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Polycystic Ovarian Syndrome (PCOS):

Non-Pharmacological Solutions for a Complex Endocrine Disorder

Polycystic Ovarian Syndrome (PCOS) is one of the most common endocrine disorders in women of childbearing age, affecting nearly 10% of women in some population; and is the leading cause of infertility. While a range of phenotypes exist for PCOS, it is typically characterized by irregular menstruation, ovarian cysts, and hyperandrogenism; which may further include anovulation, hirsutism, and acne, among other signs of hyperandrogenism. Beyond these classical endocrine-related symptoms, PCOS is commonly associated with metabolic disorders (e.g., insulin resistance, obesity and type 2 diabetes), and mood/psychiatric disorders (e.g., depression, anxiety, HPA axis stress); suggesting that PCOS is a tissue-specific manifestation of a more complex metabolic disorder. Recent research now shows that many factors that contribute to systemic metabolic diseases are also associated with increased risk for PCOS, including poor lifestyle and dietary habits, gut dysbiosis, chronic inflammation, mitochondrial dysfunction and exposure to certain environmental toxins. In this monograph, we will explore the pathophysiology of PCOS, focusing on several of the emerging metabolic relationships known to be strongly associated with this common endocrine disorder. This root-cause approach will allow clinicians to better understand therapies designed to target these underlying pathways with evidence-based lifestyle therapies. In addition, we summarize the evidence for non-pharmacological therapies which have been investigated with respect to their ability to improve PCOS-specific outcomes.

Polycystic Ovarian Syndrome (PCOS) is a multifactorial disorder in women, usually characterized by menstrual irregularities, hyperandrogenism, and the presence of multiple ovarian follicular cysts. Originally called Stein-Levinthal syndrome after the two people who described it in 1935, this condition was nominally characterized as the triad of polycystic ovaries, hirsutism, and oligo/amenorrhea.[1,2] The term "polycystic ovary syndrome" became more widely used after specific hormonal imbalances were discovered and ultrasound could routinely diagnose the ovarian morphology. While mostly considered an endocrine disorder, PCOS presents with a wide-range of phenotypes and is strongly associated with metabolic abnormalities, mostly as a consequence of insulin resistance and obesity; leading some to suggest that PCOS (at least in many women) is a metabolic disorder with endocrine consequences. In fact, like many other complex metabolic disorders (e.g., nonalcoholic fatty liver disease), there are calls for PCOS to undergo nomenclature changes that more accurately define its various phenotypes and underlying pathophysiology.[3,4] While this monograph will not discuss the details of this nomenclature debate, it is still important for the clinician to understand the challenges in interpreting the heterogeneous results of clinical research performed in subjects with the same formal diagnosis (i.e., all meeting one of the criteria used to define PCOS), that may have different types or severities of underlying metabolic dysfunction. Therefore, it is critical to understand the metabolic vulnerabilities and status of each individual before prioritizing a treatment plan designed to address their PCOS-related symptoms.

PCOS- Definitions and Prevalence

It is generally reported that PCOS affects one in ten women during their childbearing years, though prevalence has been reported to be between 5.5% and 11.5%, depending on which definition is used.[5] As a syndrome, PCOS has had several different definitions over the years, and there are still different diagnostic criteria used in various epidemiological surveys and clinical trials. However, the most comprehensive international guidelines recommend the use of the revised consensus

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Rotterdam criteria for the diagnosis of PCOS.[6] These criteria state that PCOS may be diagnosed if any two of the following are present: (1) clinical or biochemical hyperandrogenism, (2) evidence of oligo-anovulation, (3) polycystic appearing-ovarian morphology on ultrasound, with exclusion of other relevant disorders (such as thyroid disorders, hyperprolactinemia, and congenital adrenal hyperplasia).[7] The most reliable measure of biochemical hyperandrogenism is elevated total or free serum testosterone, though calculated measures of free testosterone, and in some cases, measures of dehydroepiandrosterone sulfate (DHEA-S) and/or androstenedione can be used.[8] Subjects with PCOS usually have reduced levels of sex-hormone-binding globulin (SHBG), which contributes to higher levels of free testosterone, though SHBG levels are not diagnostic for PCOS. Nonetheless, low serum SHBG levels are considered a biomarker of metabolic abnormalities and are associated with insulin resistance (IR), hyperandrogenism, and abnormal glucose and lipid metabolism in PCOS patients.[9] Clinical manifestations of elevated androgen levels in women are also helpful in the diagnosis of PCOS, which include hirsutism, acne and female pattern hair loss.

Oligo-anovulation is defined by cycles of more than 35 days or fewer than 8 cycles per year in pre-perimenopausal women. In some cases, irregular measures or timing of progesterone, luteinizing hormone (LH) or follicle-stimulating hormone (FSH) can act as surrogate markers for oligo-anovulation. Based on transvaginal ultrasonography with a transducer frequency ≥ 8 MHz, polycystic ovarian morphology is defined as ≥20 follicles per ovary and/or an ovarian volume of ≥10 cm^3, though there is some debate as to the threshold of follicles (and other morphological characteristics) that should be used to define this criterion.[7,10] Serum anti-Mullerian hormone (AMH), a peptide secreted by growing follicles, is generally much higher in subjects with PCOS and has been considered a surrogate marker for polycystic ovarian morphology (in the absence of ultrasound results). Since AMH levels begin to decline in perimenopause, and there is no consensus on the diagnostic threshold in subjects with PCOS, AMH levels are not yet considered part of the official diagnostic criteria.[11,12]AMH levels, however, may be an important biomarker to follow to understand the effects of initiating therapies designed to treat PCOS.[13]

Because the criteria for defining PCOS allows for individuals to meet the diagnosis in different ways, some have proposed the use of phenotyping to differentiate the varied presentations of PCOS.[14] Four phenotypes are usually described, based on the overlapping diagnostic criteria, as shown in Figure 1. The use of phenotyping may help clinicians prioritize therapies that are more likely to address underlying physiological drivers. For instance, phenotype D does not present with any of the classic PCOS measures of hyperandrogenism, and is even considered to be a separate condition by some members of the PCOS community.[15] While nuanced discussion of each phenotype is beyond the scope of this monograph (more than four have been proposed), using phenotyping as a general guideline may help clinicians focus more on the underlying dysfunctions (and their related pathophysiological pathways), when developing personalized therapeutic strategies in patients diagnosed with PCOS.

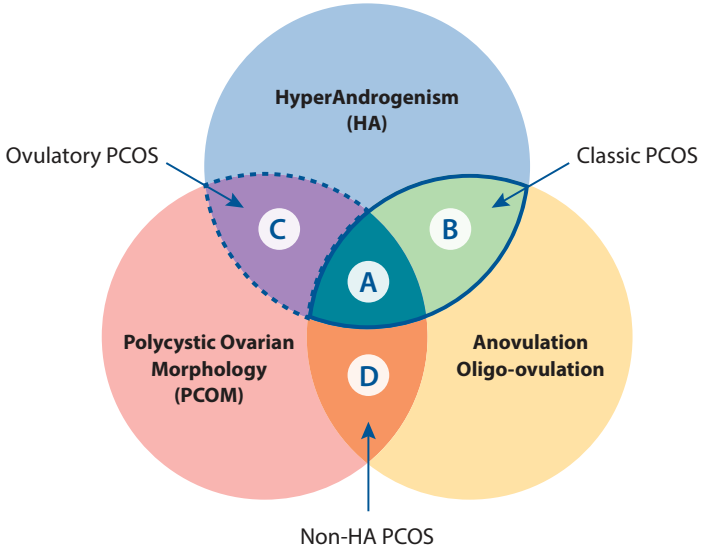


Figure 1: The Four Basic Phenotypes of PCOS. Defined by the overlapping diagnostic criteria of PCOS, these four phenotypes help distinguish subtypes of PCOS that may require different clinical approaches (see text for more details).

PCOS Pathophysiology and Risk

As the previously described diagnostic scheme suggests, the pathophysiology driving PCOS is complex and the etiology is often described as unknown.[16] Nonetheless, emerging research suggests that several underlying processes and signals drive the varied symptoms of PCOS. These encompass a bidirectional interaction between dysfunctional metabolic signals and reproductive system dysregulation, genetic or epigenetic vulnerabilities, and increased burden from environmental and lifestyle factors.[17] From these various processes and signals, the two metabolic consequences that appear to be responsible for the classic signs and symptoms of PCOS (and many of its co-morbidities) are hyperandrogenism and insulin resistance, the biological targets of most proposed therapies.[18,19] These two biological phenomena can create a feed-forward loop such that excess androgen production induces insulin resistance and visceral adiposity; whereas the compensatory hyperinsulinemia caused by peripheral insulin resistance stimulates androgen secretion and gonadotropin-sensitivity in theca cells.[20] Furthermore, most of the genes which have been identified to be associated with PCOS risk are related to hormones, receptors or enzymes in the hypothalamic-pituitary-ovarian (HPO) axis, or insulin-sensitive metabolic pathways.[21] While the hereditary

aspects of PCOS (genetic, epigenetic and gestational) are critically important for clinicians to understand, these details are beyond the scope of this monograph.[22]

Hypoandrogenism and HPO Axis

The hypothalamic-pituitary-ovarian (HPO) axis creates a highly orchestrated monthly cycle of hormonal signals that, in healthy premenstrual women, normally results in the ovulation of a maturing follicle once per month. Critical to this process is the function of theca cells, a layer of steroidogenic cells situated just inside the basal lamina of the growing follicle.[23] These theca cells produce androstenedione (AND), an androgen precursor, which diffuses into adjacent granulosa cells. Within the granulosa cells, AND is converted to estrogens through the action of aromatase, or to progesterone, depending on the hormonal signals. This androgen production is primarily regulated by luteinizing hormone (LH), but factors like insulin and insulin-like growth factor-1 (IGF-1) can enhance theca cell sensitivity to LH, promoting increased androgen production, including testosterone (T), via the enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD).[24]

In subjects with PCOS, the delicate feedback loops that maintains the hormone balance during follicle maturation is disrupted. Anti-Mullerian hormone (AMH), produced by growing follicles, is elevated in most subjects with PCOS and is thought to perpetuate this androgen-dominant cycle.[25] When AMH binds to receptors in the hypothalamus and pituitary it triggers the release of gonadotropin releasing hormone (GnRH) and LH, resulting in a higher LH-to-FSH ratio. Reduced FSH-signaling affects granulosa cell conversion of androgens to estrogens (by inhibiting aromatase, etc.), resulting in lower estradiol (E2) production and elevated AND and T levels.[26] The alteration in hormone signaling (e.g., LH, FSH, T, E2, AMH) and subsequent dysregulation of follicular growth often results in anovulation and infertility.[27]

Infertility is often the therapeutic concern for many women with PCOS, much of which focuses on modulation of the timing or concentration of hormone signals. Pharmaceutical therapies† that have been used to help induced ovulation in women with PCOS include aromatase inhibitors (e.g., letrozole), selective estrogen receptor modulators (SERMs, e.g., Clomiphene citrate- most often with metformin) and gonadotrophin therapy.[6,28,29,30] Other hormone-modulating therapies for women not intending to become pregnant include various combined oral contraceptives and anti-androgen drugs (e.g., finasteride, flutamide, spironolactone; for acne, hirsutism, androgen-pattern balding).[6,31,32]

Insulin Resistance and Related Metabolic Dysfunctions

Insulin resistance (IR) is considered a hallmark of PCOS- and is present in 65-95% of those with this diagnosis.[33] Peripheral insulin resistance reduces the rate of glucose disposal in key insulin-sensitive tissues (e.g., hepatocytes, myocytes, adipocytes), eliciting an increasing production of pancreatic insulin in an attempt to bring down serum glucose levels (i.e. compensatory hyperinsulinemia). As mentioned above, ovarian follicular cells remain sensitive to insulin under peripheral IR conditions, a signal that results in higher levels of ovarian-derived androgens and a reduction in their conversion to estrogens. In many subjects with PCOS, IR triggers a down-regulation of sex hormone binding globulin (SHBG) production from the liver, allowing for increased circulation of free androgens.[34] Insulin also appears to increase adrenal cortex sensitivity to adrenocorticotropic hormone (ACTH), resulting in excess production of both androgens (DHEA(S)) and cortisol.[35,36]

Insulin resistance is strongly associated with a number of other metabolic dysfunctions, many of which are common in subjects with PCOS. These include obesity, type 2 diabetes, fatty liver disorders, gut microbiome dysbiosis, chronic inflammation, mitochondrial dysfunction and cardiometabolic dyslipidemias. The consequences of these metabolic dysfunctions are quite far-reaching and last well beyond a woman's pre-menopausal years. Here, we discuss some of these associations and consequences.

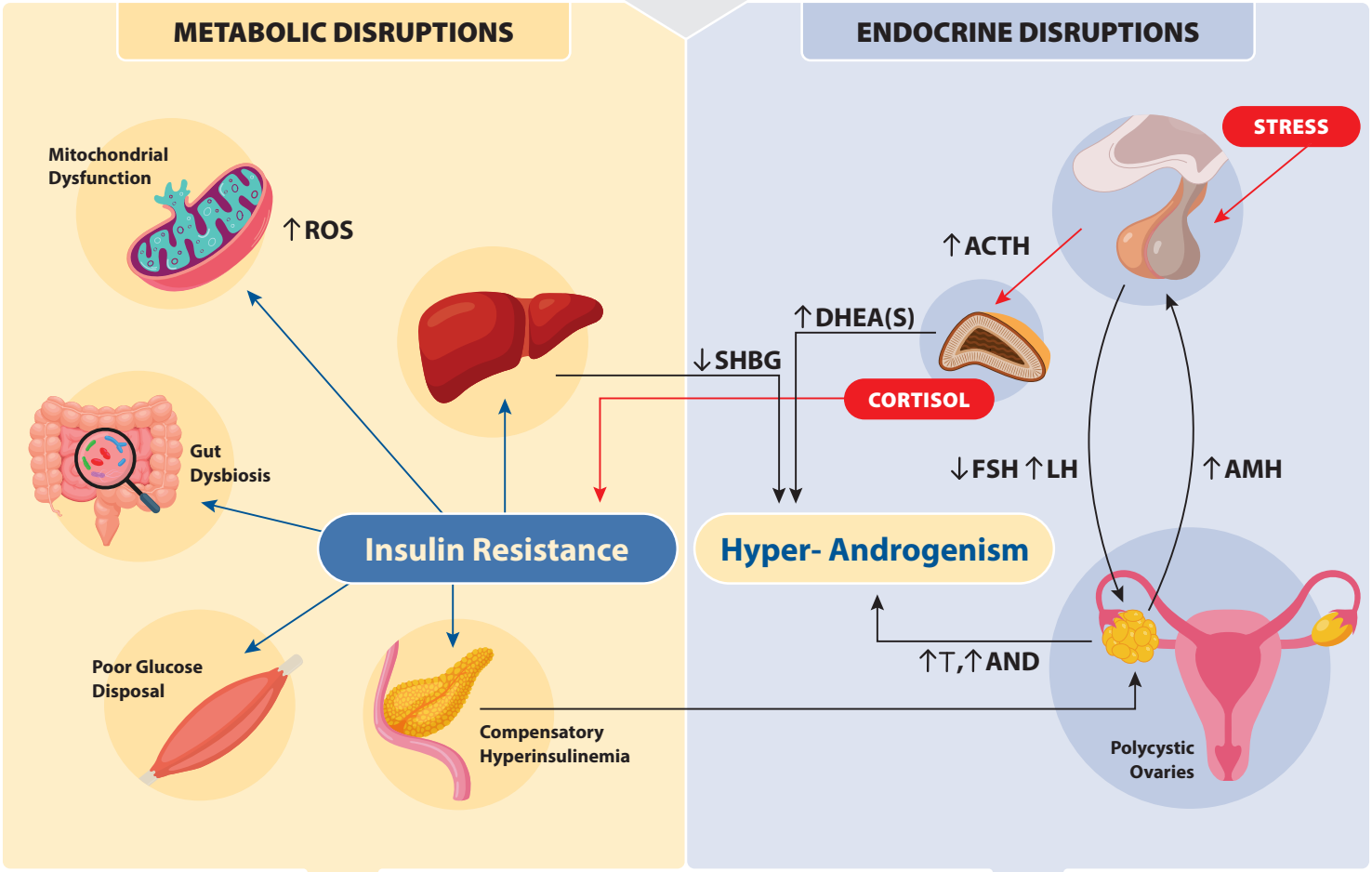
Obesity and Type 2 Diabetes

In the general population, IR is strongly associated with obesity (i.e., increased BMI, central adiposity, increased waist circumference, and increased waist-to-hip ratio); which is associated with an increased risk of type 2 diabetes. Likewise, a strong association with these IR-related conditions in also found in subject with PCOS, where studies report that 38-88% of women with PCOS are overweight or obese.[37] A recently published retrospective cohort study derived from database records (UK) showed that obesity in PCOS subjects was associated with a significantly lower chance of pregnancy (compared to normal weight women with PCOS), whereas weight loss in obese PCOS women greatly increased their chances of pregnancy (10% weight loss increased chances by 68%).[38] However, while previous intervention studies have shown successful weight loss can be achieved in obese subjects with PCOS, dropout rates are often high and there is little consensus on the best way to achieve meaningful weight loss in these subjects.[39]

† The mention of pharmaceutical therapeutics for PCOS here (and throughout this monograph) is rudimentary, and is not intended as an endorsement or discouragement of their use. Please access the references in these sections to learn more about their potential benefits, doses and side-effects. In some cases, the use of these pharmaceuticals (while recommended in some guideline documents) are considered "off-label" in subjects with PCOS.

Predisposing Factors (Antecedents and Triggers)

Western Dietary Pattern
Sedentary Lifestyle
Systemic Inflammation
Obesity/Central Adiposity
Obesogenic Toxins
Genetics- Epigenetics
Intrauterine Environment
Chronic HPA axis Stress
Sleep/Circadian Disruptions
Endocrine Disrupting Toxins



Metabolic Syndrome
Type 2 Diabetes/CVD
Fatty Liver Disease

Anovulation/Infertility
Hirsutism
Acne

Figure 2: The Basic Risks, Pathophysiology and Consequences Associated with PCOS. Here we show the basic metabolic and endocrine disruptions that are commonly associated with PCOS, rooted in Insulin Resistance and Hyperandrogenism. See text for details.

Similar to obese subjects without PCOS, subjects with PCOS also have a much higher risk of developing type 2 diabetes (4 times higher is some cohorts), usually at a much earlier age.[40] This risk for type 2 diabetes has been shown, in some studies, to be elevated even in some non-obese women with PCOS, highlighting the fact that insulin resistance and metabolic abnormalities can still be associated with PCOS independent of obesity.[41] The urgency of managing metabolic dysfunctions in subjects with PCOS, with respect to fertility/pregnancy concerns and the risk for chronic metabolic complications, is often a primary focus for clinicians. Therefore, pharmaceutical agents approved primarily for type 2 diabetes or weight loss, are commonly used in PCOS patients. These include metformin, GLP-1 receptor agonists, thiazolidinediones, orlistat, phentermine/topiramate, and SGLT-2 inhibitors.[6,42-46] Lifestyle interventions and natural ingredients with similar targets (i.e., insulin sensitivity, weight-loss, etc.) have also been investigated in subjects with PCOS with successful outcomes (see page 10).

Gut Microbiome Dysbiosis and PCOS

In the past several decades, research has uncovered a strong association between various metabolic dysfunctions, such as obesity and type 2 diabetes, and particular characteristics of a person's gut microbiome.[47,48] These characteristics includes alterations in the normal pattern of microbial organisms (i.e., dysbiosis) or microbe-derived metabolites. A variety of mechanisms appear to link these gut microbiome patterns with various metabolic dysfunctions, such as increased gut permeability, dysfunctional immune-activation, increased inflammatory signaling, alterations in bile acid metabolism, decreased synthesis of short-chain fatty acids, and alterations in polyphenol metabolism- to name just a few.[49] Not surprisingly, many of these altered gut microbial characteristics (and their related metabolic-related dysfunctions) are also associated with PCOS.[50,51] While studies are limited, the gut microbiota of women with PCOS mirrors many of the characteristics of obese women without PCOS, suggesting that a metabolic-association drives this relationship.[52-55] However, some studies suggest that a bi-directional relationship between gut microbial metabolism and hormone signaling (both androgens and estrogens) are also influencing this association, differentiating PCOS dysbiosis from simply that of obesity.[56,57] In one study, when the stools of women with PCOS were transplanted into mice, the donor mice had increased levels of both metabolic and ovarian/hormonal abnormalities, compared to mice given stools from control subjects.[58] The authors suggested that specific bile acid metabolites and immune-signaling changes (e.g., lowered IL-22) were critical in this model.

Gut microbiome modulation using diet, fecal microbial transplants (FMT), probiotics, prebiotics, and/or postbiotics is a promising therapeutic strategy to alter metabolic dysfunction caused by gut dysbiosis. Several of these strategies have been explored in studies related to PCOS, either in animal models or human clinical trials.[59] However, while studies suggest these microbiome-modulating therapies result in metabolic benefits similar to those seen in obese subjects, more research is needed to determine if specific probiotics or prebiotics can target a PCOS-specific outcome (see further below). While the use of FMTs are speculated to be helpful in subjects with PCOS (using healthy donor stool), there is little research evaluating this unapproved therapy for PCOS-specific outcomes.[60,61] Finally, PCOS is also associated with dysbiosis within the vaginal microbiota, though fewer studies have been done to investigate these characteristics or what interventions might be helpful in subjects with PCOS-related vaginal dysbiosis.[62]

Fatty Liver Disease

Recently, nomenclature changes to define non-alcoholic fatty liver diseases (NAFLD) have firmly acknowledged that these conditions are primarily metabolic diseases, driven by obesity and insulin resistance (e.g., MAFLD, metabolic dysfunction-associated fatty liver disease).[63] As such, metabolic fatty liver disorders are one of the fastest growing chronic metabolic conditions worldwide. Some have estimated that 25% of the adult population, worldwide, meets the older definition of NAFLD, with the highest prevalence reported in certain Middle Eastern and South American countries.[64,65] The prevalence is highest in severely obese subjects (>80%) and those with type 2 diabetes (>75%), though many lean subjects (i.e., non-obese and normal BMI) can also suffer from NAFLD.[66-69]

Women with PCOS are 2.5 to 4 times more likely to have clinical signs of fatty liver disease than women without PCOS, especially in those with both obesity and hyperandrogenism.[70,71] Though these two conditions share the common antecedents of obesity and insulin resistance, some research suggests that common genetic vulnerabilities exist to increase the comorbidities of PCOS and metabolic fatty liver disorders.[72] Clinicians need to be aware of this strong association (sometimes called the hepato-ovarian axis) so that they actively screen subjects with PCOS for metabolic fatty liver disorders, and vis versa.[73] While the therapeutic goals for both conditions overlap significantly (i.e., weight loss, insulin sensitivity, gut microbiome, reduce inflammation, etc.), including the lifestyle and non-pharmacological recommendations for each, a greater urgency is warranted in women diagnosed with both conditions.

Mitochondrial Dysfunction

Over the past several decades, research has revealed that mitochondrial dysfunction is routinely associated with common metabolic disorders, including metabolic syndrome, obesity, type 2 diabetes, fatty liver disorders, Alzheimer's disease, and chronic stress; suggesting that cellular energy and mitochondrial function is critically important in the etiology of these complex

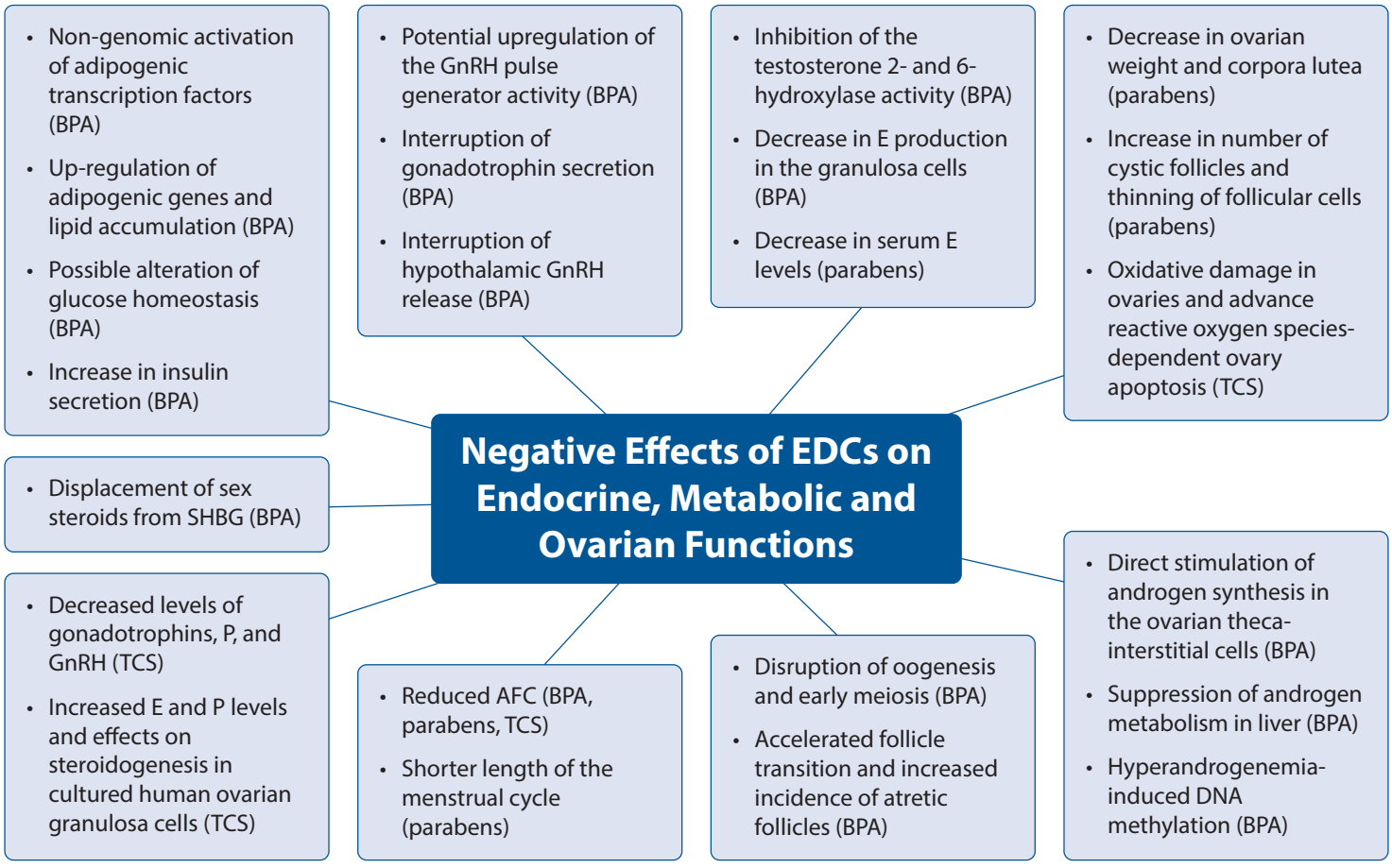
disorders.[74-78] While many mechanisms are thought to contribute to mitochondrial dysfunction, the common result is reduced cellular energy, increase reactive oxygen species (and oxidative damage), reduced beta-oxidation of fatty acids, a depletion of antioxidant reserves and a reduction in mitochondrial biogenesis.[79] Like these other complex metabolic disorders, PCOS is also strongly associated with various measures of mitochondrial dysfunction.[80,81] In particular, researchers have pinpointed mitochondrial dysfunction within ovarian follicular cells, which increases oxidative stress and impairs proper ovarian function in PCOS.[82] This relationship between mitochondrial function, metabolic efficiency and ovarian hormonal balance likely explains why many therapies known to improve mitochondrial health are often noted for improving symptoms related to PCOS.

Lifestyle Therapies for PCOS

As with most other chronic conditions which have strongly-associated metabolic dysfunctions (e.g., type 2 diabetes, fatty liver disorders), the foundation of any comprehensive and sustainable strategy for the treatment of PCOS must include lifestyle-based interventions. The most studied of these interventions focus primarily on diet, physical activity and weight loss, though a comprehensive lifestyle approach will include an emphasis on sleep/circadian rhythm, stress management, and an appropriate interface with environmental signals, both good (e.g., sunlight) and bad (e.g., endocrine disrupting chemicals- see sidebar graphic). Since many of these recommendations overlap with those for subjects with metabolic syndrome, type 2 diabetes, and fatty liver disease, we will focus on defining those that have specifically been tested in subjects with PCOS.

Diet Patterns and Interventions

Since a person's diet can impact several factors known to influence PCOS (e.g., insulin resistance, microbiome diversity, inflammation, etc.), the relationship between diet and PCOS is not at all surprising. There are two primary areas of research that confirm this relationship between dietary choices and PCOS. The first is epidemiological data (or case-controlled studies) that show an association between various dietary patterns and the incidence or severity of PCOS. The other set of data come from interventional trials in subjects with PCOS; where controlled changes to dietary patterns, caloric restriction, macronutrient



Endocrine-Disrupting Toxins and PCOS. Endocrine-disrupting chemicals (EDCs) have profound effects on human metabolism and reproductive function. Clinicians should investigate the toxic burden of EDCs in patients with PCOS, especially bisphenol-A (BPA), parabens, and triclosan (TCS). Image modified from *Life* 2023, 13(1), 138, with permission; in which the references for these relationships can be found.

intake, or meal timing show a statistical reduction in one or more PCOS-dependent measure.[83]

While observational and epidemiological data are often heterogenous and difficult to interpret, several notable dietary patterns that are commonly associated with an increased prevalence of PCOS have emerged.[84] These include diets with high glycemic impact (e.g., diets high in glycemic index foods, high carbohydrate diets, or those low in dietary fiber), diets that measure low on a Mediterranean diet (MedDiet) adherence scale or similar prudent diet score, diets that are generally pro-inflammatory and any diet that increases fat mass or contributes to insulin resistance.[85-89] Thus, the general dietary recommendation for an individual with PCOS would be founded upon a prudent dietary pattern (i.e., the MedDiet) and modified to suit the need for individuality and other health goals.

While obesity is almost always detrimental for individuals with PCOS, research suggests that some phenotypes of obesity (i.e., metabolically unhealthy obesity) may confer greater risk compared to obesity phenotypes characterized by less markers of inflammation and metabolic dysfunction (often called metabolically healthy obesity). In one study of 94 obese women with PCOS, those with an unhealthy metabolic phenotype (statistically higher levels of CRP, testosterone, insulin, HOMA-IR, visceral adiposity, and fatty liver index scores compared to those with metabolically healthy obesity- all $p < 0.001$) scored significantly lower on a MedDiet adherence scale (PREDIMED, OR=0.28, $p < 0.001$).[90]

Diets that restrict carbohydrates, especially those that are considered ketogenic, have also been investigated for their effect in patients with PCOS.[91] A recent meta-analysis of 11 such trials (for weight loss in adult PCOS patients) showed that the ketogenic diet was quite successful in reducing weight, and fat mass in obese and overweight subjects with PCOS.[92] Another meta-analysis (of 7 clinical trials) showed that a ketogenic diet (followed for at least 45 days) was able to significantly reduce LH/FSH ratios and free testosterone levels, while increasing SHBG levels in women with PCOS.[93] While there is some debate about the long-term safety (and efficacy) of a strict ketogenic diet in adults, more research is needed to suggest such a dietary therapy for adolescents with PCOS.[94]

Consistent with our overall dietary recommendation for most metabolic dysfunction, we recommend that women with PCOS be encouraged to follow a MedDiet pattern which focuses on reducing glycemic impact, an intervention that has also been studied with success in women with PCOS.[95] A similar dietary pattern known as the Dietary Approach to Stop Hypertension (DASH diet), which is nearly identical to the MedDiet (often with salt restrictions), is another dietary intervention that is likely to achieve similar results in patients with PCOS.[96,97] The so-called MIND diet (**M**edDiet/DASH Diet **I**ntervention for **N**eurodegenerative **D**elay) is also a likely candidate for an interventional dietary pattern, especially for those concerned about neurocognitive function, though few publications have investigated this specific approach.[98] We also recommend that clinicians emphasize a circadian eating pattern (often called time-restricted eating), whereby calories are consumed during daylight hours and a significant period of time (usually greater than 10 hours) between dinner and breakfast is observed (see below for circadian benefits of this eating pattern.).

Physical Activity and Exercise

As with most chronic metabolic diseases that are influenced by insulin-signaling, physical activity is an important factor in the prevention and treatment of PCOS. In general, higher levels of reported sedentary behavior increase the risk of insulin resistance, obesity and PCOS; whereas higher levels of physical activity lower these same risks.[99,100] Therefore, the primary physical activity recommendations for PCOS are very similar to those of the general public; best summarized by the position statement from Exercise and Sports Science Australia (for PCOS): *individuals with PCOS should aim to undertake between 150 to 300 min of moderate-intensity or 75 to 150 min of vigorous-intensity aerobic activity per week, or an equivalent combination of both spread throughout the week. Additionally, muscle-strengthening activities on two non-consecutive days per week are recommended to maintain health and prevent weight gain. For further health benefits and to achieve modest weight loss, individuals with PCOS should aim for a minimum of 250 min of moderate-intensity or 150 min of vigorous-intensity aerobic activity per week, or an equivalent combination of both spread throughout the week, plus muscle-strengthening activities on two non-consecutive days per week.*[101]

A variety of mechanisms are though to link regular moderate exercise with improved PCOS outcomes, including weight loss, reduced inflammation, reduced oxidative stress, decreased insulin, changes in DNA methylation, improved sleep, improved mood and decreases in measures of stress.[102,103] We should note that while there are many clinicians that advise that subjects with PCOS should avoid intense (long-term) exercise regimens or hyper-training, as this has been associated with increased risk for PCOS; the use of high-intensity intermittent training (HIIT) has been shown to be beneficial for many metabolic parameters in PCOS subjects.[104]

Sleep and Circadian Synchrony

As with many other conditions driven by chronic metabolic and hormonal dysregulations, PCOS is also commonly associated with disruptions in circadian rhythm.[105] The mechanisms that drive this association are complex, as they involve alterations in HPA axis cortisol signaling, dysregulations in melatonin signaling, sleep disturbances and lifestyle (or work) choices that alter normal light/dark signaling. Women with PCOS are more likely to be diagnosed with sleep disorders compared to age-matched controls, and have a higher incidence of obstructive

sleep apnea.[106,107] In a cohort of 724 women (87 of whom had PCOS), sleep disturbances, specifically difficulty falling asleep (odds ratio [OR] = 1.9, 95% CI 1.3–3.0) and difficulty maintaining sleep (OR = 1.9, 95% CI 1.1–3.3), were twice as common in women with PCOS compared to those without.[108] In a survey of 436 subjects with PCOS and 715 control subjects, there was a significant correlation between night shift work and PCOS (OR 1.80, 95% CI 1.18–2.75).[109] This association was observed among those with rotating, but not permanent, night shifts (rotating vs. day work: OR 2.00, 95% CI =1.16–3.44); and a significant association existed between night shift work for >2 years with PCOS (OR 2.08, 95% CI 1.15–3.73).

Women with PCOS should be specifically asked about their quantity and quality of sleep, and evaluated for sleep disorders when indicated. PCOS patients should also be evaluated for circadian cortisol production by measuring diurnal salivary levels (with cortisol awakening response, if possible). This will not only allow the clinician to evaluate the robustness of the diurnal pattern (circadian signal from the HPA axis), but also evaluate how other stressors may be influencing cortisol production. PCOS patients may also be advised to consider circadian eating patterns (i.e., time-restricted eating), where they consume their calories over an 8-10 hour time period (during daylight hours) and fast for the remaining period (16-14 hours). This pattern of eating has been shown in both human and animal studies to reinforce the proper circadian expression of peripheral clock genes, as well as reproductive system signaling.[110,111]

Non-pharmacological Approaches

Inositol(s)

Perhaps the most studied non-pharmacological nutrient therapy for PCOS is inositol, a compound that is critical for both insulin-mediated signaling and hormone-mediated ovarian function.[112] The unique structure of inositol (hexahydroxycyclohexane) allows for nine different isomers, based on the spacial positions of each of the six hydroxy groups, of which myo-inositol and D-chiro-inositol are the most abundant and important for biological signaling (see Figure 3). Though inositol can be synthesized from glucose-6-phosphate (or recycled from phosphorylated inositol signaling molecules), a significant amount of inositol is also consumed in the diet, primary from nuts, seeds, and legumes (as phytates), cantaloupe and most citrus fruits.[113,114]

Inositol isomers are used for many biological activities and are incorporated into several important molecules, including phospholipids (e.g., phosphatidylinositol), and secondary signaling molecules critical for the endocrine signaling of insulin, FSH and TSH (e.g., inositol-1,4,5-triphosphate). Myo-inositol (Myo-I) is the primary isomer in biological tissues, though the

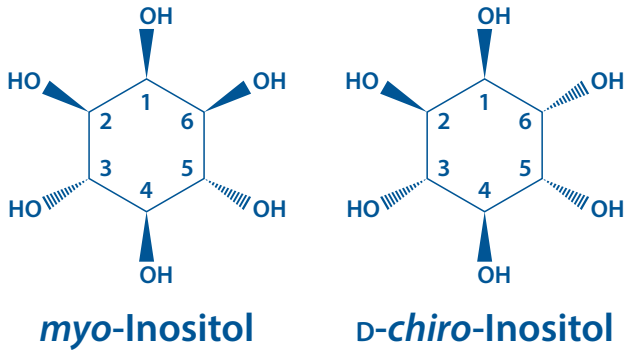


Figure 3: Stereochemical structures of Myo-Inositol and D-chiro-Inositol. These nearly identical molecules only differ in their stereochemistry, where the solid wedges depict the -OH groups coming toward the viewer, and the dashed wedges depict -OH groups positioned away from the viewer.

ratio between Myo-I and D-Chiro Inositol (DCI) differs in various tissues from approximately 65:35 (e.g., adipocytes) to 99:1 (e.g., heart, brain, ovaries).[112] Myo-I can be converted by tissue-specific epimerases into DCI, an action that is modulated by insulin-signaling.[112] Under insulin-resistant conditions (which triggers compensatory hyperinsulinemia), these ratios can shift dramatically. In tissues that rely heavily on insulin-dependent glucose disposal (e.g., fat, liver, muscle), this ratio shifts almost completely toward Myo-I; while in ovarian tissues (which do not lose their sensitivity to insulin) the oppositive seems to occur, causing a dramatic shift toward DCI.[115,116,117] This insulin-dependent shift toward a DCI-dominant inositol pool within ovarian follicles is considered to be a critical factor in altering FSH signaling and promoting androgen synthesis in PCOS.[118] This phenomenon suggested a potential role for inositol supplementation in modulating both insulin signaling and ovarian hormone function in women diagnosed with PCOS.

Over the past several decades, researchers have performed numerous clinical studies using both Myo-I and DCI, alone or in combinations of various ratios, in subjects with PCOS. The early studies were summed up in a document from the International Consensus Conference on Myo-inositol and d -chiro-inositol in Obstetrics and Gynecology, published in 2013.[119] They concluded that "*the experimental data actually give convincing support to the notion that both MI and DCI are involved in several biological pathways, namely those related to the transduction of insulin signal. Clinical data demonstrated that inositol(s) supplementation could fruitfully affect different pathophysiological aspects of disorders pertaining Obstetrics and Gynecology. The treatment of PCOS women as well as the prevention of GDM [Gestational Diabetes] seem those clinical conditions which take more advantages from MI supplementation, when used at a dose of 2 g twice/day. The clinical experience with MI is largely superior to the one with DCI. However, the existence of tissue-specific ratios, namely in the ovary, has prompted researchers to recently develop a treatment based on a MI/DCI combination (ratio 40:1). Such approach seems promising.*"[119] Since

2013, numerous other reviews have been published evaluating the clinical trials using inositol supplementation in subjects with PCOS.[120,121,122] A recent review and meta-analysis of 26 clinical trials showed that inositol supplementation was both a safe and effective treatment for improving menstrual cycle regularity, decreasing free and total testosterone, androstenedione, glucose and insulin in subjects with PCOS (compared to placebo); and was statistically non-inferior compared to the standard-of-care therapy- metformin.[123]

Both Myo-I and DCI, when used as oral therapeutics, have resulted in positive outcome in subjects with PCOS; however most clinical trials have been performed with Myo-I (alone) or in a 40:1 ratio with DCI (considered to be the "normal" plasma ratio of these isomers) following Nordio et al.[124,125] Many of these studies delivering this combination used a product called Inofolic® Combi (LO.LI. Pharma) which provided 550 mg of Myo-I, 13.8 mg of DCI and 200 mcg of folic acid in a soft gel capsule (given twice per day in most studies).[126] This 40:1 ratio of Myo-I:DCI proved superior to other isomer ratios in one animal model of PCOS.[127] The only human clinical trial to compare different inositol isomer ratios in subjects with PCOS was performed by Nordio et al ("Sapienza" University, Rome, Italy.), and published in 2019.[128] In this study, 56 women with PCOS were divided into seven groups (eight women in each group), each given 2 grams BID of one of the following Myo-I:DCI mixtures: 0:1 (i.e., DCI alone), 1:3.5, 2.5:1, 5:1, 20:1, 40:1 and 80:1. Supplementation was given for 3 months and participants were evaluated for ovulation (each month by progesterone assay) and improvements in hormone and metabolic parameters (e.g., FSH, LH, SHBG, FreeT, Insulin, HOMA-IR, etc.). The greatest level of improvements across nearly every parameter measured was realized by the women taking the 40:1 mixture, followed by those in the 20:1 and 80:1 groups. Progesterone increased in these three groups the greatest and menses were recorded in 5 (of 8) women in the 40:1 arm, 4 (of 8) in the 80:1 arm and 3 (of 8) women in the 20:1 arm. No women in the 0:1, 1:3.5, or 2.5:1 arm achieved menses or meaningful increases in progesterone throughout the 3-month study.

Inositol supplementation should be considered a safe and effective first-line therapy in women with PCOS, with or without concomitant insulin resistance. Supplementation with a 40:1 mixture of Myo-I: DCI, which mimics the ratio found in healthy human plasma, is commonly recommended based on the limited dose-comparison studies performed in animals and humans with PCOS.[129] Successful outcomes have been reported for inositol doses from 1100 mg/day to 4000 mg/day; though the 4000 mg/day dose is most commonly used.[123] Individuals with a higher BMI are likely to benefit from these higher doses, while individuals with a lower BMI may achieve benefits at the lower end of this dose-range.

Vitamin D

After inositol, vitamin D is the most frequently studied non-pharmacological therapy used in research studies for PCOS.[130] There is extensive research showing the importance of vitamin D signaling in the function of reproductive tissues of both males and females, especially in the process of follicle recruitment and maturation, and estrogen synthesis.[131,132] Lower vitamin D status is more common and is correlated with greater metabolic dysfunctions in women with PCOS.[133,134] In a secondary analysis of the Pregnancy in PCOS I (PPCOS I) study, baseline serum 25-OH vitamin D (25OHD) levels were reported in 540 women with PCOS given one of three ovulation induction therapies (clomiphene citrate, metformin, or both).[135] Evidence of ovulation was observed in 74% of the cohort (402 of 540) over the 6-month trial duration. Vitamin D deficient women were less likely to achieve ovulation compared to those with 25OHD levels ≥20 ng/mL (P = .006). The overall live birth rate was almost 19% (112 of 540). Compared to a live birth rate of 26% in those with sufficient levels of vitamin D (38 of 148), the likelihood of live birth declined progressively in those with vitamin D insufficiency (33 of 207; OR, 0.74; 95% CI, 0.57, 0.96), deficiency (25 of 143; OR, 0.61; 95% CI, 0.35, 1.08), and severe deficiency (6 of 42; OR, 0.48; 95% CI, 0.19, 1.23). According to these authors: "*These observations allow us to propose that a circulating 25OHD level of 45 ng/mL (112.5 nmol/L) or higher be considered as "optimal" for women attempting to conceive.*"

We recommend that all subjects with PCOS be evaluated for their vitamin D status. Clinicians should consider oral vitamin D3 supplementation for anyone with a baseline 25OHD level <30 ng/ml, with a goal of reaching a target 25OHD level of between 40-70 ng/ml. The level of oral vitamin D3 needed to reach this target level is likely to be higher in subjects with a higher BMI. Numerous clinical trials, with positive outcomes in subjects with PCOS, have safely used doses of 4,000 IU/day or weekly doses of 50,000 IU (though a wide-range of doses have been successfully used).[136,137]

Omega-3 Fatty Acids

Optimizing omega-3 status (i.e., achieving an omega-3 index >8%) is strongly recommended for reducing cardiovascular and all-cause mortality.[138] Supplementation of long-chain omega-3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are also commonly recommended in subjects with obesity, type 2 diabetes, insulin resistance, and chronic inflammation, all conditions which are higher in subjects with PCOS. Based simply on these risks alone, women with PCOS are likely to benefit from improved omega-3 status by increasing EPA and DHA consumption through diet and/or supplementation. In the past few decades, omega-3 supplementation has been evaluated in women with PCOS with mixed outcomes, depending on what outcomes were measured (i.e., metabolic, hormonal, fertility, etc.).[139] A

meta-analysis of 10 RCTs where omega-3 supplementation was given to women with PCOS revealed a reduction in C-reactive protein, serum malondialdehyde, luteinizing hormone and serum total testosterone; and an increase in total antioxidant capacity and serum sex hormone binding globulin.[140] Others studies have shown that omega-3 fatty acid supplementation improves biomarkers of lipid and insulin metabolism in subjects with PCOS, such as total cholesterol, LDL cholesterol, triglycerides, adiponectin and HOMA-IR.[141] Furthermore, omega-3 supplementation has been shown to reduce measures of depression, anxiety and perceived stress, all of which are more common in women with PCOS that control subjects.[142,143]

Antioxidant Supplementation

According to several observational studies, women with PCOS have a lower total antioxidant capacity and higher biomarkers of oxidative stress.[144] While obesity, insulin resistance, and inflammation contribute much to the systemic burden of oxidative stress, ovarian cells are particularly sensitive to oxidation (perhaps as a protection against pregnancy during biological stress).[145] Therefore, the role of antioxidant supplementation has been explored as a means to reduce oxidative stress and metabolic abnormalities, and improve ovarian function in women with PCOS.[146,147] Common antioxidants used in these studies include vitamin E, coenzyme-Q10, zinc, selenium, N-acetyl cysteine, lipoic acid and melatonin; all with promising, though sometimes, heterogeneous results.[148-152]

The antioxidant **N-acetyl cysteine (NAC)**, the acetylated form of the amino acid L-cysteine, has emerged as a promising therapeutic antioxidant in PCOS subjects.[153,154] NAC is a multifunctional antioxidant molecule having both direct antioxidant activity (via thiol interactions with specific reactive oxygen or nitrogen species, and chelation of active redox metals) and indirectly (as a precursor for glutathione synthesis, and releasing free thiols).[155] NAC's thiol reducing capacity can also break disulfide crosslinked mucus proteins, hence its approval as a mucolytic agent. NAC is used for a wide-range of health concerns, including both male and female infertility, as well as PCOS.[156,157] NAC supplementation in PCOS subjects (commonly at 1.2 grams/day, most studies performed in Iran or Egypt) generally improves metabolic parameters, reduces total testosterone and has been shown to improve ovulation (when compared to ovulation induction therapy alone).[158,159]

Zinc is an important antioxidant mineral cofactor, involved in both insulin signaling and oocyte quality.[160,161,162] Based on several observational studies, women with PCOS are more likely to have a low zinc status than age-matched control subjects.[163,164] In women with PCOS, low zinc status is associated with higher levels of hyperandrogenism, hirsutism, fasting insulin and other biomarkers of metabolic dysregulation.[165] While there are limited studies using zinc (alone) in subjects with PCOS to know the specific role of zinc supplementation in subject with PCOS, clinicians should evaluate the zinc status of subjects with PCOS and ensure proper zinc intake (via diet or supplementation) is recommended.

Insulin-Sensitizing Nutraceuticals

Since insulin resistance is one of the hallmarks of PCOS, many studies have evaluated nutraceutical compounds that are known to improve insulin signaling or glucose disposal in subject with various (non-PCOS) metabolic disorders. Here we summarize the data of those that have specifically been investigated in subjects with PCOS (or similar animal models).

Alpha-Lipoic Acid (LA, a.k.a. thioctic acid) is a natural and versatile antioxidant able to "recharge" vitamin C, vitamin E, and glutathione via its three-fold water-soluble, fat-soluble, and sulfhydryl properties. These activities affect a variety of cell-signaling pathways altering glucose metabolism or the pathways leading to complications of hyperglycemia. LA is endogenously synthesized in the mitochondria via lipoic acid synthase and is an essential cofactor for alpha-ketoacid dehydrogenase. LA exists in an oxidized (disulfide) and a reduced form (dithiol: dihydrolipoic acid, DHLA), both of which have antioxidant properties. Exogenous LA in the diet is made available to tissues, where a substantial portion is converted to DHLA via lipoamide dehydrogenase. LA has been used in a number of clinical trials in adult subjects with type 2 diabetes, insulin resistance and obesity.[166] Both the antioxidant and insulin-potentiating activities of LA make it a good candidate for use as a therapeutic agent for PCOS. In fact, numerous clinical trials have been conducted using LA, alone or in combination with inositol, in subjects with PCOS.[167] These clinical trials, though often small and/or uncontrolled, show a consistent benefit in metabolic biomarkers in subjects presenting with insulin resistance and, when combined with myo-inositol, show beneficial changes in endocrine biomarkers or oocyte quality.[168,169] LA has also been combined with metformin and pioglitazone in subjects PCOS, with favorable clinical benefits.[170,171] The minimum dose of LA that was shown to be beneficial was 400 mg/day, though most studies used 600-1200 mg/day.

Berberine, and related alkaloids, have a long history of medicinal use in the herbal traditions of both the East and West. These alkaloids are found in the roots, rhizomes and stem bark of numerous plants like *Berberis aquifolium* (Oregon grape), *Berberis vulgaris* (barberry), *Berberis aristata* (tree turmeric), *Hydrastis canadensis* (goldenseal), *Xanthorhiza simplicissima* (yellowroot) and *Coptis chinensis* (Chinese goldthread, the most common source for commercial berberine HCl/sulfate ingredients). While its most noted historical quality is as a compound with antimicrobial, antifungal and immune enhancing properties, it has recently been discovered to be a potent modulator of insulin-signaling improving a host of biomarkers of metabolic dysregulation.[172-175] Consequently, berberine is considered to be a promising therapeutic agent in

subjects with PCOS.[176,177] While few studies have been performed using only berberine, the use of 1000-1500 mg of berberine/day appears to improve the metabolic and endocrine outcomes when added to common therapies for PCOS (i.e., metformin, letrozole, etc.).[178] A recent meta-analysis of 10 studies, showed that berberine improved ovulation and pregnancy rates in Chinese subjects with PCOS given oral ovulatory therapeutics.[179] While most studies have been performed on Chinese subjects, one study of 50 women with PCOS from Italy, who were given 500 mg twice/day, suggests that these results may be more universal in women with PCOS.[180]

Chromium plays an important function in insulin receptor signaling and has been successfully used to improve biomarkers of glucose disposal and insulin metabolism in some, though not all, cohorts of insulin resistant or type 2 diabetic subjects.[181] Chromium supplementation (as picolinate) has also been investigated for its metabolic benefit in subjects with PCOS with heterogenous results. In fact, three meta-analyses have been performed on the clinical trials using chromium supplementation in women with PCOS.[182,183,184] As with chromium studies in non-PCOS subjects, many metabolic parameters remained unchanged; however, in PCOS subjects chromium supplementation generally improved HOMA-IR and lowered free testosterone levels. Clinicians should ensure PCOS patients are consuming adequate chromium levels to ensure optimal insulin-dependent glucose disposal. Supplementation with 400-800 mcg/day of chromium is a simple way to ensure adequate chromium levels in most subjects.

Herbal therapies for PCOS

Herbal/botanical remedies are commonly used for both menstrual and fertility-related difficulties within most indigenous and traditional medicine systems. However, clinical studies using these remedies is subjects diagnosed with Western definitions of PCOS are less common. Many herbal therapies that have shown positive benefits in clinical trials are likely to be agents that are already known to have antioxidant, anti-inflammatory, or insulin-sensitizing benefits. These include **curcumin**, **resveratrol**, **quercetin**, and **cinnamon**.[185-188] The list is much longer if we consider animal models.[189] Surprisingly, those herbal remedies most known for their regulation of menstrual cycle irregularities or hyperprolactinemia, such as *Vitex agnus castus* (Chaste berry) or *Paeonia lactiflora* (White Peony) have limited research in subjects with PCOS.[190]

Microbiome Modulation through Prebiotics and Probiotics

Modulating the signals coming from the gut microbiome by means of added microbes (i.e., live- probiotics or dead-postbiotics) or the use of specific nutrients designed to promote the growth of metabolically-favorable commensal organisms (i.e., prebiotics) is well-established as a way to influence host metabolism; especially related to obesity, insulin resistance and metabolic syndrome.[191] This same therapeutic approach has also been demonstrated to be beneficial in women with PCOS; for both metabolic-related and endocrine-related outcomes. However, the wide range of studies performed to address the benefits of these therapeutic agents, as summarized in several recent reviews and meta-analyses, are often heterogenous and difficult to apply across different available products.[192,193,194] Nonetheless, several studies are worth noting as clinicians evaluate how to leverage the potential benefit of microbiome modulation in subjects with PCOS.

Several studies have shown the potential benefit of prebiotics in subjects with PCOS, particularly inulin and resistant starches. Inulin, a fructan polysaccharide most often derived from chicory, is a commonly used prebiotic known for its ability to promote metabolically-favorable commensal organisms which results in the production of short-chain fatty acids (e.g., butyrate) and a host of beneficial metabolic outcomes.[195] In one study of 75 women with PCOS, subjects were randomized to receive 10g/day of one of two forms of inulin-like fructans (or maltodextrin placebo). After 12 weeks, the women receiving the inulin supplementation had statistical improvements in metabolic outcomes, androgen status and reported lower incidence of irregular menses and oligomenorrhoea.[196,197] Another study in women with PCOS showed significant changes to gut microbiota and intestinal-derived inflammatory signals after 3 months of inulin supplementation (15 g/day).[198] Inulin has also been shown to have benefits in several animal models of PCOS, even showing similar results to metformin in one study.[199,200] Resistant starch describes a broad category of polysaccharides from a variety of sources that are characterized by their resistance to human digestion allowing them to be fermented by colonic bacteria and promote healthy metabolic signals.[201] In one study, resistant dextrin, given at 20 grams/day for 3 months, was shown to improve metabolic biomarkers when given to women with PCOS.[202] Other forms of resistant starch also show promising microbiome-related mechanisms in PCOS-related animal models.[203,204]

Probiotics have also been used in subjects with PCOS, with mixed results. This is primarily due to the fact that clinical trials using probiotics are performed with different bacterial species, combinations of species, doses and duration (or in combination with other agents already known to affect PCOS); all of which make it difficult to form clear recommendations for the use of specific probiotics in women with PCOS. In general, when the use of probiotics improves biomarkers of dysbiosis or inflammation, they are also likely to improve biomarkers of metabolism and, when measured, PCOS-related endocrine biomarkers.[205] Clinicians should prioritize gut microbial health in all women with PCOS, utilizing diet, lifestyle and appropriate supplementation of prebiotics and probiotics (called synbiotics, when delivered together) to improve microbial metabolic signaling.

Summary

PCOS is a complex multifactorial metabolic disorder whose etiology involves genetic, epigenetic, environmental and lifestyle components. While hyperandrogenism and insulin resistance are often the core dysfunctions driving PCOS symptoms, other contributing factors include mitochondrial dysfunction, gastrointestinal dysbiosis, fatty liver disease, lipid abnormalities, inflammation and oxidative stress. However, since many of these dysfunctions are metabolically-linked, a concerted effort should be made to address a patient's underlying dysfunctions (e.g., obesity, insulin resistance, inflammation, circadian disruptions, etc.) will benefit most of these abnormalities. For most women with PCOS, lifestyle-based therapies such as diet, physical activity, stress management, sleep and avoidance of endocrine-disrupting toxins are foundational for their overall health and PCOS-related signs and symptoms. Furthermore, the use of non-pharmacological therapies to improve insulin sensitivity, reduce oxidative burden and promote ovarian function in women with PCOS shows great promise. Based on the available evidence, inositol, vitamin D and N-acetyl cysteine and zinc show the greatest promise and can be used in combination. All subjects with PCOS should be evaluated for adequate omega-3 status, as well as assessed for other common nutrient deficiencies and insufficiencies. Increasing dietary and supplementary polyphenol intake is also likely to benefit subjects with PCOS, and should be encouraged to promote a healthy gut microbiome, reduce inflammation and increase antioxidant capacity. Depending on the patient goals, many of these therapies have also been tested as safe and effective adjunct therapies during ovulation induction therapies.

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