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Chapter

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Abstract

Polycystic ovary is part of an endocrine syndrome in which different pathophysiological mechanisms lead to a similar reproductive outcome: anovulation, irregularity of the menstrual cycle, and infertility. Hormonal and metabolic disorders are associated with reproductive adverse outcomes, which represent a vicious circle with dysfunctional ovaries. Hyperandrogenemia, disorder of carbohydrate metabolism, and sex hormone synthesis led to reproductive abnormalities. One of the most crucial questions still remains, whether the polycystic ovary is the cause or the consequence of all known disorders. Inositols are in the treatment of PCOS capable of restoring ovulation with the impact on the carbohydrate metabolism, by increasing the sensitivity of cells to insulin, which releases the sex hormones-binding protein and improves hyperandrogenemia. Nine stereoisomers of inositol are known, myo-inositol and chiro-inositol are the most studied in the reproductive age. By normalizing the level of androgens in the blood, the growth of and the balance of sex hormones is established. A variety of metabolic pathways of these molecules are recognized in different tissues, such as fat, muscle, or ovarian tissue. Still, it is not clear which isomer has better reproductive or metabolic effects, and there are controversies about their effectiveness in the treatment of reproductive disorders.

Keywords: D-chiro inositol, inositol, myoinositol, polycystic ovary, pregnancy

1. Introduction

Polycystic ovarian syndrome (PCOS) is defined as a heterogeneous disorder involving a variety of serious clinical manifestations, hormonal disorders, unbalanced metabolic functions, and psychological disorders in women of reproductive age with the main impact on infertility [1]. Rotterdam criteria covered all aspects of this well-acknowledged syndrome with a wide variety of clinical presentations. The four phenotypes of PCOS syndrome are defined, and there is one fundamental criterion—hyperandrogenemia with or without hyperandrogenism in three phenotypes [2]. Evidence suggests the role of different external and internal factors as disorders in the metabolism of carbohydrates with hyperinsulinemia and insulin

resistance (IR). Metabolic disorders have an impact on hormonal production, causing serious hormonal dysfunction and disorder that interferes with the dysfunction of ovaries. Polycystic ovary is characterized by a significantly larger content of primordial follicles, therefore enhanced number of granulosis cells that synthesize up to 75 times more anti-Müllerian hormones in granulosa cells of normal ovaries [3]. In women of reproductive age, anovulation, and irregular, extended menstrual cycles are the main causes of infertility. Anovulation is treated primarily by ovulation induction with clomiphene citrate or aromatase inhibitors. Failure of ovarian response in PCO patients on clomiphene induction has introduced gonadotropins as an optional medicamentous treatment to achieve ovulation. The use of gonadotropins includes significant risks of hyperstimulation, thrombo-embolic incidents, or ovarian torsions with possible ovarian necrosis. Niche for the introduction of new promising molecules for therapeutic indications as a useful, effective, and safe tool for restoring spontaneous ovulation has put inositols in focus. Inositols became very interesting and promising molecules due to their biochemical pathways, which are safe in their action and have an effective role in the metabolism of carbohydrates, just like insulin. Inositols are messenger molecules with an impact on enhanced insulin effect in the cells, and specific foods contain them in significant quantities. A positive effect of inositols on ovarian function, ovulation, carbohydrate metabolism, infertility treatment, the incidence of gestational diabetes, and eutrophic newborns after infertility treatment was observed. Inositols improve development of follicules and oocytes, oocyte maturation, fertilization, implantation, and post-implantation development, bringing positive outcomes in IVF procedures in patients with PCOS by reducing the occurrence of gestational diabetes. Reproductive abnormalities are more common in women with higher BMI and continue to rise with higher BMI. Obese women are more likely to have anovulatory cycles and menstrual irregularities. Obesity complicated by metabolic abnormalities can further worsen the outcome of treatment in PCOS patients. Inositols as "messenger" molecules that have a role in insulin signaling with increase effects of insulin intracellularly through phosphoglycan mediators. Naturally, inositols are synthesized mostly in the kidney, with the highest concentration in the brain, where they play a substantial role in neurotransmitters and some steroid hormones binding to their receptors. There are nine isomers of inositol, and differences in the action of these molecules in different tissues, such as fat, muscle, or ovarian tissue, have also been noted. It is not entirely clear which isomer has better reproductive or metabolic effects, and there are controversies and discussions about their effectiveness in the treatment of reproductive disorders, which we will present in this chapter.

2. Metabolic consequences and effect of insulin and inositol

Mechanisms of insulin action and signal transmission to cells of various tissues that use glucose as an energy source are well known. Insulin forms a complex with the insulin receptor that activates phosphatidyl inositol-3 kinase, which is an enzyme that increases the concentration of *phosphoinositides* (PIPs) in cells that integrate molecules into the cell membrane. Four cellular processes are activated in this way: glucose uptake, glycogenesis, antigluconeogenesis, and antilipolysis [4]. Phosphatidyl inositol-3 kinase also activates another messenger of insulin, phosphatidyl inositol, which also stimulates all metabolic activities of insulin and therefore acts synergistically with insulin and enhances its action.

Inositols generally enhance the effects of insulin and regulate intracellular functions: *phosphoinositides* (PIPs)—integrate molecules into the cell membrane *inositol polyphosphates* (InsPs)—are cytoplasmic "second" messengers and *inositol phosphoglycans* (IPGs) regulate mitochondrial metabolism [5]. Inositols are very stable polar molecules that belong to the family of nine *hexahydroxycyclohexane stereoisomers*. They were initially isolated from muscles and later from numerous cells and tissues of mammals. In addition to insulin signal transmission, they are very active in cellular processes such as calcium transport, association of cytoskeletal proteins, lipid metabolism, modulation of the serotoninergic pathway, cell growth and differentiation, and oocyte maturation. Inositol isomers have been proven to have an important role in various medical conditions such as neurodegenerative diseases (scyllo inositol), diabetes (D-chiro inositol, DCI), malignant diseases, metabolic syndrome, or PCO syndrome (myo-inositol, MI).

Myo-inositol is the most widespread molecule among inositols in nature and mammalian cells (99%); the rest is D-chiro inositol (1%). Myo-inositol is converted into DCI by epimerization (the metabolic pathway is stimulated by the tissue-specific NAD/NADH epimerase that is dependent on insulin), which significantly changes the concentration ratio of these molecules in tissues such as the liver, adipose tissue, or muscle, respectively, to the brain. Myo-inositol is found in cells in free form or bound in cell membranes (in the composition of *phosphoinositide*), i.e., it is a protector of hyperosmotic stress in cells. As a second messenger, it regulates the activity of a number of hormones, such as follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and insulin. In mammals, a tissue-specific epimerase converts MI to DCI in an insulin-dependent manner, and different ratios of these epimers have been confirmed in different tissues. D-chiro inositol is an important molecule in the insulin signaling pathway and, together with galactosamine, significantly affects molecular mechanisms (activation of molecules that alleviate the consequences of glucose metabolism disorders). However, paradoxically in women with IR and pre-eclampsia the concentration of DCI is elevated and it is suspected that DCI contributes to IR [6, 7].

3. Ovary, insulin resistance, and hyperandrogenemia

Ovarian function disorders in PCOS represent a growing heterogeneous, multifaceted complex entity, combined with a metabolic disorder caused by deterioration in the metabolism of carbohydrates and insulin resistance (IR). Insulin resistance is defined as a subnormal biological response to normal insulin levels, decreased sensitivity, or response to metabolic action of insulin. Clinically, insulin resistance can be described as the insufficient cell response of a known quantity of endogenous or exogenous insulin to increase glucose uptake and reduced sensitivity or answer to the metabolic effect of insulin. The significant association of PCO syndrome and IR is seemingly, obvious highly, complex and problematic. The frequency of association between IR and PCO according to the literature, depends on the studied population and different criteria used in the published studies. Although IR is significantly associated with PCO syndrome, it is not established as diagnostic criteria for PCO syndrome. Similarly, there are no general recommendations for screening for IR in patients with PCO syndrome. Obesity is frequently associated with PCO syndrome and plays a key role in hyperinsulinemia and IR. There are debates about interactions and relations of obesity, IR, and PCO syndrome. Accumulation of adipocytes

in adipose tissue plays an important role in the endocrine function of the ovary. The enhanced production of leptin in adipocytes inhibits the expression of aromatase mRNA in granulosa cells, causing interruption of androgen conversion to estrogen. Production of adiponectin in adipocytes has insulin-sensitizing, anti-diabetic, and anti-inflammatory effects. It is unclear whether obesity is the cause of IR or worsens already-existing IR in PCO syndrome. Published data indicate that obesity is an additional circumstance that aggravates IR in patients with already previously existing IR [8, 9]. Systemic hyperinsulinism can be endogenous (obesity, gestational diabetes, diabetes type 2, extreme IR caused by mutation of the insulin receptor gene, autoantibodies on insulin receptor, insulinoma) or exogenous (diabetes mellitus type 1). HOMA-IR is a frequent parameter used in estimating IR in large epidemiological population studies and is calculated as a ratio of fasting insulin and glucose values. HOMA-IR can be increased in code-slender or obese patients with PCO syndrome in comparison to patients without PCO syndrome. According to the published results [9], cut-off value of HOMA-IR is 3.15 for patients with PCO syndrome, while in the general population, the defined cut-off value of HOMA-IR is 2.5. The assessment of insulin resistance is one of the major questions for clinicians, remaining how to distinguish wide variability in insulin-sensitive female patients in relation to insulin insensitivity and what criteria are acceptable in different ethnic groups. The fact is that not all women with IR have PCO syndrome, and the same IR is not present in all women with PCO syndrome. Insulin levels depend on several factors, such as metabolism and clearance of insulin, enzymatic degradation of the insulin - receptor complex, obesity, age, and androgen level. It should be taken into concern during the evaluation of the patient. To conclude, there might be some additional factor that leads to hyperandrogenism, which is an obligatory diagnostic criterion included in PCO syndrome and associated with hyperinsulinemia or some additional trigger. Hyperandrogenism has an important impact on the mechanism of PCOS, including IR, inflammation, and oxidative stress. Hyperandrogenism aggravates IR through different routes, by inhibiting insulin degradation in the liver and reduction of insulin sensitivity. Still, we need to define the necessary criterion for the diagnosis of HA as an additional trigger for the development of PCO syndrome with hyperinsulinemia.

Insulin resistance and compensatory hyperinsulinism, contribute to increased production of androgens. Insulin has a modality as gonadotropin in the ovary triggering ovarian steroidogenesis, stimulating androgen synthesis in the adrenal glands, and modulates pulsatility of LH by increasing LH binding sites and androgen-producing response to LH. Hyperandrogenemia is responsible for reproductive disorders like ovulatory dysfunction, anovulation, oligomenorrhea, infertility, acne, and alopecia, which are clinical signs of PCO syndrome. Hyperandogenemia is associated with obesity and increased visceral fat, which are not rare in patients with PCO syndrome. An increase in fat tissue promotes IR, HA, and hyperinsulinemia, and again, we are in the same circle of alternating disorders [1]. The genomic, transcriptomic, and proteomic profile of visceral fatty tissue in women with PCO syndrome is different from that of healthy women, more similar to a man's fatty tissue, and hence suggests the metabolic effect of HA on typical obesity in PCO syndrome [10]. Beyond the multifactorial pathophysiology of PCOS, one of the hypotheses suggests a pathogenetic role of high levels of androgens that are the main cause of abdominal and visceral fatty tissue accumulation. Both stimulate IR and compensatory hyperinsulinism. Hyperinsulinism is responsible for androgen synthesis in the ovary and adrenal glands and promotes leptin-mediated inflammation in women with PCO syndrome, so the vicious circle closes [1]. Heterogeneity of PCO syndrome most likely arises from

obesity, abdominal fat, and IR. Central hepatic insulin resistance can be present in slender patients with PCO syndrome, while the peripheral IR is tied to adipose tissue and muscles and is characteristic of female patients who are obese.

Since some inositols (MI, DCI) are mediators of insulin, they can potentially change the metabolism of various tissues. Inositols are credited with acting as a second messenger, in response to external or endocrine signals, reducing IR, improving ovarian function by reducing androgen levels, and alleviating metabolic, menstrual, and cutaneous hyperandrogenic features of PCOS. Noticeably, inositols are involved in the action of several endocrine systems (insulin, thyroid hormone, gonadotropins) and lipids with hormone-like activity signals (as prostaglandins). Myo-inositol and DCI are the two most abundant members of nine stereoisomeric inositols. The specificities of the inositols metabolism in the ovary and differences compared to other tissues are known. D-chiro inositol is synthesized *in vivo* by epimerization from MI, and the degree of epimerization defines insulin-dependent epimerase. The ratio of MI and DCI concentration in ovarian tissues is 70:1, while the ratio in women with PCOS is pathologically decreased. Type 2 diabetes mellitus, with its pathophysiological pathways, can dramatically impair DCI levels, resulting in low intracellular levels of DCI due to reduced activity of epimerase (hyperinsulinemia reduces activity of epimerase). In PCO syndrome, ovaries are in a state in which tissue-selective resistance to metabolic effects of insulin seems to be contradictory and associated with ovarian sensitivity to insulin unlikely liver, fat, and muscle tissue. In PCO syndrome with pronounced hyperinsulinemia, in the ovary, there is an accelerated conversion of MI to DCI (increased ratio, excessive synthesis of DCI) due to accelerated epimerization. As a consequence, deficiency of MI in the ovary causes specific reproductive disturbances [11]. This phenomenon, called the D-chiro inositol paradox, is a rebound effect on hyperinsulinemia. The mechanism involves tissue-specific metabolic pathways that decrease the synthesis of DCI in the liver, muscles, and fat tissue as a response to hyperinsulinemia; under the same circumstances, DCI synthesis increases within the ovaries while synthesis of MI is lacking. Therefore, DCI is effective in treating and reducing IR but not in reducing ovarian disorders in PCO patients [12].

4. Treatment of reproductive disorders with inositols

Reproductive abnormalities in PCO syndrome may appear with different clinical presentations. Disorders of ovulation, oligomenorrhea, amenorrhea, and menstrual cycle irregularities are the most common infertility causes in patients with PCO syndrome [13]. The different clinical PCOS phenotypes require proper assessment of the biochemical and medical features to adopt the best pharmacological treatment and therapeutic strategy in patients with PCO syndrome. The aim of the pharmacological treatment may be hyperandrogenemia, oligo-ovulation, or IR. The therapeutic management and selection of the best therapy should be done upon the clinical features of the target patient and her priorities—regulation of menstrual disturbance, ovulation abnormalities, oligo-ovulation, hyperandrogenism or IR, or altogether. Before drug administration, diet and healthy lifestyle advice must be given to all patients with PCOS regardless of their weight.

Physical activity and exercise (150 min of moderate or 75 min of intense exercise per week) play a key role in weight reduction and the prevention of obesity. That approach has been proven to be the best way to improve insulin sensitivity. Weight

reduction with calorie intake restriction and the introduction of physical activity would be the first step in treatment for obese women diagnosed with PCO syndrome. With weight loss, the free testosterone level decreases, as does the incidence of metabolic syndrome. Changes in lifestyle and diet lead to lower insulin and free androgen levels, inducing the restoration of body composition, hyperandrogenism, and IR. There is very much to discover, for a better understanding of pathogenesis, how the reduction of fat tissues modifies all metabolic pathways and improvements in the metabolism of glucose or lipids, reproductive outcome, mood, quality of life, and therapy satisfaction [14]. Weight reduction leads to better self-esteem.

Hyperinsulinemia and IR are key goals of treatment in patients with PCO syndrome and include the administration of insulin-sensitizing drugs (e.g. metformin). There is still no definite cure or medication for this heterogeneous endocrine disorder with various clinical appearances. The routine approach after advising on lifestyle modification and weight loss is symptomatic therapy with different agents including oral antidiabetics, progesterone, contraceptives, or antiandrogens. The efficiency and efficacy of some other drugs as thiazolidinediones, berberine, or inositols have not been enough investigated and the effects of these drugs are still unclear in infertility treatment.

Classic gynecological treatment in reproductive-age patients with PCO syndrome implies regulation of the menstrual cycle with the administration of combined oral hormonal contraception or progestins. Induction of ovulation is required in PCOS patients with anovulatory cycles if there is a desire for motherhood. The first-line treatment for ovulation induction is clomiphene citrate and letrozole, especially if it is a fertility problem tied only to anovulation without co-existing male factor or tubal obstruction. In the cases of combined male–female infertility, couples are treated with assisted reproductive technologies (*in vitro* fertilization) and stimulation of ovaries with gonadotropins. The use of gonadotropins carries significant risks of ovarian hyperstimulation, and that's why they have indicated targeted protocols for ovarian stimulation. From the point of patient safety, treatment with an antagonist for ovarian stimulation and the "freez all" approach are the best options if there is ovarian hyperstimulation. Ovarian stimulation in agonistic protocol in combination with metformin can reduce the risk of hyperstimulation [15]. Some patients are resistant to ovulation inductors due to persistent hyperandrogenemia, hyperestrogenemia, and IR. This abnormality is further worsened in overweight PCOS patients, especially with visceral and abdominal fatty tissues, since SHBG level is reduced due to hyperinsulinemia, and androgen levels are increasing. Since hyperinsulinemia stimulates androgen synthesis in patients with PCO syndrome, the attention of researchers is focused on inositol phosphoglycans as post-receptor mediators or secondary messengers of insulin signals [16].

Administration of MI in patients with PCO syndrome after 12 weeks has a positive effect on the hormonal status of the patients. It has proven a significant reduction in the concentration of LH, PRL, androgens, and of LH/FSH ratio and ratio glucose/insulin. Increased sensitivity to insulin had been observed by reduction of HOMA-IR. In PCO patients treated with *in vitro* fertilization procedure and inositols it has been observed shorter duration of ovarian stimulation and minor total dose of applied gonadotropins. Severe ovarian hyperstimulation syndrome is significantly more often present in patients with PCO syndrome and associated with increased number of antral follicles. The positive effect of MI was observed in improved response to ovarian stimulation. The group of PCOS patients treated with

MI had significantly more large follicles (> 16 mm) on hCG day administration, compared to control group, which had significantly more small follicles (< 12 mm). The application of MI contributes not only to the safety of infertility treatment but also to the better quality of the follicles. The final reproductive outcome, the proportion of conceived pregnancies and births, decreased incidence of gestational diabetes, and giving birth to eutrophic children are also significantly better in MI users [17, 18]. In patients with PCO syndrome, a higher concentration of DCI in the urine (higher elimination) and at the same time a reduced concentration in the plasma was observed compared to healthy eumenorrhoic women, while no such differences were observed for MI. According to the previously published data, MI supplementation improves many ovarian functions, oocyte quality, ovulation, higher chance of conception with better reproductive outcomes, as well as minor total gonadotropin dose during ovarian stimulation. Both stereoisomers MI and DCI additionally improve the regulation of glucose metabolism, lipid metabolism, and clinical signs of hyperandrogenemia [19–21]. Thus, significant dysregulation of inositol metabolism was demonstrated in patients with PCO syndrome, which indicates a connection with hyperinsulinemia and IR.

Already initial studies have proven the significant effectiveness of DCI in reducing lipid biomarkers, increasing insulin sensitivity, reducing androgen levels, and increasing the frequency of ovulation. These effects are mainly related to the positive systemic effect of DCI on the metabolic syndrome, but not to the effect on the ovary [22, 23], while MI has proven direct positive effects on ovarian function at the cellular level. Administration of higher doses of DCI (2.4 g/day), seems to have a negative effect on ovarian function, more significantly in PCO patients who do not have diagnosed IR. The release of a larger amount of DCI-phosphoglycan with hyperinsulinemia stimulates the biosynthesis of testosterone in ovarian theca cells, which increases hyperandrogenemia and leads ovarian follicles to growth arrest, atresia, or even to anovulation [24, 25].

Newer published studies indicate on positive effects of combined preparations which contain both stereoisomers MI and DCI on ovarian function in patients with PCO syndrome treated with *in vitro* fertilization procedures. The analysis of seven factors of oocyte quality has proved the positive impact of the combined administration of MI and DCI on oocyte quality, with preparations that contained a higher concentration of DCI in relation to MI. The known physiological ratio of MI and DCI in the plasma is 40:1 (in the ovary 100:1), but it seems that the effect on cell quality is still more dependent on the concentration of DCI than on the physiological ratio. The effect of these changes on developing embryos after ICSI fertilization remains unclear, as well as the effect on conception [26]. Studies on the preventive effect of inositol on the occurrence of gestational diabetes in the population of patients with PCO syndrome have also been published [27, 28]. The effect of MI and DCI was evaluated in the population of pregnant women diagnosed with gestational diabetes in mono-formulations or in combination. It was found that there is a significant beneficial effect on metabolic factors and reduction of IR in pregnant women diagnosed with gestational diabetes, and according to the results of the study, it has been shown that DCI has a more significant effect than MI [29, 30]. The choice of medical treatment including inositols depends on undelaying endocrinological and metabolic disorders and treatment goals. The applications of inositols showed clinical benefits in almost all outcomes compared to metformin independently and in combination with other forms of treatment for reproductive disorders.

5. Conclusions

Inositols are very interesting and promising molecules with consideration on their role in carbohydrate metabolism and consideration on their impact on cells with increasing effects of insulin intracellulary. They act as "messenger" molecules, increasing the sensitivity of insulin in targeted cells. Inositols have a positive impact on follicle maturation, they can improve the mechanism of dominant follicle selection by a reduction in free androgen levels by increasing aromatase activity and induction of SHBG production. According to literature data, it has been proved that inositol had favorable effect on several results of outcomes in patients with PCO syndrome. Present scientific results proved that inositols have a beneficial impact on ovarian function establishing ovulation, a positive effect on carbohydrate metabolism, on occurrences of gestational diabetes, and giving birth to eutrophic newborns. The positive effects of both stereoisomers MI and DCI as well as their combinations, on metabolic parameters, IR, regulation of glucose metabolism, and reproductive disorders have been scientifically proven. According to the data published so far, DCI has its greatest effectiveness in states with pronounced IR. Myo-inositol, on the other hand, has proven positive direct effects on ovarian function at the cellular level. Replacement of a single isomer, or their combination, is based on different actions and biological roles. The choice of treatment depends on which underlying disorder we want to treat. The clinical application of these substitutes has proven the beneficial effects of these molecules independently and in combination with other forms of treatment for reproductive disorders.

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Conflict of interest

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