Part IV

PCO, Metabolism, Vitamin D, Myoma and Endometriosis

13 The Ratio of MI to DCI and Its Impact in the Treatment of Polycystic Ovary Syndrome: Experimental and Literature Evidences

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13.1 Introduction

In the last decades, scientific interest has been directed to study the inositol family (INS) to understand their role in health and diseases. Among them, myo-inositol (MI) and D-chiro-inositol (DCI) play a key function owing to their involvement as second messengers of insulin in various insulin-dependent processes. Nowadays, an alteration in insulin signaling is recognized as the main driver in the pathophysiology of polycystic ovary syndrome (PCOS) [1]. PCOS is the most common reason of infertility, affecting approximately up to 10 % of women in reproductive age. Although MI and DCI exert different physiological functions, their respective roles in the etiology and treatment of PCOS are still debated.

13.2 Inositol(s) Story

Myo-inositol (MI), the first molecule to be known among INS (in the year 1850), can exist in nine possible stereoisomeric forms, consequently to the epimerization of the six OH- groups [2]. Natural sources for INS are dietary intake and endogenous biosynthesis. In food, these compounds are found especially in citrus fruits (with the exception of lemon), beans, and whole grains [3]. Among the nine stereoisomers, only MI shows a wide distribution in organisms, and it participates to the regulation of several hormone signals including insulin, follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and serotonin. D-Chiro-inositol

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(DCI), another biologically relevant stereoisomer, is enzymatically converted from MI through an insulin-dependent epimerase. Despite their similarities, MI and DCI display different biological function. Considering that glucose metabolism is regulated by insulin whereas the activation of glucose transporters and glucose utilization are due to MI, the glycogen synthesis is controlled through DCI [4–6]. On the other hand, MI in the ovary is devoted to glucose uptake and FSH signaling, while DCI mediates insulin-induced testosterone synthesis. MI constitutes almost all (>99 %) INS in the intracellular pool of most tissues, whereas the remainder is DCI. Noteworthy, every tissue has its own specific MI:DCI ratio, which translates into the different tissue function [7]. Accordingly, in order to set a proper treatment for PCOS, it is necessary to restore and maintain the appropriate MI:DCI ratio. In this chapter, we will report, for the first time, MI and DCI plasma ratio in healthy subjects, discussing the trials that have investigated a therapeutic option based on this ratio and some results of the international consensus conference held on myo-inositol and D-chiro-inositol in obstetrics and gynecology.

13.3 MI:DCI Physiological Plasma Ratio

We identified two studies [8, 9], both from the same group, reporting the pharmacokinetic (PK) profile of a pharmaceutical preparation of MI. In these studies were measured MI plasma levels, but also DCI levels were recorded. We had permission from the authors to access their data to calculate the physiological plasma ratio from each study. Study 1 was performed in 20 volunteers (eight males, 12 females), aged between 18 and 35 years, with a body mass index (BMI) ranging between 21 and 25 kg/m^2. Study 2 was performed in 12 volunteers (all women) aged between 20 and 40 years with a BMI between 18 and 24. By pooling data from the two studies, we have found a MI:DCI ratio of 40:1 (Fig 13.1).

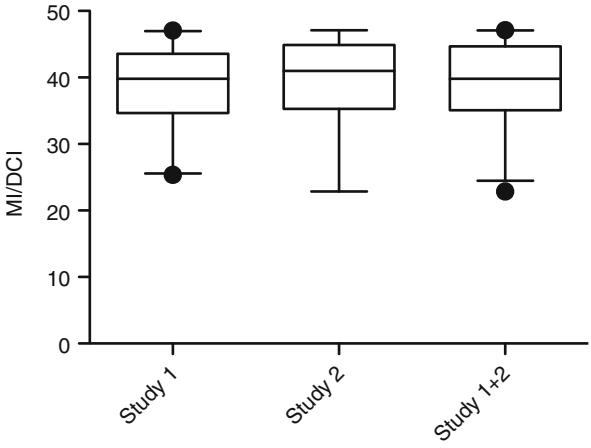

Fig. 13.1 Plasma ratio (40:1) and pharmacokinetics of MI and DCI. Data of two different studies in human volunteers

Recently, three different studies evaluated the efficacy of a treatment based on the physiological plasma ratio between MI and DCI of 40:1 in PCOS women. The idea behind this therapy is that an *INS dysregulation plays a central role in nurturing PCOS*. Indeed, epimerase dysregulation changes the MI:DCI ratio, which in turn could impair hormone signaling, namely, of both insulin and FSH.

Various evidences supported a deficiency concerning the availability and/or utilization of MI and/or DCI in tissues of PCOS women, and this impairment likely contributes to the insulin resistance typical of that syndrome [6, 10]. Unlike other tissues, such as muscles and liver, the ovaries are not insulin resistant. Because the epimerase activity, regulating the MI:DCI ratio, is insulin dependent, PCOS patients are affected by a boosted MI to DCI epimerization into the ovary, leading to overproduction of DCI and MI deficiency [11], as shown by two independent laboratories [6, 10]. Thus, a specific MI depletion and a DCI overload characterize the ovary of PCOS women. The poor oocyte quality observed in PCOS patients can be explained by this imbalance, responsible also for the impaired FSH signaling [12, 13].

Literature evidences have already shown that *MI supplementation is able to correct PCOS metabolic aspects*. Two trials demonstrated that the same effect was obtained even in a more effective way by administering MI and DCI in a physiological ratio (40:1). Indeed, the improved parameters were diastolic blood pressure, fasting glucose, fasting insulin, and both insulin and glucose AUCs [14, 15]. Additional improved parameters were those linked to the CVD, namely, HOMA index, triglycerides, and both HDL and LDL cholesterol. Noteworthy, ovulation was restored in the majority of the women.

Furthermore, by moving from the metabolic aspects of the syndrome to the reproductive ones, a trial has shown that the treatment of PCOS women undergoing ICSI, with a MI:DCI 40:1 based therapy, retains the beneficial effects of MI treatment alone, outperforming the DCI treatment [12].

In particular, the treatment is able to improve ovarian response and oocyte and embryo quality. Recently, the interest of the scientific world on MI and DCI has pushed the PRESIS to organize an international consensus conference in order to clarify this issue and lay the foundations of future researches.

13.4 Conference Aim and Methods

Since the knowledge of the differences between MI and DCI is not well established among researchers, as it is proven by a systematic and a Cochrane review mixing trials performed using MI or DCI, the PREIS School (Permanent International and European School in Perinatal Neonatal and Reproductive Medicine) has organized the "2013 Florence International Consensus Conference on Myo and D-CHIRO-INOSITOL in Obstetrics and Gynecology and Assisted Reproduction Technology (ART)" aimed at elucidating some controversial points with the contribution of opinion leaders in the fields of cell biology, mammalian embryology, human endocrinology, metabolism, obstetrics, and

gynecology. Two separate panels of this Committee worked on the roles of MI:DCI in metabolic syndrome (mainly PCOS) therapy and of MI in ART and drew up two lists of hot topics. Our review reports only the published results in the paper on myo-inositol and ART [16].

The following is a set of research questions concerning ART:

1. Physiological involvement of INS in oocyte maturation
2. INS involvement in the physiology of spermatozoa function
3. Usefulness of the treatment with INS during ART cycles
4. Comparison of the clinical efficacy between supplementation with MI and/or DCI

13.5 MI and ART

13.5.1 Physiological Involvement of INS in Oocyte Maturation

13.5.1.1 Role of MI in Oogenesis and Early Embryogenesis

In mammalian females including humans, an elevated MI content in the follicular fluid fosters oocyte quality and pregnancy outcome [17, 18]. MI activity is in connection with the InsP3 function on the modulation of intracellular calcium ion concentration, influenced by LH and FSH hormones [16]. In oocytes, MI, among different functions at the ovarian level, positively affects the maturation process [16]. The decrease of intracellular MI stores impairs oocyte maturation, and MI supplementation in culture medium has been shown to increase the development of fertile eggs [16]. The implantation rate and post-implantation viability of embryos rise if the oocytes are cultured in a medium containing MI and then fertilized in vitro and transferred for promoting pregnancy [16–19]. During in vitro fertilization (IVF) cycles, the treatment of women with MI before the hormonal stimulation has reduced the FSH quantity to be administered and the number of days required for the appropriate stimulation. All these parameters are positively related to the possibility of pregnancy and improved quality of oocytes and embryos and, probably, the implantation rate [12, 16]. Thus, MI administered 3 months before ovulation induction can produce a rise in the number of high-quality embryos obtained in IVF cycles.

13.5.1.2 MI and Oogenesis: A Lesson from Polycystic Ovary Syndrome

Further proofs confirming the essential role of MI in follicular fluid for safeguarding egg quality derived from the PCOS studies were previously examined. It is therefore clear that MI depletion in a PCOS ovary impairs dominant follicle recruitment and appropriate oocyte growth/maturation. These data support the fundamental observations by Chiu et al. [18] showing that proper content of MI in follicular fluid indicates a required condition to ensure egg quality.

13.5.2 INS Involvement in the Physiology of Spermatozoa Function

In agreement with the MI high levels in female generative system, the same condition can be found in mammalian male, where MI content is more elevated in reproductive organs than in blood serum and increases from the caput to the cauda epididymis [16]. In males, FSH-responsive Sertoli cells are the main producers of MI, which is implicated in processes such as the regulation of spermatozoa motility, capacitation, and acrosome reaction. MI increases sperm cell parameters in male patients suffering from oligoasthenoteratozoospermia (OAT), a severe pathology impairing sperm cell number, morphology, and function [16]. This evidence suggests that MI use in the treatment of semen samples during IVF cycles can raise fertilization rate and embryo quality, in this way, giving higher chances of pregnancy. Treating OAT patients' sperm cells with MI gives the following changes: the presence of amorphous material and semen viscosity decreases, midpiece volume improves, and mitochondrial cristae morphology is restored, regularizing the mitochondria structures [16]. At the functional level, a key step is the direct MI action on mitochondria, raising the membrane potential [16]. High values of mitochondrial membrane potential attest to the integrity of this structure, meaning, optimal levels of activity and proper cell viability. Therefore, MI treatment of sperm cells from both OAT patients and normal subjects enhances the recovery of cells usable in IVF cycles after swim-up [16], supporting its use as supplement in sperm cells manipulation in the procedures of medical-assisted reproduction.

13.5.3 Usefulness of INS Treatment During ART Cycles

As shown before, the pre-treatment of the PCOS patients with MI looks really very encouraging. The MI effect has been verified also in non-PCOS women needing fertility treatment owing to male or tubal anomalies [16]. All these data are in keeping with the evidence by Chiu et al. [18] that the gonadotropin quantity necessary for ovarian stimulation is lower in patients with follicular fluid characterized by higher MI levels.

13.5.4 Comparison of the Clinical Efficacy Between Supplementation with MI and/or DCI

As highlighted previously, for exerting its physiological function, the ovary would not need high doses of DCI. Moreover, the poor oocyte quality in PCOS ovary could be caused by decreased energy metabolism, and in turn, it is an effect of the down-regulation of the genes controlling glucose uptake [13, 20]. These findings agree with those obtained by Unfer et al. [12], showing that MI but not DCI exerts an action at the ovarian level and leading to the previously quoted DCI paradox. Therefore, the positive MI activity on oocyte quality could be related with its

function in glucose cell uptake, which ameliorates the energy status of the ovary, and in FSH signaling and induction of calcium release, which allows proper germ cell maturation.

Conclusions

Experimental data demonstrated that in the baseline condition, the MI:DCI ratio in healthy volunteers is set at 40:1. According to the International Consensus Conference, it is now clear that both MI and DCI are involved in various physiological and pathological functions (mainly the transduction of insulin and FSH signal), although with differentiated roles. INS supplementation could exert a positive action in different pathophysiological features in obstetrics and gynecology. The MI supplementation is very promising, with clear benefits, in the treatment of PCOS women and also in the prevention of gestational diabetes mellitus. A much larger amount of clinical data are available for MI in comparison with DCI, but the existence of tissue-specific ratios also in the ovary has suggested to develop a treatment based on both MI:DCI combination (ratio 40:1), in agreement with the "DCI paradox" [11].

On the other hand, INS by itself or through its derivatives exerts a pivotal role in reproduction, namely, in oocyte and spermatozoa development. MI depletion induces a defect in glucose uptake, reducing glucose availability in the ovary for both oocytes and follicular cells. The impairment of sugar availability in oocytes compromises their quality [21]. MI treatment in ART has demonstrated undeniable positive effects, and the use of MI, alone or in combination with DCI, at the 40:1 ratio, should be definitely considered a predictive factor for the improvement of ART outcome.

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14 Metabolic Healthy Obesity and Metabolic Obesity with Normal Weight and CVD Risk in Women

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Obesity is defined as the excess of body fat and results from interactions between genes and the environment. The factors contributing to obesity are unsuitable nutrition and food overproduction, poor physical activity, mental stress, psychoemotional disorders, and metabolic and hormonal disturbances [1].

Among gene candidates predisposing to obesity mutations and polymorphism of the gene of insulin receptor, polymorphism of the gene of PPARγ receptor, polymorphism of the gene of glucocorticoid receptor, and polymorphism of the gene of β3-adrenergic receptor are mentioned [2–5].

To evaluate obesity, body mass index (BMI) is useful (BMI = body weight in *kg* and high m^2 ratio). Obesity is diagnosed when BMI is above 30 kg/m^2, whereas overweight is when BMI is above 25 kg/m^2. Also fatty tissue percentage (>25 % of body mass in males and >30 % in females) is useful in obesity evaluation. To estimate fat distribution, waist-to-hip ratio (WHR, >1.0 in males and >0.8 in females) and waist circumference (>80 cm in females and >94 cm in males) can be used. More accurate methods used to evaluate fat mass are dual-energy X-ray absortiometry and computed tomography.

In order to evaluate abdominal fat, a dual-energy X-ray absorptiometry (DXA) is used where androidal deposit is assessed at L_2–L_4 level [6]. The "gold standard" to determine the visceral and subcutaneous abdominal fat is through computer tomography. The evaluation is performed at the level of the intervertebral lumbar disc L_4–L_5 [7].

The adipose tissue is not only a fat storage but also an active endocrine organ which produces and secretes many hormones and protein factors and plays an important role in metabolic homeostasis. Adipocytes contain over 20 hormone receptors and products or release numerous protein and non-protein substances

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which play a significant role in the immune system (TNFα, Il-6, TGFβ), blood pressure (angiotensinogen), blood coagulation (PAI-1), glycemic homeostasis (adiponectin, resistin, visfatin, leptin), and angiogenesis (VEGF) [8].

The visceral adipose tissue (VAT) differs from the subcutaneous fat (SCAT) anatomically, hormonally, and metabolically; excessive amount of the former type has been postulated as the key causative factor for metabolic disturbances. Visceral fat is characterized by high density of β-adrenergic, resistin, androgen, and glucocorticoid receptors, which impair insulin sensitivity. Adipocytes in this localization are also resistant to insulin lipogenic effects and more lipolytic. Additionally, adipocytokines are released directly to the portal venous system and influence and affect carbohydrates and lipids metabolism. Visceral fat may enhance truncal SCAT lipolysis as well. Production of inflammatory markers (IL-6) and prothrombotic factors (PAI-1) is higher in visceral adipose tissue than in subcutaneous adipose tissue.

Preadipocytes of the subcutaneous adipose tissue have a greater differentiation and may replenish VAT. Localized subcutaneous adipocytes secrete relatively more atheroprotective adiponectin and leptin while less resistin compared to that of visceral adipose tissue cells, which leads to insulin sensitivity improvement is associated with female phenotype characterized by higher subcutaneous fat accumulation.

Fat distribution depends on gender, age, and ethnicity. For example, Asian and Japanese people have lower deposits of the visceral fat than Caucasians. In men, visceral fat deposits reach 20 % of the whole fat pool; in pre-menopausal women, from 5 to 8 %.

Depending on the body fat distribution and metabolic disturbances presence, there may be mentioned healthy controls, healthy obesity, obesity with metabolic disorders, and obesity with metabolic disorders and normal weight people.

Depending on biological age and gender (20–35 % in women, 29 % in men), more often in women and elderly ones, people with a BMI >30.0 kg/m^2 show the metabolic healthy obesity (MHO) phenotype without insulin resistance, dyslipidemia, or hypertension. MHO people have waist circumference ≤80 cm, adipose tissue mass >35 %, fasting glucose level <100 mg/dl, serum triglycerides level ≤150 mg/dl, HDL cholesterol >50 mg/dl, and blood pressure ≤130/85 mmHg. Fat accumulates mainly in the region of the hips, buttocks, and thighs with slim waist. This phenotype is characterized by the early development of obesity (before 20 years of age, in 13 % – already in childhood) and increased subcutaneous fat content, excluding the pathological deposition of fat in the liver, muscles, and visceral area. Histologically fatty tissue in people with MHO is characterized by decreased size and number of adipocytes. A relationship between the onset and duration of obesity and insulin sensitivity of tissues as the adaptation mechanism has been postulated [9]. In individuals with this obesity phenotype, an excess of energy delivered with food is directed to subcutaneous fat deposits and/or burnt in the hepatic mitochondria or the muscles. Therefore, the positive energy balance does not increase risk of metabolic disorders. The significantly elevated subcutaneous fat deposit and its ratio to visceral fat deposit reveals protective effect against atherosclerosis and metabolic syndrome [10, 11]. The candidate genes postulated to these modifications are endocanabinoid receptor gene (CNR1), 1 adiponectin receptor gene