydroepiandrosterone-sulfate (DHEA-S), and SHBG levels.[77]

## Metformin

It is estimated that IR is associated with 50-70% of PCOS cases, although the prevalence of IR widely changes according to the measurement methods employed.[78] As described above PCOS is a heterogeneous disorder characterized by varying degrees of IR which have hormonal and metabolic effects independent of BMI and glucose tolerance state.[79] Indeed, the co-existence of a higher BMI in PCOS subjects with IR further exacerbates such effects, thus leading to the increased cardiometabolic risk and worsening degrees of hormonal and reproductive disorders.[25] Most importantly, it appears that the metabolic abnormalities characterizing overt PCOS take place early in life, with adolescents affected by PCOS displaying higher degrees of (hepatic) IR, as compared with their BMI-matched counterparts without PCOS.[80] Metformin exerts pleiotropic effects on several tissues, including the liver, skeletal muscle and adipose tissue. Recent evidence also supports both direct and indirect effects on ovarian steroidogenesis.[81] The earliest data of an insulin-sensitizing effect of metformin, with a concomitant reduction in serum T levels and increased SHBG, were reported by Nestler *et al.*[82, 83] The improvement of insulin sensitivity by metformin is primarily due its effects on the liver, as metformin activates the cellular energy sensor adenosine monophosphate-activated protein kinase (AMPK) and reduces the expression of gluconeogenic enzymes. These events lead to the breakdown of energy stores, which, in turn, reduce the hepatic glucose output[84] and, concomitantly, increase hepatic insulin sensitivity.[85] Metformin also suppresses lipolysis and lipogenesis in the adipose tissue, thus improving the lipidic and glycemic toxicity burden to the insulin-mediated glucose uptake in the skeletal muscle.[84] With regard to the effects of metformin on androgen levels, it is estimated that metformin reduces circulating T levels by approximately 20-25%,[81] primarily as a consequence of reduced hyperinsulinemia.[82, 83] Importantly, such effect occurs early after metformin initiation and before any noticeable change in individual insulin sensitivity, thus suggesting possible direct effects of metformin at the ovarian level.[86, 87] Supporting evidence of such an effect stems from data obtained in cultured human thecal cells, where exposure to metformin led to decreased steroidogenesis secondary to inhibition of the mitochondrial respiratory chain that subsequently would reduce 3β-hydroxysteroid dehydrogenase type 2 (HSD3B2) and 17α-Hydroxylase/17,20 lyase (CYP17A1) activity, which is reported to be overexpressed in PCOS.[88, 89] The question as to whether metformin, alone or in combination with other insulin sensitizing drugs, exerts beneficial effects on fertility in women with PCOS has been thoroughly investigated in at least two large meta-analyses of randomized clinical trials. However, results were not conclusive.[38] Similar lack of benefit has been reported also with regard to the treatment of acne and hirsutism.[90] In summary, the use of metformin in adult women is supported by international guidelines for the treatment of the metabolic disturbances underlying PCOS,[17] *i.e.* altered glucose tolerance in women with (pre)diabetes and comorbid PCOS and in those with PCOS that are at high risk of developing diabetes because of ethnic background and/or genetic/familial predisposition.[91] In contrast, the effects on body composition and weight loss are not so robust and are not currently supported by solid data to support inclusion in treatment guidelines. On top of that, it should also be pointed out that no firm evidence currently exists in adolescents, although a limited number of trials are currently ongoing or awaiting completion,[92, 93] as reported in Table II.[92-96]

TABLE II.—*Double-blinded randomized clinical trials of insulin sensitizing drugs and GLP-1 RA conducted in adolescents/young adults with PCOS over the last decade.*[92-96]

| Study | Subjects | Drug | Comparator | Main Results | NCT |
|---|---|---|---|---|---|
| Ladson (2011)[93] | Overweight/obese adolescents and young adults with PCOS<br>N.=114; age: 29.0±5.4 years; BMI at baseline: 38.0±8.0 kg/m$^2$<br>PCOS definition: 1990 NIH Criteria | Metformin 2 g daily (500 mg/day step-up increase) + lifestyle intervention as in Knowler *et al.*[97] | Placebo + lifestyle intervention (150 min/wk exercise combined with a low-calorie diet: ≥7% weight loss goal) | Only 38 completed the study; drop-out rate: 66.7%<br>No significant difference in ovulation rates or ISI<br>Testosterone levels significantly lower compared with baseline in the metformin group at 3 months, but not at 6 months<br>No differences in weight loss between groups, but significant weight change from baseline within the metformin group was observed at 6 months (-3.4 (95% CI: -5.3, -1.5) kg, P<0.05) | NCT00151411 |
| Hoeger (2015)[92] | Overweight/obese adolescents with PCOS<br>N.=36; age: 15.1±1.6 years; BMI at baseline: 38.0±8.0 kg/m$^2$<br>PCOS definition: unknown | Metformin 2 g daily + oral contraceptive (drospirenone/ethinyl estradiol, Yasmin®) + lifestyle intervention | Placebo + oral contraceptive + lifestyle intervention | Pending analysis completion | NCT00283816 |
| Tfayli (2011)[95] | Overweight/obese adolescents with PCOS<br>N.=46; age: 16.0±0.3 years; BMI at baseline: 33.3±5.1 kg/m$^2$<br>PCOS definition: 1990 NIH Criteria | Rosiglitazone 4 mg daily | Drospirenone (DRSP)/ethinyl estradiol (EE) (3 mg/30 µg) daily | Rosiglitazone significantly improved hepatic and peripheral insulin sensitivity<br>Rosiglitazone was effective but inferior to drospirenone/EE in reducing hyperandrogenemia<br>Rosiglitazone led to significantly decreased visceral adiposity, lower fasting and stimulated insulin levels during OGTT, lower triglycerides and higher adiponectin levels<br>DRSP/EE treatment led to increased total cholesterol, high-sensitivity C-reactive protein and leptin concentrations<br>Neither IMT nor PWV did change with any of the drugs | NCT00640224 |
| Frøssing (2018)[96] | Overweight/obese women with PCOS, BMI >25 kg/m$^2$ and/or presence of IR<br>N.=72; age: 29.9 [24.7-34.2] years; BMI at baseline: 33.3±5.1 kg/m$^2$<br>PCOS definition: 2003 Rotterdam Criteria | Liraglutide 1.8 mg/day | Placebo | Liraglutide treatment significantly reduced body weight (-5.6%), liver fat content (-44%), VAT (-18%) and NAFLD prevalence (-75%) - all P<0.01 | NCT02073929 |
| Long (2018)[94] | Overweight/obese women with PCOS, BMI ≥24 kg/m$^2$ and/or waist circumference ≥85 cm<br>N.=30 - expected; age: 14 to 50 years<br>PCOS definition: 2003 Rotterdam Criteria | Metformin 1.5 g + exenatide 10 µg daily OR metformin 1.5 g + liraglutide 1.8 mg daily | Metformin 1.5 g + oral contraceptive (Diane 35®) | Pending | NCT03151005 |

Data are presented as proportions, median [interquartile range] or mean±SD, as appropriate.
GLP-1 RA: glucagon-like peptide receptor agonist; BMI: Body Mass Index; NCT: ClinicalTrials.gov registration number; SHBG: sex hormone binding globulin; DRSP: drospirenone; EE: ethynyl estradiol; IS: insulin sensitivity; IR: insulin resistance; ISI: Insulin Sensitivity Index; HEC: hyperinsulinemic euglycemic clamp; OGTT: oral glucose tolerance test; PWV: pulse wave velocity; IMT: intima media thickness; NAFLD: nonalcoholic fatty liver disease; VAT: visceral adipose tissue.

However, it should be kept in mind that the possible long-term positive effect of metformin on the cardiovascular risk profile shown in other hallmark studies,[97] would play in favor of an early use also in subjects with PCOS, in whom therapeutic LSM are impracticable or ineffective. The evidence of beneficial effects of metformin on hyperandrogenism (including acne alopecia, hirsutism), menses abnormalities, infertility and live birth rates in young women with PCOS is very limited[93, 98] and should be matter of further investigation.[94] Finally, when it comes to weigh benefits against potential side effects, it should be borne in mind that 25% of individuals treated with metformin suffer of gastro-intestinal, dose-dependent side effects (mainly diarrhea and gastric spasms), which, despite proper dose tapering may eventually lead to therapy discontinuation.[99] The mechanisms responsible for metformin intolerance lack of conclusive evidence, although it is hypothesized that high concentrations may induce increased of serotonin production from enterochromaffin cells in the gut, particularly in individuals carrying mutations in the Organic Cation Transporter (OCT) and Serotonin Transporter (SERT) loci.[100, 101]

## Thiazolidinediones

Thiazolidinediones (TZDs) are "insulin sensitizers" that have been demonstrated to be effective in some aspects of PCOS. TZDs are peroxisome proliferator–activated receptor gamma (PPAR-γ) agonists. PPAR-γ represents a nuclear receptor family of ligand-inducible transcription factors primarily expressed in the liver and skeletal muscle and involved in the regulation of adipogenesis, lipid metabolism, cell proliferation, inflammation and insulin sensitivity.[102, 103] When used alone or in combination with estro-progestinic drugs, TZDs have dose-dependent beneficial hormonal and metabolic effects in adult women with PCOS, including improvement of ovulatory dysfunction, hirsutism, hyperandrogenemia, and IR.[104-108] Among the active principles developed so far, troglitazone and rosiglitazone have been removed from the market due to reports of idiosyncratic liver toxicity and increased cardiovascular events.[109] The only TZD available on the market and currently routinely used for the treatment of type 2 diabetes is pioglitazone, which holds similar insulin-sensitizing property without major adverse effects, except an increased rate of drug-induced fluid retention. Some reports have associated the use of pioglitazone with an increased risk osteoporotic fractures and bladder cancer in individuals carrying additional predisposing genetic and environmental co-factors. A teratogenic risk of TZDs prevents their prescription in women desiring pregnancy.[110] As a result, large interventional trials in PCOS are lacking and data on adolescents are limited[95, 111] (Table II). Hence, given the unfavorable risk-benefit ratio, TZDs are currently not recommended for treatment in PCOS.[17] However, they can be considered for use in selected cases of women not seeking pregnancy, intolerant to metformin and with symptoms and signs of PCOS poorly controlled by other established therapies.

## GLP-1 receptor agonists

Glucagon-like peptide 1 receptor agonists (GLP-1 RAs) represent a relatively recent class of drugs, currently approved for the treatment of diabetes mellitus, that exert a glucose-lowering effect by combining reduced gastric emptying, increased satiety, regulation of hypothalamic and cerebral responses to feeding, improvement of glucose-dependent insulin secretion from pancreatic islets and have other multifaceted effects on the liver, skeletal muscle and adipocytes that eventually lead to improved glucose homeostasis and more favorable lipid profiles. Since the earliest registration trials, GLP-1 RAs have been shown to be effective in reducing whole-body IR, primarily due to significant and consistent improvements in body weight and visceral fat content since treatment initiation.[112] Given the beneficial effects on many factors contributing to IR, and having a negligible risk of hypoglycemia, GLP-1 RAs represent an attractive treatment option to manage the various aspects of IR, including PCOS. In a 24-week open label trial involving 60 obese adult women with PCOS, the combination of the GLP-1 RA exenatide (10 μg/day) with metformin (2 g/day) improved menstrual

cyclicity, ovulation rate, androgen levels, insulin sensitivity measures, abdominal fat content and weight loss, as compared with exenatide and metformin used as single therapies.[113] Hormonal and reproductive effects appeared to be mediated by the decreased insulin levels, although mechanistic insights regarding a direct effect of GLP-1 and related gastrointestinal peptides on the hypothalamic-pituitary-adrenal axis have been recently postulated.[40] However, this study had a relatively high drop-out rate (nearly 30%) and the limited duration did not allow to generalize the observations and did not provide any cue on the long-term efficacy of the combined GLP-1 RA-metformin therapy. More recently, add-on liraglutide (1.2 mg/day) has been reported to be effective in reducing body weight in a 12-week, open label, prospective study involving 40 obese, adult women with PCOS, who had been pretreated with metformin for at least 6 months.[114] This study was, however, affected by the limited sample size and the unblinded treatment assignment[96] (Table II). Gastrointestinal intolerance, including nausea and vomiting, represents the most frequent and well-known side effect of GLP-1 RA therapy, which may be improvement by careful stepwise dose titration. The current availability of extended release formulations of GLP-1 RA will represent a new opportunity to explore their effects on metabolic and fertility outcomes of PCOS. Indeed, long-acting GLP-1 RA formulations are characterized by a considerably better tolerability profile, which would reduce side effects and favor overall treatment compliance. The current level of evidence is not sufficient to support a wide use of GLP-1 RA in PCOS, although it seems reasonable to forecast the potential therapeutic use of GLP-1 RA primarily in obese PCOS women.[115] Therefore, further larger double-blinded, randomized trials of longer duration are warranted. Adolescents/young adults unfortunately, still remain under-represented in PCOS trials.

## Inositols

New treatment strategies have been developed to improve insulin sensitivity in PCOS, and among these, inositols are becoming increasingly attractive. Inositols are natural compounds contained in many plants and foods and are mainly synthetized in humans from D-glucose in two inositol stereoisomers: myo-inositol (MYO) and D-chiro-inositol (DCI).[116, 117] MYO is the precursor of the second messenger inositol triphosphate (IP3), which is involved in insulin, follicle-stimulating hormone (FSH) and thyroid-stimulating hormone (TSH) signalling pathways. Moreover, additional roles have been evidenced for MYO as the ability to counteract oxidative stress[118] and reduce ovarian stromal blood flow.[119] Red blood cells (RBCs) from PCOS women have been reported to be more sensitive to oxidative injuries, and an *in-vitro* study showed that some of these abnormalities were restored after 12 weeks of treatment with MYO.[118] DCI is a derivative of MYO which is synthesized via an insulin-dependent pathway by an epimerase.[120, 121] DCI decreases aspartate transaminase (AST), alanine transaminase (ALT), reduces inflammation and attenuates oxidative stress in treated cholestatic rats.[122] MYO and DCI are physiologically present in plasma in a 40:1 ratio[123] and both act as insulin sensitizers.[124] MYO supports cellular glucose intake and DCI promotes glycogen synthesis.[125] An interesting study demonstrated that in the endometrium from insulin-resistant obese PCOS patients, sodium-myoinositol cotransporter-1 (SMIT-1), a specific MYO transporter, was reduced, thus limiting MYO uptake in endometrial cells.[126] In this same study, in an endometrial stromal cell line simulating a PCOS-like condition by exposing the cells to T, insulin and tumor necrosis factor alpha (TNFα), AMPK activation was found to be reduced and was restored after the administration of MYO confirming its insulin-sensitizing effect. Noticeably, also glucose transporter type 4 (GLUT-4) levels, which were decreased in the PCOS-induced condition, were restored after MYO administration.[126] These results were similar to those found using metformin as insulin-sensitizing agent in the same *in-vitro* conditions.[126] As myo-inositol oxygenase (MIOX) is the first enzyme involved in MYO catabolism it was hypothesized that it could be altered in PCOS patients leading to MYO deficiency, however, a recent study reported that this enzyme was unchanged in serum from PCOS subjects excluding its role in MYO deficiency,

opening a view on alternative ways of clearance of MYO from the body.[127] Recent reviews extensively documented the emerging roles of inositols in PCOS treatment.[128-131] Here the most recent clinical and laboratory studies have been reported (Table III).[123, 132-143] A meta-analysis of 10 RCTs, reported the effects of MYO treatment in PCOS in 573 patients showing that MYO improved HOMA-IR, increased E2, and slightly reduced T levels.[132] Another meta-analysis including 6 randomized clinical trials compared MYO with metformin treatment in 355 PCOS patients. The analysis evidenced no differences between the two treatments relative to the effects on fasting insulin, HOMA-IR, BMI, T, SHBG, as well as on other hormonal levels. An increased risk of adverse events was reported in women receiving metformin, and MYO had a better tolerability.[133] Furthermore, a recent study on 53 women with PCOS compared MYO with metformin to evaluate their role in glycemic control, insulin and lipid metabolism. Patients were randomly treated with MYO or metformin for 3 months; fasting plasma glucose, insulin, and HOMA-IR as well as serum triglycerides and VLDL-cholesterol were reduced by MYO treatment. Furthermore, MYO treatment increased PPAR-γ expression, a key regulator of adipocyte differentiation, fat storage, and energy homeostasis.[134] The evaluation of the effects of 4 g MYO+400 μg folic acid alone or in combination with an oral contraceptive pill (OCP) containing drospirenone in a population of 61 adolescents with PCOS led to important results concerning the prevention and correction of metabolic disorders. In particular, the treatment with MYO reduced body weight and BMI, as well as blood glucose, insulin, HOMA-IR, and C-peptide. MYO reduced also hormonal parameters such as T and luteinizing hormone (LH). The treatment with OCP increased slightly weight and BMI, and the combination with MYO and OCP reduced insulin, HOMA-IR and C-peptide. With regard to hormonal parameters, the combined treatment led to a decrease in T, FAI, DHEA-S, LH and AMH and to an increase in SHBG thus enhancing antiandrogenic effects.[135] Besides these results, some PCOS patients seem to be resistant to MYO; in these patients combining MYO with alpha-lactalbumin (α-LA), a molecule that increases MYO bioavailability, seems to be promising. One study reports that in 14 out of 37 PCOS patients resistant to treatment, the association of MYO (2 g) with α-LA (50 mg) twice daily for 3 months restored ovulation in 86% of the patients.[136]

MYO has been studied in combination with tyrosine, selenium and chromium treatments also, and the efficacy has been reported in relationship with the different PCOS phenotypes.[137] In detail, in this study, 186 PCOS patients were divided in 4 groups based on clinical and biochemical parameters. The administration of MYO (2 g), L-tyrosine (0.5 mg), folic acid (0.2 mg), selenium (55 μg), and chromium (40 μg) daily for 6 months restored ovulation in 65% of the patients belonging to the phenotypic group characterized by androgen excess, ovulatory dysfunction and PCOS morphology. Glucose and HOMA-IR improved too. In the group with androgen excess and ovulatory dysfunction, 80% of patients ovulated after six months on treatment and clinical and biochemical hyperandrogenism improved. In the group characterized by androgen excess and PCOS morphology a decrease in BMI was observed also. Finally, in the group with ovulatory dysfunction and PCOS morphology, 60% of the women regularized their menstrual periods and ovulated. The combined daily treatment of MYO (2 g), with gymnemic acid (75 mg) and L-methylfolate (400 μg) was compared with treatment with MYO (2 g) and folic acid (400 μg) in 100 PCOS patients for 6 months. Gymnemic acid is structurally similar to glucose and is involved in glucose homeostasis.[138] L-methylfolate is a natural folate that is used to reduce hyperhomocysteinemia and increases insulin sensitivity.[138] The combined treatment was more effective in improving BMI, menstrual cycle regularity, glycemia and insulinemia. Furthermore, the combination decreased T, total cholesterol and homocysteine levels while SHBG increased.[138] The administration of MYO in combination with DCI in different ratios has been also evaluated. This treatment was hypothesized based on the belief that in the PCOS ovary the MYO/DCI ratio is altered.[120, 121] Since the insulin-responsive tissues present a specific ratio MYO/DCI, the physiological balance might be restored.[124] The most recent studies concerning the combined treat-

TABLE III.—*Main findings relative to studies using inositols as single treatment or in association with other treatments.*[123, 132-143]

| Study | Subjects | Drug | Comparator | Main results |
|---|---|---|---|---|
| Zeng (2018)[132] | Normal/overweight/obese PCOS patients included in 10 randomized controlled trials<br>N.=573; age: from 13 to 38 years<br>BMI >30 kg/m$^2$ (3 studies)<br>25 <BMI<30 kg/m$^2$ (3 studies)<br>BMI <25 kg/m$^2$ (3 studies)<br>BMI not reported (1 study)<br>PCOS definition: unknown | MYO 0.55 g + DCI 0.0138 g + folic acid 200 µg twice daily OR MYO from 1.2 g to 4 g + folic acid from 200 µg to 400 µg daily OR MYO from 0.2 g to 2 g OR MYO 4 g + folic acid 400 µg + DRSP 3 mg/ EE 30 µg daily | Placebo OR folic acid from 200 µg to 400 µg daily OR metformin 1.5 g daily OR DRSP 3 mg/ EE 30 µg daily | Improvement in HOMA-IR<br>Increase in E2 levels<br>Slight reduction in testosterone levels |
| Facchinetti (2018)[133] | Normal/overweight PCOS patients included in 6 randomized controlled trials<br>N.=355; age: from 15 to 45 years<br>Mean BMI >25 kg/m$^2$ except for 1 study on normal-weighted subjects<br>PCOS definition: 2003 Rotterdam Criteria or Androgen Excess Society Guidelines | MYO from 2 g to 4 g daily | Metformin from 1.5 g to 2 g daily | No differences between the two treatments relative to fasting insulin, HOMA-IR, BMI, testosterone, SHBG and other hormonal levels<br>Increased risk of adverse side effects under MET treatment<br>Better tolerability for MYO |
| Shokrpour (2019)[134] | Overweight PCOS patients<br>N.=53; age: from 18 to 40 years<br>BMI at baseline:<br>MYO group: 28.1±3.1 kg/m$^2$<br>MET group: 27.3±3.3 kg/m$^2$<br>PCOS definition: 2003 Rotterdam Criteria | MYO 4 g + folic acid 400 µg daily | Metformin 1.5 g daily | Reduction in FPG, insulin, HOMA-IR, serum triglycerides and VLDL-cholesterol and increase in PPAR-γ expression levels under MYO treatment |
| Pkhaladze (2016)[135] | Normal/slightly overweight adolescents with PCOS<br>N.=61; age: from 13 to 19 years<br>BMI at baseline:<br>MYO group: 22.3±3.08 kg/m$^2$<br>MYO+OCP group: 22.24±3.26 kg/m$^2$<br>OCP group: 22.74±3.75 kg/m$^2$<br>PCOS definition: 2003 Rotterdam Criteria | MYO 4 g + folic acid 400 µg daily<br>OR<br>MYO 4 g + folic acid 400 µg + DRSP 3 mg/ EE 30 µg daily | DRSP 3 mg/ EE 30 µg daily | Reduction of weight, BMI, blood glucose, insulin, HOMA-IR, C-peptide, testosterone, LH under MYO treatment alone<br>Slight increase in weight and BMI under DRSP/EE treatment<br>Reduction of insulin, HOMA-IR and C-peptide, testosterone, FAI, DHEA-S, LH, AMH, and increase in SHBG under combined treatment with MYO+ DRSP/EE |
| Montanino Oliva (2018)[136] | Overweight PCOS patients<br>N.=37; age: 27.1±2.4 years<br>BMI: 25.9±1.2 kg/m$^2$<br>PCOS definition: 2003 Rotterdam Criteria | MYO 4 g + α-lactalbumin 100 mg daily | MYO 4 g daily | Restoration of ovulation in 86% of the patients resistant to MYO alone treatment |
| Montanino Oliva (2019)[137] | Overweight PCOS patients<br>N.=186; age: from 16 to 38 years<br>BMI at baseline:<br>Phenotype A: 26.52±3.67 kg/m$^2$<br>Phenotype B: 26.39±5.09 kg/m$^2$<br>Phenotype C: 25.84±3.13 kg/m$^2$<br>Phenotype D: 26.52±2.98 kg/m$^2$<br>PCOS definition: 2003 Rotterdam Criteria | MYO 2 g + L-tyrosine 0.5mg + folic acid 0.2 mg + selenium 5 µg + chromium 40 µg daily | Baseline with no treatment | Phenotype A (androgen excess, ovulatory dysfunction, PCO morphology): restoration of ovulation in 65% patients, improvement in glucose levels and HOMA-IR<br>Phenotype B (androgen excess, ovulatory dysfunction): restoration of ovulation in 80% patients, improvement in clinical and biochemical hyperandrogenism<br>Phenotype C (androgen excess, PCO morphology): decrease in BMI<br>Phenotype D (ovulatory dysfunction, PCO morphology): restoration of ovulation in 60% patients |

*(To be continued)*

TABLE III.—*Main findings relative to studies using inositols as single treatment or in association with other treatments.*[123, 132-143] *(continues).*

| Study | Subjects | Drug | Comparator | Main results |
|---|---|---|---|---|
| Stracquadanio (2018)[138] | Overweight PCOS patients<br>N.=100<br>Age:<br>MYO+gym+L-met group: 27±3 years<br>MYO group: 28±4 years<br>BMI at baseline:<br>MYO+gym+L-met group: 27±0.8 kg/m$^2$<br>MYO group: 27±0.9 kg/m$^2$<br>PCOS definition: 2003 Rotterdam Criteria | MYO 2 g + gymnemic acid 75 mg + L-methylfolate 400 µg | MYO 2g + folic acid 400 µg | Greater effectiveness of the combination in improving BMI, menstrual cycles, glycemia, and insulinemia<br>Reduction of T, total cholesterol and homocysteine levels and increased SHBG |
| Januszewski (2019)[139] | Overweight/obese PCOS patients<br>N.=70; age: 28.4±5.1 years<br>BMI at baseline: >25 kg/m$^2$<br>PCOS definition: 2003 Rotterdam Criteria | MYO:DCI in a 10:1 ratio daily (total of 1100 g inositols) + of folic acid 400 µg + vitamin D 1000 IU + vitamin $B_6$ 1.4 mg + vitamin $B_5$ 6 mg + vitamin $B_{12}$ 2.5 µg twice a day | Baseline with no treatment | Reduction in body weight, T, FSH, LH, insulin, glucose levels<br>Increase in SHBG<br>Improvement of skin conditions |
| Benelli (2016)[123] | Obese young PCOS patients<br>N.=46<br>Age:<br>treated group: 23±6.8 years<br>placebo group: 25±7.3 years<br>BMI at baseline:<br>Treated group: 32±4.8 kg/m$^2$<br>Placebo group: 31±4.6 kg/m$^2$<br>PCOS definition: 2003 Rotterdam Criteria | MYO:DCI in a 40:1 ratio (MYO 550 mg + DCI 13.8 mg + 200 µg folic acid twice a day | Placebo: folic acid 200 µg twice a day | Reduction in fasting insulin, HOMA-IR, LH, and T<br>Increase in 17-β-estradiol and SHBG |
| Nordio (2019)[140] | Normal weighted PCOS patients<br>N.=56<br>Age:<br>0:1 group: 30.5±2.9 years<br>1:3.5 group: 28.9±3.4 years; 2.5:1 group: 29.3±3.1 years<br>5:1 group: 31.2±2.7 years<br>20:1 group: 30.6±3.0 years<br>40:1 group: 31.1±3.2 years<br>80:1 group: 29.7±2.8 years<br>BMI at baseline:<br>0:1 group: 23.48±3.0 kg/m$^2$<br>1:3.5 group: 24.52±2.8 kg/m$^2$<br>2.5:1 group: 22.87±2.9 kg/m$^2$<br>5:1 group: 24.12±2.7 kg/m$^2$<br>20:1 group: 23.21±3.1 kg/m$^2$<br>40:1 group: 24.08±3.0 kg/m$^2$<br>80:1 group: 22.98±2.9 kg/m$^2$<br>PCOS definition: 2003 Rotterdam Criteria | MYO/DCI 2 g in 7 different ratios: 0:1, 1.3:5, 2.5:1, 5:1, 20:1, 40:1, 80:1 twice a day | NR | The 40:1 ratio was the most effective in decreasing LH, postprandial insulin levels and increasing SHBG, E2, progesterone, and normalizing ovulation<br>A modification of the ratio in favor of DCI reduced the beneficial effects of treatment |
| Troisi (2019)[141] | Young overweight PCOS patients<br>N.=30<br>Age:<br>PCOS group: 19.7±1.9 years<br>CTRL group: 21.9±2.9 years<br>BMI at baseline:<br>PCOS group: 28.4±1.7 kg/m$^2$<br>CTRL group: 21.6±1.6 kg/m$^2$<br>PCOS definition: 2003 Rotterdam Criteria and adolescent criteria for the diagnosis of PCOS | MYO 1.75 g + DCI 0.25 g + glucomannan 4 g daily | Baseline with no treatment OR control group | Fifteen metabolites mainly involved in carbohydrate and lipid metabolic pathways were identified and discriminated PCOS from CTRL at baseline<br>A subgroup of these metabolites showed changes in PCOS patients after treatment<br>Improvement in metabolism, BMI, menstrual cycles, the number of antral follicles, ovary volume, hirsutism, and acne under the combined treatment |

*(To be continued)*

TABLE III.—*Main findings relative to studies using inositols as single treatment or in association with other treatments.*[123, 132-143] *(continues).*

| Study | Subjects | Drug | Comparator | Main results |
|---|---|---|---|---|
| Advani (2020)[142] | Lean/obese women with PCOS<br>N.=51<br>Age:<br>*Lean group*<br><21 yr: 17.50±0.50 years<br>21-25 years: 22.50±0.50 years<br>26-35 years: 30.00±0.88 years<br>>35 years: 36.50±0.50 years<br>*Obese group*<br><21 years: 19.00±0.57 years<br>21-25 years: 23.00±0.70 years<br>26-35 years: 29.38±0.47 years<br>>35 years: 36.50±0.50 years<br>BMI at baseline:<br>Lean group: 21.88±0.30 kg/m$^2$<br>Obese group: 27.03±0.56 kg/m$^2$<br>PCOS definition: 2003 Rotterdam Criteria | MYO:DCI 600 mg + NAC 300 mg + Biotin 5 mg + 10% lycopene 5 mg + chromium picolinate 200 µg + folic acid 120 µg + vitamin D 400 IU twice a day | Baseline with no treatment | Reduction in body weight and BMI in obese subjects<br>Reduction of hirsutism and acne in both obese and lean PCOS patients |
| Le Donne (2019)[143] | Overweight/obese PCOS patients<br>N.=43<br>Age:<br>Only diet group: 29.7±0.8 years<br>Diet+MYO+folic acid group: 25.5±3.4 years<br>Diet+MYO+DCI+folic acid group: 24.1±5.1 years<br>BMI at baseline:<br>Only diet group: 31.9±5.2 kg/m$^2$<br>Diet+MYO+folic acid group: 32.4±5.5 kg/m$^2$<br>Diet+MYO+DCI+folic acid group: 31.8±6 kg/m$^2$<br>PCOS definition: 2003 Rotterdam Criteria | MYO 1.1 g + DCI 27.6 mg + folic acid 400 µg + controlled diet daily | Controlled diet only<br>OR MYO 4 g + folic acid 400 µg + controlled diet daily | Improvement in terms of body weight, BMI, waist and hip circumferences in all groups<br>Restoration of menstrual cyclicity in the group treated with the combined treatment MYO + DCI + folic acid + controlled diet |

PCOS: polycystic ovary syndrome; BMI: Body Mass Index; MYO: myo-inositol; DCI: D-chiro-inositol; DRSP: drospirenone; EE: ethinyl estradiol; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; E2: 17-β-estradiol; SHBG: sex hormone binding globulin; MET: metformin; FPG: fasting blood glucose; VLDL: very low-density lipoprotein; PPAR-γ: peroxisome proliferator activated receptor gamma; OCP: oral contraceptive pill; FAI: Free Androgen Index; DHEA-S: dehydroepiandrosterone sulfate; AMH: anti-Müllerian hormone; gym: gymnemic acid; L-met: L-methylfolate; T: testosterone.

ment are reported hereafter. The combination of MYO and DCI in a 10:1 ratio has been reported to be effective in improving metabolic and hormonal parameters in 70 women with PCOS after 6 months on treatment.[139] In particular, a significant reduction in body weight, as well as a decrease in T, FSH and LH levels was reported after 3 months on treatment and was confirmed after 6. Furthermore, SHBG increased after 6 months on treatment while insulin and glucose levels decreased. The treatment improved skin conditions also.[139] In a group of 46 young obese PCOS an improvement of hormonal and metabolic parameters was observed. In detail, the combined treatment with MYO and DCI in a 40:1 ratio for six months lead to a reduction in fasting insulin, HOMA-IR, LH, and T, and an increase in 17-β-estradiol (E2) and SHBG.[123] To better clarify the effectiveness of different ratios between MYO and DCI a recent study evaluated 7 different combinations of ratios.[140] A population of 56 patients was subdivided into 7 groups treated with a MYO/DCI ratio of 0:1, 1:3.5, 2.5:1, 5:1, 20:1, 40:1, 80:1, respectively given twice daily for 3 months. The most effective formulation was the 40:1 ratio MYO/DCI which decreased LH, and increased SHBG, E2, progesterone, and normalized ovulation. Furthermore, the 40:1 ratio decreased basal and postprandial insulin levels. Interestingly, a modification of the ratio in favor of DCI determined a reduction in the beneficial effects of treatment.[140] These results were consistent with findings obtained in a PCOS mouse models where the 40:1 ratio was the most

effective in reverting PCOS phenotype in terms of theca/granulosa cell layer thickness.[144] A combined treatment of MYO (1.75 g), DCI (0.25 g) and glucomannan (4 g) daily for 3 months was investigated in a population of PCOS patients using a metabolomic approach. Glucomannan is a vegetal compound which inhibits cholesterol synthesis in liver and enhances its clearance, and in addition gives a feeling of satiety. A group of 15 metabolites, mainly involved in carbohydrate and lipid metabolic pathways, were identified and discriminated cases from controls at baseline, and a subgroup of these metabolites showed changes in the PCOS patients after treatment. Furthermore, the combined treatment improved metabolism, BMI, menstrual cycles, the number of antral follicles, ovary volume, hirsutism and acne.[141] MYO and DCI in combination with antioxidants (N-acetylcysteine and lycopene), chromium picolinate and vitamins (vitamin D, biotin and folic acid) were used in a very recent observational study. These compounds were administered to 35 obese and 16 lean PCOS patients revealing a reduction in body weight and BMI in obese subjects. Furthermore, this treatment reduced hirsutism and acne both in lean and obese PCOS patients.[142] Other studies have focused more on clinical and body composition outcomes in PCOS associated with overweight and obesity. A recent study in 43 PCOS patients, subdivided the women in 3 groups that were re-evaluated after 6 months of treatment: the first group underwent a controlled diet only, the second group was treated with controlled diet and MYO (4 g) and folic acid (400 μg) daily in addition, and the third group with controlled diet and MYO (1.1 g), DCI (27.6 mg) and folic acid (400 μg) daily in association. Body composition in terms of body weight, BMI, waist and hip circumferences improved in all groups. However, only the administration of the combined treatment MYO+DCI was effective in the restoration of menstrual cyclicity.[143]

## Alpha-lipoic acid

Emerging data in the Literature support alpha-lipoic acid (ALA) as another compound effective in treating insulin resistant conditions, as PCOS often is on a systemic basis. PCOS is also characterized by chronic low-grade inflammation and patients have abnormal high levels of reactive oxygen species (ROS).[145] Increased oxidant status appears to worsen in IR state.[146] ALA is a potent natural antioxidant lipophilic compound, produced by plants and animals, and is a catalytic factor for the oxidative decarboxylation of pyruvate and α-ketoglutarate. It acts as a pivotal cofactor for mitochondrial enzymes activity and favors the efficacy of other antioxidants such as glutathione.[147] ALA, similarly to insulin, plays a role in the regulation of glucose and lipid metabolism by stimulating glucose uptake through an intracellular redistribution of glucose transporter type 1 (GLUT-1) and GLUT-4 glucose transporters.[148] ALA has been demonstrated to stimulate glucose utilization through the increase of AMP) in skeletal muscles increasing GLUT-4.[149, 150] Growing evidence suggests that ALA may improve reproductive function and metabolic parameters (Table IV).[146, 151-157] A controlled-release ALA preparation at a high daily dosage of 1200 mg (600 mg twice daily) was administered for 16 weeks to normal weight (BMI: 18.5 to 26.6 kg/m$^2$), non-diabetic PCOS young women (age 23 to 34 years). A significant improvement in insulin sensitivity was described by the euglycemic, hyperinsulinemic clamp gold standard technique, and an improvement in menstrual cyclicity was reported also. A significant reduction in triglyceride levels was further reported, although no change in the distribution of the lipoprotein fractions was measured. Moreover, no change in circulating oxidative stress markers was detected.[151] In a recent observational study ALA was administered at a low dosage (400 mg daily) for 12 weeks in obese (BMI >25 kg/m$^2$) young PCOS patients (24.5±1.3 years). Among these patients, about 62% reported to have a type 1 or 2 diabetes familiarity. Treatment reduced significantly insulin, glucose, BMI and HOMA-IR. In particular, the PCOS patients with diabetic relatives showed a reduction in triglycerides and AST as well. Interestingly, in all PCOS patients no changes occurred on all hormonal parameters associated with fertility such as LH, FSH, and androstenedione.[152] Administration of ALA combined with inositols was recently used

TABLE IV.—*Main findings relative to studies using alpha-lipoic acid as single treatment or in association with inositols.*[146, 151-157]

| Study | Subjects | Drug | Comparator | Main results |
|---|---|---|---|---|
| Masharani (2010)[151] | Non-obese women with PCOS<br>N.=6<br>Age: from 23 to 34 years<br>BMI at baseline: from 18.5 to 26.6 kg/m$^2$; mean 22±1.4 kg/m$^2$<br>PCOS definition: Rotterdam Criteria | CRLA 600 mg twice daily for 16 weeks | Baseline with no treatment | Improvement in insulin sensitivity and in menstrual cyclicity. Reduction in triglyceride levels. No change in the distribution of the lipoprotein fractions and in circulating oxidative stress markers |
| Genazzani (2018)[152] | Obese PCOS patients with PCOS<br>N.=32<br>Age: 24.5±1.3 years<br>BMI at baseline: 32.5±1.9 kg/m$^2$<br>PCOS definition: American Society for Reproductive Medicine and the European Society for Human Reproduction and Embryology consensus meeting to diagnose the presence of PCOS | ALA 400 mg *per os* every day for 12 weeks | Baseline with no treatment | Insulin, glucose, BMI and HOMA-IR were reduced. A reduction in triglycerides and AST. No changes occurred on all hormonal parameters associated with fertility such as LH, FSH, and androstenedione |
| Cianci (2015)[146] | Women of reproductive age with vs without PCOS<br>N.=46: 26 *vs.* 20<br>Age: from 16 to 32 years; mean 23.8±2.5 years<br>BMI at baseline: 33.3±5.1 kg/m$^2$<br>PCOS definition: Rotterdam Criteria | DCI 1000 mg + ALA 600 mg daily OR untreated<br>Treatment was taken for 180 days | Baseline with no treatment AND non-PCOS patients | Improvement in HOMA-IR Index, insulin levels, frequency of menstrual cycles and ovulation. Reduction in ovarian cyst count and BMI |
| Fruzzetti (2019)[153] | Women of reproductive age with *vs.* without PCOS.<br>N.=71: 41 *vs.* 30<br>Age: from 18 to 30 years; mean 22.3±5.0 *vs.* 23.1±5.4 years<br>BMI at baseline: 26.2±5.3 *vs.* 27.2±3.9 kg/m$^2$<br>PCOS definition: Rotterdam Criteria | ALA 600 mg + DCI 1000 mg daily for 180 days | Baseline with no treatment AND non-PCOS patients | Cycle length reduced after treatment. BMI, after an initial reduction at 6 months on treatment, returned to the baseline. HOMA-IR and fasting insulin unchanged, but insulin response to OGTT improved |
| De Cicco (2017)[154] | Overweight–obese women with PCOS<br>N.=40<br>Age: from 18 to 35 years; mean 25.57±5.72 years<br>BMI at baseline: 30.03±4.47 kg/m$^2$<br>PCOS definition: Rotterdam Consensus Conference Criteria | ALA 800 mg + MYO 2000 mg daily for six months. | Baseline with no treatment | Improved frequency of menses. Free androgen index, mean androstenedione and DHEAS levels were lowered, SHBG levels increased, hirsutism and BMI improved, AMH, ovarian volume and number of total antral follicular diminished. No change in glucose, insulin or lipid parameters was observed |
| Fruzzetti (2020)[155] | Women of reproductive age with vs without PCOS<br>N.=74: 44 *vs.* 30<br>Age: 21±4.7 *vs.* 23.1±5.4 years<br>BMI at baseline: 27.05±4.17 *vs.* 27.17±3.93 kg/m$^2$<br>PCOS definition: Rotterdam Criteria | ALA 800 mg + MYO 2000 mg per day divided into two oral administrations for at least 6 months | Baseline with no treatment AND non-PCOS patients | Improvement in menstrual cycle length, restoration of ovulation, reduction in BMI. No change in the HOMA-IR index, and in insulin levels. In the women with insulin resistance, HOMA-IR and insulin decreased at variance with the women with normal insulin sensitivity |
| Genazzani (2019)[156] | Women of reproductive age with PCOS<br>Group A: N.=24; BMI at baseline: 28.4±1.7 kg/m$^2$<br>Group B: N.=24; BMI at baseline: 32.8±1.7 kg/m$^2$<br>Group C: N.=28; BMI at baseline: 29.8±1.5 kg/m$^2$<br>Age: 25.6±1.3 years<br>PCOS definition: ESHRE Criteria | Group A: MYO 1 g/day *per os* for 12 weeks<br>Group B: ALA 400 mg/day *per os* for 12 weeks<br>Group C: MYO 1 g/day + ALA 400 mg/day *per os* for 12 weeks | Baseline with no treatment | MYO improved reproductive hormones and reduced insulin levels in response to OGTT in PCOS patients with no family history of diabetes; ALA reduced insulin levels in response to OGTT and metabolic parameters; MYO + ALA improved both hormonal and metabolic parameters and reduced insulin levels in response to OGTT in all patients |
| Cirillo (2020)[157] | Women of reproductive age with *vs.* without PCOS<br>N.=44: 23 *vs.* 21<br>Age: 17.22±0.72 *vs.* 16.97±0.63 years<br>BMI at baseline: 27.51±1.15 *vs.* 23.19±1.52 kg/m$^2$<br>PCOS definition: Rotterdam Criteria | ALA 400 mg + MYO 1 gr twice daily for 3 months and once daily for further 3 months | Baseline with no treatment AND non-PCOS patients | HMGB1 increased in PCOS and normalized after treatment. Treatment reduced insulin, HOMA-IR and 17-OHP |

PCOS: polycystic ovary syndrome; BMI: Body Mass Index; CRLA: controlled-release alpha-lipoic acid; ALA: alpha-lipoic acid; MYO: myo-inositol; DCI: D-chiro-inositol; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; AST: aspartate aminotransferase: LH: luteinizing hormone: FSH: follicle-stimulating hormone: DHEA-S: dehydroepiandrosterone sulfate; AMH: anti-Müllerian hormone; OGTT: oral glucose tolerance test; 17-OHP: 17-hydroxyprogesterone; SHBG: sex hormone binding globulin.

as a dietary supplement in insulin-resistant patients as both increase insulin sensitivity (Table IV). In a long-term prospective study, a combination of ALA and DCI (600 mg ALA and 1000 mg DCI) was administered for 6 months daily to PCOS women of reproductive age. Following treatment, a significant improvement in the HOMA-IR index and insulin levels was observed in addition with improvement in the frequency of menstrual cycles and ovulation. In addition, a reduction in ovarian cyst count and BMI was reported.[146] A further recent study used the same combination of ALA and DCI as in this previous study, including a heathy control group matched for age and BMI, and observed an improvement in menstrual cycle length, restored ovulation in most, a reduction in BMI but no significant change in the HOMA-IR index, and in insulin levels. However, in the women with IR, HOMA-IR and insulin decreased significantly at variance with the women with normal insulin sensitivity.[153] A pilot cohort study used a combination of ALA and MYO (800 mg ALA and 2000 mg MYO) given daily for six months to overweight-obese PCOS women only. Following treatment PCOS patients reported a significant increase in the frequency of menses. The free androgen index (FAI), the mean androstenedione and DHEA-S levels were lowered significantly, SHBG levels significantly increased, hirsutism and BMI improved, AMH, ovarian volume and number of total antral follicular diminished. No change in glucose, insulin or lipid parameters was observed.[154] A further long-term retrospective study reported on the effects of a combined treatment with ALA and MYO (800 mg of ALA + 2000 mg of MYO) administered daily for 24 months to a group of young normal weight PCOS women. A healthy control group matched for age and BMI was included. Cycle length was significantly reduced after treatment. BMI, after an initial reduction at 6 months on treatment, returned to the baseline levels; HOMA-IR and fasting insulin were unchanged, but the insulin response to oral glucose tolerance test (OGTT) was improved.[155] In a recent retrospective study, the effects of ALA alone (400 mg daily), MYO alone (1 g daily) and a combination of ALA + MYO (400 mg of ALA + 1 g of MYO daily) were evaluated in overweight/obese young PCOS patients after 3 months of treatment. MYO improved reproductive hormones and reduced insulin levels in response to OGTT in PCOS patients with no family history of diabetes; ALA reduced insulin levels in response to OGTT and metabolic parameters in all patients; MYO + ALA improved both hormonal and metabolic parameters and reduced insulin levels in response to OGTT in all patients.[156] In a further recent observational study, a combination of ALA (400 mg) + MYO (1 g) taken twice daily for 3 months and once daily for further 3 months was administered to adolescents with PCOS. This combined treatment was effective on high mobility group box 1 (HMGB1), a small protein involved in both inflammation and hyperglycemia/IR. In particular, HMGB1 was increased in PCOS compared with controls and normalized after treatment. Moreover, the treatment reduced insulin, HOMA-IR and 17-hydroxyprogesterone.[157] Globally, data relative to the use of ALA alone or in combination with inositols are encouraging, however, data are still scarce and incomplete as the few studies differ with regard to study population (age, BMI), dosage, type and associations of molecules containing ALA. Very few studies, have been conducted in adolescents. Most studies include adult women with an age range over 18 years. Additional studies are required on adolescent women, in order to assess the effectiveness of diet supplements (nutraceutical approach) in preventing negative impacts of PCOS on fertility in adult age.

## Conclusions

Current treatment of PCOS is increasingly moving towards treatments that consider the mechanisms leading to the clinical and biochemical changes that characterize and complicate over time this syndrome. Thus, whereas initially the correction of symptoms with LSM interventions and the use of OCs represented the mainstay, the use of metformin and new insulin sensitizers, and among these latter nutraceutical agents is increasing and promising as they have an effect both on insulin sensitivity and inflammation, and ultimately on androgen production. There

is increasing evidence for the use of nutraceutical agents that are devoid of the side-effects. Unfortunately, we are still missing significant studies in adolescents. This represents the most important age, as if PCOS were fully understood, treatment acting on the etiological causes would be more effective and would prevent full-blown pictures and the subsequent risk of infertility.

## References


**1.** Escobar-Morreale HF. Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. Nat Rev Endocrinol 2018;14:270–84.

**2.** The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long term health risks related to polycystic ovary syndrome (PCOS). The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group. Hum Reprod 2004;19:41–7.

**3.** Lizneva D, Suturina L, Walker W, Brakta S, Gavrilova-Jordan L, Azziz R. Criteria, prevalence, and phenotypes of polycystic ovary syndrome. Fertil Steril 2016;106:6–15.

**4.** Yildiz BO, Bozdag G, Yapici Z, Esinler I, Yarali H. Prevalence, phenotype and cardiometabolic risk of polycystic ovary syndrome under different diagnostic criteria. Hum Reprod 2012;27:3067–73.

**5.** Boyle JA, Cunningham J, O'Dea K, Dunbar T, Norman RJ. Prevalence of polycystic ovary syndrome in a sample of Indigenous women in Darwin, Australia. Med J Aust 2012;196:62–6.

**6.** Escobar-Morreale HF, Carmina E, Dewailly D, Gambineri A, Kelestimur F, Moghetti P, *et al.* Epidemiology, diagnosis and management of hirsutism: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome Society. Hum Reprod Update 2012;18:146–70.

**7.** Moghetti P, Toscano V. Treatment of hirsutism and acne in hyperandrogenism. Best Pract Res Clin Endocrinol Metab 2006;20:221–34.

**8.** Carmina E, Azziz R, Bergfeld W, Escobar-Morreale HF, Futterweit W, Huddleston H, *et al.* Female pattern hair loss and androgen excess: A report from the multidisciplinary Androgen Excess and PCOS Committee. J Clin Endocrinol Metab 2019;104:2875–91.

**9.** Tosi F, Fiers T, Kaufman JM, Dall'Alda M, Moretta R, Giagulli VA, *et al.* Implications of androgen assay accuracy in the phenotyping of women with polycystic ovary syndrome. J Clin Endocrinol Metab 2016;101:610–8.

**10.** Dewailly D, Lujan ME, Carmina E, Cedars MI, Laven J, Norman RJ, *et al.* Definition and significance of polycystic ovarian morphology: a task force report from the Androgen Excess and Polycystic Ovary Syndrome Society. Hum Reprod Update 2014;20:334–52.

**11.** Teede H, Misso M, Tassone EC, Dewailly D, Ng EH, Azziz R, *et al.* Anti-Müllerian Hormone in PCOS: A review informing international guidelines. Trends Endocrinol Metab 2019;30:467–78.

**12.** Apter D, Sipilä I. Development of children and adolescents: physiological, pathophysiological, and therapeutic aspects. Curr Opin Obstet Gynecol 1993;5:764–73.

**13.** Fanelli F, Gambineri A, Belluomo I, Repaci A, Di Lallo VD, Di Dalmazi G, *et al.* Androgen profiling by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in healthy normal-weight ovulatory and anovulatory late adolescent and young women. J Clin Endocrinol Metab 2013;98:3058–67.

**14.** Codner E, Villarroel C, Eyzaguirre FC, López P, Merino PM, Pérez-Bravo F, *et al.* Polycystic ovarian morphology in postmenarchal adolescents. Fertil Steril 2011;95:702–6.e1, 2.

**15.** Carmina E, Oberfield SE, Lobo RA. The diagnosis of polycystic ovary syndrome in adolescents. Am J Obstet Gynecol 2010;203:201.e1–5.

**16.** Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, *et al.* 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.e25.

**17.** Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, *et al.*; Endocrine Society. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2013;98:4565–92.

**18.** Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, *et al.*; International PCOS Network. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod 2018;33:1602–18.

**19.** Ibáñez L, Oberfield SE, Witchel S, Auchus RJ, Chang RJ, Codner E, *et al.* An international consortium update: pathophysiology, diagnosis, and treatment of polycystic ovarian syndrome in adolescence. Horm Res Paediatr 2017;88:371–95.

**20.** Witchel SF, Oberfield SE, Peña AS. Polycystic Ovary Syndrome: Pathophysiology, Presentation, and Treatment With Emphasis on Adolescent Girls. J Endocr Soc 2019;3:1545–73.

**21.** Lim SS, Davies MJ, Norman RJ, Moran LJ. Overweight, obesity and central obesity in women with polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update 2012;18:618–37.

**22.** Tosi F, Di Sarra D, Kaufman JM, Bonin C, Moretta R, Bonora E, *et al.* Total body fat and central fat mass independently predict insulin resistance but not hyperandrogenemia in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2015;100:661–9.

**23.** Ezeh U, Yildiz BO, Azziz R. Referral bias in defining the phenotype and prevalence of obesity in polycystic ovary syndrome. J Clin Endocrinol Metab 2013;98:E1088–96.

**24.** Tosi F, Bonora E, Moghetti P. Insulin resistance in a large cohort of women with polycystic ovary syndrome: a comparison between euglycaemic-hyperinsulinaemic clamp and surrogate indexes. Hum Reprod 2017;32:2515–21.

**25.** Cassar S, Misso ML, Hopkins WG, Shaw CS, Teede HJ, Stepto NK. Insulin resistance in polycystic ovary syndrome: a systematic review and meta-analysis of euglycaemic-hyperinsulinaemic clamp studies. Hum Reprod 2016;31:2619–31.

**26.** Tosi F, Negri C, Perrone F, Dorizzi R, Castello R, Bonora E, *et al.* Hyperinsulinemia amplifies GnRH agonist stimulated ovarian steroid secretion in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2012;97:1712–9.

**27.** Tosi F, Negri C, Brun E, Castello R, Faccini G, Bonora E, *et al.* Insulin enhances ACTH-stimulated androgen and glucocorticoid metabolism in hyperandrogenic women. Eur J Endocrinol 2011;164:197–203.

**28.** Nestler JE, Powers LP, Matt DW, Steingold KA, Plymate SR, Rittmaster RS, *et al.* A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese wom-

en with the polycystic ovary syndrome. J Clin Endocrinol Metab 1991;72:83–9.

**29.** Stepto NK, Moreno-Asso A, McIlvenna LC, Walters KA, Rodgers RJ. Molecular Mechanisms of Insulin Resistance in Polycystic Ovary Syndrome: Unraveling the Conundrum in Skeletal Muscle? J Clin Endocrinol Metab 2019;104:5372–81.

**30.** Johnson T, Kaplan L, Ouyang P, Rizza P. National Institutes of Health evidence-based methodology workshop on polycystic ovary syndrome. NIH EbMW Reports. Bethesda, MD: National Institutes of Health; 2012. p. 1–14.

**31.** Moghetti P, Tosi F, Bonin C, Di Sarra D, Fiers T, Kaufman JM, *et al.* Divergences in insulin resistance between the different phenotypes of the polycystic ovary syndrome. J Clin Endocrinol Metab 2013;98:E628–37.

**32.** Moghetti P, Tosi F, Castello R, Magnani CM, Negri C, Brun E, *et al.* The insulin resistance in women with hyperandrogenism is partially reversed by antiandrogen treatment: evidence that androgens impair insulin action in women. J Clin Endocrinol Metab 1996;81:952–60.

**33.** Holmäng A, Svedberg J, Jennische E, Björntorp P. Effects of testosterone on muscle insulin sensitivity and morphology in female rats. Am J Physiol 1990;259:E555–60.

**34.** Tosi F, Dal Molin F, Zamboni F, Saggiorato E, Salvagno GL, Fiers T, *et al.* Serum androgens are independent predictors of insulin clearance but not of insulin secretion in women with PCOS. J Clin Endocrinol Metab 2020;105:dgaa095. [Epub ahead of print]

**35.** Buffington CK, Kitabchi AE. Evidence for a defect in insulin metabolism in hyperandrogenic women with polycystic ovarian syndrome. Metabolism 1994;43:1367–72.

**36.** Coviello AD, Legro RS, Dunaif A. Adolescent girls with polycystic ovary syndrome have an increased risk of the metabolic syndrome associated with increasing androgen levels independent of obesity and insulin resistance. J Clin Endocrinol Metab 2006;91:492–7.

**37.** Dokras A. Noncontraceptive use of oral combined hormonal contraceptives in polycystic ovary syndrome-risks versus benefits. Fertil Steril 2016;106:1572–9.

**38.** Morley LC, Tang T, Yasmin E, Norman RJ, Balen AH. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. Cochrane Database Syst Rev 2017;11:CD003053.

**39.** Luque-Ramírez M, Nattero-Chávez L, Ortiz Flores AE, Escobar-Morreale HF. Combined oral contraceptives and/or antiandrogens versus insulin sensitizers for polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update 2018;24:225–41.

**40.** Moffett RC, Naughton V. Emerging role of GIP and related gut hormones in fertility and PCOS. Peptides 2020;125:170233.

**41.** Domecq JP, Prutsky G, Mullan RJ, Hazem A, Sundaresh V, Elamin MB, *et al.* Lifestyle modification programs in polycystic ovary syndrome: systematic review and meta-analysis. J Clin Endocrinol Metab 2013;98:4655–63.

**42.** Sedighi S, Amir Ali Akbari S, Afrakhteh M, Esteki T, Alavi Majd H, Mahmoodi Z. Comparison of lifestyle in women with polycystic ovary syndrome and healthy women. Glob J Health Sci 2014;7:228–34.

**43.** Abazar E, Taghian F, Mardanian F, Forozandeh D. Effects of aerobic exercise on plasma lipoproteins in overweight and obese women with polycystic ovary syndrome. Adv Biomed Res 2015;4:68.

**44.** Norman RJ, Homan G, Moran L, Noakes M. Lifestyle choices, diet, and insulin sensitizers in polycystic ovary syndrome. Endocrine 2006;30:35–43.

**45.** Patel S. Polycystic ovary syndrome (PCOS), an inflammatory, systemic, lifestyle endocrinopathy. J Steroid Biochem Mol Biol 2018;182:27–36.

**46.** Brennan L, Teede H, Skouteris H, Linardon J, Hill B, Moran L. Lifestyle and Behavioral Management of Polycystic Ovary Syndrome. J Womens Health (Larchmt) 2017;26:836–48.

**47.** Cappelli V, Musacchio MC, Bulfoni A, Morgante G, De Leo V. Natural molecules for the therapy of hyperandrogenism and metabolic disorders in PCOS. Eur Rev Med Pharmacol Sci 2017;21(Suppl):15–29.

**48.** Lim SS, Hutchison SK, Van Ryswyk E, Norman RJ, Teede HJ, Moran LJ. Lifestyle changes in women with polycystic ovary syndrome. Cochrane Database Syst Rev 2019;3:CD007506.

**49.** de Lima Nunes R, Dos Santos IK, Cobucci RN, Pichini GS, Soares GM, de Oliveira Maranhão TM, *et al.* Lifestyle interventions and quality of life for women with polycystic ovary syndrome: A systematic review and meta-analysis protocol. Medicine (Baltimore) 2019;98:e18323.

**50.** Goodman NF, Cobin RH, Futterweit W, Glueck JS, Legro RS, Carmina E; American Association of Clinical Endocrinologists (AACE); American College of Endocrinology (ACE); Androgen Excess and PCOS Society. American College of Endocrinology (ACE); Androgen Excess and PCOS Society. American Association of Clinical Endocrinologists, American College of Endocrinology, and Androgen Excess and PCOS Society Disease State Clinical Review: guide to the best practices in the evaluation and treatment of polycystic ovary syndrome - part 2. Endocr Pract 2015;21:1415–26.

**51.** Bastianelli C, Farris M, Rosato E, Brosens I, Benagiano G. Pharmacodynamics of combined estrogen-progestin oral contraceptives 3. Inhibition of ovulation. Expert Rev Clin Pharmacol 2018;11:1085–98.

**52.** Amiri M, Kabir A, Nahidi F, Shekofteh M, Ramezani Tehrani F. Effects of combined oral contraceptives on the clinical and biochemical parameters of hyperandrogenism in patients with polycystic ovary syndrome: a systematic review and meta-analysis. Eur J Contracept Reprod Health Care 2018;23:64–77.

**53.** Yildiz BO. Approach to the patient: contraception in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2015;100:794–802.

**54.** Al Khalifah RA, Florez ID, Dennis B, Thabane L, Bassilious E. Metformin or Oral Contraceptives for Adolescents With Polycystic Ovarian Syndrome: A Meta-analysis. Pediatrics 2016;137:e20154089.

**55.** Harris-Glocker M, Davidson K, Kochman L, Guzick D, Hoeger K. Improvement in quality-of-life questionnaire measures in obese adolescent females with polycystic ovary syndrome treated with lifestyle changes and oral contraceptives, with or without metformin. Fertil Steril 2010;93:1016–9.

**56.** Halperin IJ, Kumar SS, Stroup DF, Laredo SE. The association between the combined oral contraceptive pill and insulin resistance, dysglycemia and dyslipidemia in women with polycystic ovary syndrome: a systematic review and meta-analysis of observational studies. Hum Reprod 2011;26:191–201.

**57.** Amiri M, Ramezani Tehrani F, Nahidi F, Kabir A, Azizi F, Carmina E. Effects of oral contraceptives on metabolic profile in women with polycystic ovary syndrome: A meta-analysis comparing products containing cyproterone acetate with third generation progestins. Metabolism 2017;73:22–35.

**58.** Naka KK, Kalantaridou SN, Bechlioulis A, Kravariti M, Kazakos N, Katsouras CS, *et al.* Effect of ethinylestradiol/cy-

proterone acetate on endothelial function in young non-obese women with polycystic ovary syndrome: a pilot study. Gynecol Endocrinol 2011;27:615–21.

**59.** de Bastos M, Stegeman BH, Rosendaal FR, Van Hylckama Vlieg A, Helmerhorst FM, Stijnen T, *et al.* Combined oral contraceptives: venous thrombosis. Cochrane Database Syst Rev 2014;(3):CD010813.

**60.** Burchall GF, Piva TJ, Linden MD, Gibson-Helm ME, Ranasinha S, Teede HJ. Comprehensive Assessment of the Hemostatic System in Polycystic Ovarian Syndrome. Semin Thromb Hemost 2016;42:55–62.

**61.** Bird ST, Hartzema AG, Brophy JM, Etminan M, Delaney JA. Risk of venous thromboembolism in women with polycystic ovary syndrome: a population-based matched cohort analysis. CMAJ 2013;185:E115–20.

**62.** World Health Organization Medical Eligibility Criteria for Contraceptive Use. 5th edition. Geneva; 2015 [Internet]. Available from: https://apps.who.int/iris/bitstream/handle/10665/181468/9789241549158_eng.pdf?sequence=9 [cited 2020, Jun 5].

**63.** Meczekalski B, Pérez-Roncero GR, López-Baena MT, Chedraui P, Pérez-López FR. The polycystic ovary syndrome and gynecological cancer risk. Gynecol Endocrinol 2020;36:289–93.

**64.** Gierisch JM, Coeytaux RR, Urrutia RP, Havrilesky LJ, Moorman PG, Lowery WJ, *et al.* Oral contraceptive use and risk of breast, cervical, colorectal, and endometrial cancers: a systematic review. Cancer Epidemiol Biomarkers Prev 2013;22:1931–43.

**65.** Mueck AO, Seeger H, Rabe T. Hormonal contraception and risk of endometrial cancer: a systematic review. Endocr Relat Cancer 2010;17:R263–71.

**66.** Havrilesky LJ, Moorman PG, Lowery WJ, Gierisch JM, Coeytaux RR, Urrutia RP, *et al.* Oral contraceptive pills as primary prevention for ovarian cancer: a systematic review and meta-analysis. Obstet Gynecol 2013;122:139–47.

**67.** Beral V, Doll R, Hermon C, Peto R, Reeves G; Collaborative Group on Epidemiological Studies of Ovarian Cancer. Ovarian cancer and oral contraceptives: collaborative reanalysis of data from 45 epidemiological studies including 23,257 women with ovarian cancer and 87,303 controls. Lancet 2008;371:303–14.

**68.** Del Pup L, Codacci-Pisanelli G, Peccatori F. Breast cancer risk of hormonal contraception: counselling considering new evidence. Crit Rev Oncol Hematol 2019;137:123–30.

**69.** Cibula D, Gompel A, Mueck AO, La Vecchia C, Hannaford PC, Skouby SO, *et al.* Hormonal contraception and risk of cancer. Hum Reprod Update 2010;16:631–50.

**70.** Zimmerman Y, Eijkemans MJ, Coelingh Bennink HJ, Blankenstein MA, Fauser BC. The effect of combined oral contraception on testosterone levels in healthy women: a systematic review and meta-analysis. Hum Reprod Update 2014;20:76–105.

**71.** Batukan C, Muderris II, Ozcelik B, Ozturk A. Comparison of two oral contraceptives containing either drospirenone or cyproterone acetate in the treatment of hirsutism. Gynecol Endocrinol 2007;23:38–44.

**72.** Trenor CC 3rd, Chung RJ, Michelson AD, Neufeld EJ, Gordon CM, Laufer MR, *et al.* Hormonal contraception and thrombotic risk: a multidisciplinary approach. Pediatrics 2011;127:347–57.

**73.** Bhattacharya SM, Jha A. Comparative study of the therapeutic effects of oral contraceptive pills containing desogestrel, cyproterone acetate, and drospirenone in patients with polycystic ovary syndrome. Fertil Steril 2012;98:1053–9.

**74.** Mastorakos G, Koliopoulos C, Deligeoroglou E, Diamanti-Kandarakis E, Creatsas G. Effects of two forms of combined oral contraceptives on carbohydrate metabolism in adolescents with polycystic ovary syndrome. Fertil Steril 2006;85:420–7.

**75.** Mastorakos G, Koliopoulos C, Creatsas G. Androgen and lipid profiles in adolescents with polycystic ovary syndrome who were treated with two forms of combined oral contraceptives. Fertil Steril 2002;77:919–27.

**76.** Creatsas G, Koliopoulos C, Mastorakos G. Combined oral contraceptive treatment of adolescent girls with polycystic ovary syndrome. Lipid profile. Ann N Y Acad Sci 2000;900:245–52.

**77.** Romualdi D, De Cicco S, Busacca M, Gagliano D, Lanzone A, Guido M. Clinical efficacy and metabolic impact of two different dosages of ethinyl-estradiol in association with drospirenone in normal-weight women with polycystic ovary syndrome: a randomized study. J Endocrinol Invest 2013;36:636–41.

**78.** Vigil P, Contreras P, Alvarado JL, Godoy A, Salgado AM, Cortés ME. Evidence of subpopulations with different levels of insulin resistance in women with polycystic ovary syndrome. Hum Reprod 2007;22:2974–80.

**79.** Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes 1989;38:1165–74.

**80.** Lewy VD, Danadian K, Witchel SF, Arslanian S. Early metabolic abnormalities in adolescent girls with polycystic ovarian syndrome. J Pediatr 2001;138:38–44.

**81.** McCartney CR, Marshall JC. CLINICAL PRACTICE. Polycystic Ovary Syndrome. N Engl J Med 2016;375:54–64.

**82.** Nestler JE, Jakubowicz DJ. Lean women with polycystic ovary syndrome respond to insulin reduction with decreases in ovarian P450c17 alpha activity and serum androgens. J Clin Endocrinol Metab 1997;82:4075–9.

**83.** 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;335:617–23.

**84.** Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia 2017;60:1577–85.

**85.** Miller RA, Chu Q, Xie J, Foretz M, Viollet B, Birnbaum MJ. Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. Nature 2013;494:256–60.

**86.** Kurzthaler D, Hadziomerovic-Pekic D, Wildt L, Seeber BE. Metformin induces a prompt decrease in LH-stimulated testosterone response in women with PCOS independent of its insulin-sensitizing effects. Reprod Biol Endocrinol 2014;12:98.

**87.** Mansfield R, Galea R, Brincat M, Hole D, Mason H. Metformin has direct effects on human ovarian steroidogenesis. Fertil Steril 2003;79:956–62.

**88.** Hirsch A, Hahn D, Kempná P, Hofer G, Nuoffer JM, Mullis PE, *et al.* Metformin inhibits human androgen production by regulating steroidogenic enzymes HSD3B2 and CYP17A1 and complex I activity of the respiratory chain. Endocrinology 2012;153:4354–66.

**89.** Attia GR, Rainey WE, Carr BR. Metformin directly inhibits androgen production in human thecal cells. Fertil Steril 2001;76:517–24.

**90.** Cosma M, Swiglo BA, Flynn DN, Kurtz DM, Labella ML, Mullan RJ, *et al.* Clinical review: Insulin sensitizers for the treatment of hirsutism: a systematic review and metaanalyses of randomized controlled trials. J Clin Endocrinol Metab 2008;93:1135–42.

**91.** Sam S, Ehrmann DA. Metformin therapy for the reproductive and metabolic consequences of polycystic ovary syndrome. Diabetologia 2017;60:1656–61.

**92.** Hoeger K. Impact of Metformin in Teens With Polycystic Ovary Syndrome (PCOS) on Oral Contraceptive Therapy. ClinicalTrials.gov; 2015 [Internet]. Available from: https://clinicaltrials.gov/ct2/show/study/NCT00283816 [cited 2020, Jun 5].

**93.** Ladson G, Dodson WC, Sweet SD, Archibong AE, Kunselman AR, Demers LM, *et al.* The effects of metformin with lifestyle therapy in polycystic ovary syndrome: a randomized double-blind study. Fertil Steril 2011;95:1059–66.e1, 7.

**94.** Long M. The Effect of GLP-1 Agonists Versus OCs on Reproductive Disorders and Cardiovascular Risks in Overweight PCOS. ClinicalTrials.gov; 2018 [Internet]. Available from: https://clinicaltrials.gov/ct2/show/study/NCT03151005 [cited 2020, Jun 5].

**95.** Tfayli H, Ulnach JW, Lee S, Sutton-Tyrrell K, Arslanian S. Drospirenone/ethinyl estradiol versus rosiglitazone treatment in overweight adolescents with polycystic ovary syndrome: comparison of metabolic, hormonal, and cardiovascular risk factors. J Clin Endocrinol Metab 2011;96:1311–9.

**96.** Frøssing S, Nylander M, Chabanova E, Frystyk J, Holst JJ, Kistorp C, *et al.* Effect of liraglutide on ectopic fat in polycystic ovary syndrome: A randomized clinical trial. Diabetes Obes Metab 2018;20:215–8.

**97.** Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, *et al.*; Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346:393–403.

**98.** Diamanti-Kandarakis E, Christakou CD, Kandaraki E, Economou FN. Metformin: an old medication of new fashion: evolving new molecular mechanisms and clinical implications in polycystic ovary syndrome. Eur J Endocrinol 2010;162:193–212.

**99.** Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: an update. Ann Intern Med 2002;137:25–33.

**100.** Dujic T, Zhou K, Tavendale R, Palmer CN, Pearson ER. Effect of Serotonin Transporter 5-HTTLPR Polymorphism on Gastrointestinal Intolerance to Metformin: A GoDARTS Study. Diabetes Care 2016;39:1896–901.

**101.** Dujic T, Zhou K, Donnelly LA, Tavendale R, Palmer CN, Pearson ER. Association of Organic Cation Transporter 1 With Intolerance to Metformin in Type 2 Diabetes: A GoDARTS Study. Diabetes 2015;64:1786–93.

**102.** DeFronzo RA, Inzucchi S, Abdul-Ghani M, Nissen SE. Pioglitazone: the forgotten, cost-effective cardioprotective drug for type 2 diabetes. Diab Vasc Dis Res 2019;16:133–43.

**103.** Perry CG, Petrie JR. Insulin-sensitising agents: beyond thiazolidinediones. Expert Opin Emerg Drugs 2002;7:165–74.

**104.** Aroda VR, Ciaraldi TP, Burke P, Mudaliar S, Clopton P, Phillips S, *et al.* Metabolic and hormonal changes induced by pioglitazone in polycystic ovary syndrome: a randomized, placebo-controlled clinical trial. J Clin Endocrinol Metab 2009;94:469–76.

**105.** Cataldo NA, Abbasi F, McLaughlin TL, Basina M, Fechner PY, Giudice LC, *et al.* Metabolic and ovarian effects of rosiglitazone treatment for 12 weeks in insulin-resistant women with polycystic ovary syndrome. Hum Reprod 2006;21:109–20.

**106.** Lemay A, Dodin S, Turcot L, Déchêne F, Forest JC. Rosiglitazone and ethinyl estradiol/cyproterone acetate as single and combined treatment of overweight women with polycystic ovary syndrome and insulin resistance. Hum Reprod 2006;21:121–8.

**107.** Rautio K, Tapanainen JS, Ruokonen A, Morin-Papunen LC. Endocrine and metabolic effects of rosiglitazone in overweight women with PCOS: a randomized placebo-controlled study. Hum Reprod 2006;21:1400–7.

**108.** Azziz R, Ehrmann D, Legro RS, Whitcomb RW, Hanley R, Fereshetian AG, *et al.*; PCOS/Troglitazone Study Group. Troglitazone improves ovulation and hirsutism in the polycystic ovary syndrome: a multicenter, double blind, placebo-controlled trial. J Clin Endocrinol Metab 2001;86:1626–32.

**109.** Gerstein HC, Miller ME, Byington RP, Goff DC Jr, Bigger JT, Buse JB, *et al.*; Action to Control Cardiovascular Risk in Diabetes Study Group. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 2008;358:2545–59.

**110.** Conway G, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Franks S, Gambineri A, *et al.*; ESE PCOS Special Interest Group. The polycystic ovary syndrome: a position statement from the European Society of Endocrinology. Eur J Endocrinol 2014;171:1–29.

**111.** Valsamakis G, Lois K, Kumar S, Mastorakos G. Metabolic and other effects of pioglitazone as an add-on therapy to metformin in the treatment of polycystic ovary syndrome (PCOS). Hormones (Athens) 2013;12:363–78.

**112.** Garber A, Henry R, Ratner R, Garcia-Hernandez PA, Rodriguez-Pattzi H, Olvera-Alvarez I, *et al.*; LEAD-3 (Mono) Study Group. Liraglutide versus glimepiride monotherapy for type 2 diabetes (LEAD-3 Mono): a randomised, 52-week, phase III, double-blind, parallel-treatment trial. Lancet 2009;373:473–81.

**113.** Elkind-Hirsch K, Marrioneaux O, Bhushan M, Vernor D, Bhushan R. Comparison of single and combined treatment with exenatide and metformin on menstrual cyclicity in overweight women with polycystic ovary syndrome. J Clin Endocrinol Metab 2008;93:2670–8.

**114.** Jensterle Sever M, Kocjan T, Pfeifer M, Kravos NA, Janez A. Short-term combined treatment with liraglutide and metformin leads to significant weight loss in obese women with polycystic ovary syndrome and previous poor response to metformin. Eur J Endocrinol 2014;170:451–9.

**115.** Macut D, Bjekić-Macut J, Rahelić D, Doknić M. Insulin and the polycystic ovary syndrome. Diabetes Res Clin Pract 2017;130:163–70.

**116.** Bizzarri M, Carlomagno G. Inositol: history of an effective therapy for Polycystic Ovary Syndrome. Eur Rev Med Pharmacol Sci 2014;18:1896–903.

**117.** Croze ML, Soulage CO. Potential role and therapeutic interests of myo-inositol in metabolic diseases. Biochimie 2013;95:1811–27.

**118.** Andrisani A, Donà G, Sabbadin C, Tibaldi E, Dessole F, Bosello Travain V, *et al.* Ameliorative effect of myo-inositol on red blood cell alterations in polycystic ovary syndrome: in vitro study. Gynecol Endocrinol 2018;34:233–7.

**119.** Özay AC, Emekçi Özay Ö, Okyay RE, Gülekli B. The effect of myoinositol on ovarian blood flows in women with polycystic ovary syndrome. Gynecol Endocrinol 2019;35:237–41.

**120.** 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;61:111–7.

**121.** Carlomagno G, Unfer V, Roseff S. The D-chiro-inositol paradox in the ovary. Fertil Steril 2011;95:2515–6.

**122.** Zhao SS, Li NR, Zhao WL, Liu H, Ge MX, Zhang YX, *et al.* D-chiro-inositol effectively attenuates cholestasis in bile duct ligated rats by improving bile acid secretion and attenuating oxidative stress. Acta Pharmacol Sin 2018;39:213–21.

**123.** Benelli E, Del Ghianda S, Di Cosmo C, Tonacchera M. A Combined Therapy with Myo-Inositol and D-Chiro-Inositol Improves Endocrine Parameters and Insulin Resistance in PCOS Young Overweight Women. Int J Endocrinol 2016;2016:3204083.

**124.** Bevilacqua A, Bizzarri M. Inositols in Insulin Signaling and Glucose Metabolism. Int J Endocrinol 2018;2018: 1968450.

**125.** Bevilacqua A, Bizzarri M. Physiological role and clinical utility of inositols in polycystic ovary syndrome. Best Pract Res Clin Obstet Gynaecol 2016;37:129–39.

**126.** Cabrera-Cruz H, Oróstica L, Plaza-Parrochia F, Torres-Pinto I, Romero C, Vega M. The insulin-sensitizing mechanism of myo-inositol is associated with AMPK activation and GLUT-4 expression in human endometrial cells exposed to a PCOS environment. Am J Physiol Endocrinol Metab 2020;318:E237–48.

**127.** Mertoglu C, Gunay M, Gul V, Kulhan M, Aktas M, Coban TA. Does myo-inositol oxygenase, the only enzyme to catalyze myo-inositol in vivo, play a role in the etiology of polycystic ovarian syndrome? Gynecol Endocrinol 2018;34:418–21.

**128.** Wojciechowska A, Osowski A, Jóźwik M, Górecki R, Rynkiewicz A, Wojtkiewicz J. Inositols' Importance in the Improvement of the Endocrine-Metabolic Profile in PCOS. Int J Mol Sci 2019;20:E5787.

**129.** Gateva A, Unfer V, Kamenov Z. The use of inositol(s) isomers in the management of polycystic ovary syndrome: a comprehensive review. Gynecol Endocrinol 2018;34:545–50.

**130.** Le TN, Wickham EP, Nestler JE. Insulin sensitizers in adolescents with polycystic ovary syndrome. Minerva Pediatr 2017;69:434–43.

**131.** Sortino MA, Salomone S, Carruba MO, Drago F. Polycystic Ovary Syndrome: Insights into the Therapeutic Approach with Inositols. Front Pharmacol 2017;8:341.

**132.** Zeng L, Yang K. Effectiveness of myoinositol for polycystic ovary syndrome: a systematic review and meta-analysis. Endocrine 2018;59:30–8.

**133.** Facchinetti F, Orrù B, Grandi G, Unfer V. Short-term effects of metformin and myo-inositol in women with polycystic ovarian syndrome (PCOS): a meta-analysis of randomized clinical trials. Gynecol Endocrinol 2019;35:198–206.

**134.** Shokrpour M, Foroozanfard F, Afshar Ebrahimi F, Vahedpoor Z, Aghadavod E, Ghaderi A, *et al.* Comparison of myo-inositol and metformin on glycemic control, lipid profiles, and gene expression related to insulin and lipid metabolism in women with polycystic ovary syndrome: a randomized controlled clinical trial. Gynecol Endocrinol 2019;35:406–11.

**135.** Pkhaladze L, Barbakadze L, Kvashilava N. Myo-Inositol in the Treatment of Teenagers Affected by PCOS. Int J Endocrinol 2016;2016:1473612.

**136.** Montanino Oliva M, Buonomo G, Calcagno M, Unfer V. Effects of myo-inositol plus alpha-lactalbumin in myo-inositol-resistant PCOS women. J Ovarian Res 2018;11:38.

**137.** Montanino Oliva M, Zuev V, Lippa A, Carra MC, Lisi F. Efficacy of the synergic action of myoinositol, tyrosine, selenium and chromium in women with PCOS. Eur Rev Med Pharmacol Sci 2019;23:8687–94.

**138.** Stracquadanio M, Ciotta L, Palumbo MA. Effects of myo-inositol, gymnemic acid, and L-methylfolate in polycystic ovary syndrome patients. Gynecol Endocrinol 2018;34:495–501.

**139.** Januszewski M, Issat T, Jakimiuk AA, Santor-Zaczynska M, Jakimiuk AJ. Metabolic and hormonal effects of a combined Myo-inositol and d-chiro-inositol therapy on patients with polycystic ovary syndrome (PCOS). Ginekol Pol 2019;90:7–10.

**140.** Nordio M, Basciani S, Camajani E. The 40:1 myo-inositol/D-chiro-inositol plasma ratio is able to restore ovulation in PCOS patients: comparison with other ratios. Eur Rev Med Pharmacol Sci 2019;23:5512–21.

**141.** Troisi J, Cinque C, Giugliano L, Symes S, Richards S, Adair D, *et al.* Metabolomic change due to combined treatment with myo-inositol, D-chiro-inositol and glucomannan in polycystic ovarian syndrome patients: a pilot study. J Ovarian Res 2019;12:25.

**142.** Advani K, Batra M, Tajpuriya S, Gupta R, Saraswat A, Nagar HD, *et al.* Efficacy of combination therapy of inositols, antioxidants and vitamins in obese and non-obese women with polycystic ovary syndrome: an observational study. J Obstet Gynaecol 2020;40:96–101.

**143.** Le Donne M, Metro D, Alibrandi A, Papa M, Benvenga S. Effects of three treatment modalities (diet, myo-inositol or myoinositol associated with D-chiro-inositol) on clinical and body composition outcomes in women with polycystic ovary syndrome. Eur Rev Med Pharmacol Sci 2019;23:2293–301.

**144.** Bevilacqua A, Dragotto J, Giuliani A, Bizzarri M. Myo-inositol and D-chiro-inositol (40:1) reverse histological and functional features of polycystic ovary syndrome in a mouse model. J Cell Physiol 2019;234:9387–98.

**145.** Murri M, Luque-Ramírez M, Insenser 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;19:268–88.

**146.** Cianci A, Panella M, Fichera M, Falduzzi C, Bartolo M, Caruso S. d-chiro-Inositol and alpha lipoic acid treatment of metabolic and menses disorders in women with PCOS. Gynecol Endocrinol 2015;31:483–6.

**147.** Bast A, Haenen GR. Lipoic acid: a multifunctional antioxidant. Biofactors 2003;17:207–13.

**148.** Konrad D, Somwar R, Sweeney G, Yaworsky K, Hayashi M, Ramlal T, *et al.* The antihyperglycemic drug alpha-lipoic acid stimulates glucose uptake via both GLUT4 translocation and GLUT4 activation: potential role of p38 mitogen-activated protein kinase in GLUT4 activation. Diabetes 2001;50:1464–71.

**149.** Shen QW, Zhu MJ, Tong J, Ren J, Du M. Ca2+/calmodulin-dependent protein kinase kinase is involved in AMP-activated protein kinase activation by alpha-lipoic acid in C2C12 myotubes. Am J Physiol Cell Physiol 2007;293:C1395–403.

**150.** Lee WJ, Song KH, Koh EH, Won JC, Kim HS, Park HS, *et al.* Alpha-lipoic acid increases insulin sensitivity by activating AMPK in skeletal muscle. Biochem Biophys Res Commun 2005;332:885–91.

**151.** 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;4:359–64.

**152.** Genazzani AD, Shefer K, Della Casa D, Prati A, Napolitano A, Manzo A, *et al.* Modulatory effects of alpha-lipoic acid (ALA) administration on insulin sensitivity in obese PCOS patients. J Endocrinol Invest 2018;41:583–90.

**153.** Fruzzetti F, Capozzi A, Canu A, Lello S. Treatment with d-chiro-inositol and alpha lipoic acid in the management of polycystic ovary syndrome. Gynecol Endocrinol 2019;35:506–10.

**154.** De Cicco S, Immediata V, Romualdi D, Policola C, Tropea A, Di Florio C, *et al.* Myoinositol combined with alpha-

lipoic acid may improve the clinical and endocrine features of polycystic ovary syndrome through an insulin-independent action. Gynecol Endocrinol 2017;33:698–701.

**155.** Fruzzetti F, Fidecicchi T, Palla G, Gambacciani M. Long-term treatment with α-lipoic acid and myo-inositol positively affects clinical and metabolic features of polycystic ovary syndrome. Gynecol Endocrinol 2020;36:152–5.

**156.** Genazzani AD, Prati A, Marchini F, Petrillo T, Napolitano A, Simoncini T. Differential insulin response to oral glucose tolerance test (OGTT) in overweight/obese polycystic ovary syndrome patients undergoing to myo-inositol (MYO), alpha lipoic acid (ALA), or combination of both. Gynecol Endocrinol 2019;35:1088–93.

**157.** Cirillo F, Catellani C, Lazzeroni P, Sartori C, Tridenti G, Vezzani C, *et al.* HMGB1 is increased in adolescents with polycystic ovary syndrome (PCOS) and decreases after treatment with myo-inositol (MYO) in combination with alpha-lipoic acid (ALA). Gynecol Endocrinol 2020;1–6. [Epub ahead of print]

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