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D-Chiro-Inositol in Clinical Practice: A Perspective from the Experts Group on Inositol in Basic and Clinical Research (EGOI)

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Abstract
Background: D-Chiro-inositol is a natural molecule that, in association with its well-studied isomer myo-inositol, may play a role in treating various metabolic and gynecological disorders. ***Objectives:*** This perspective seeks to explore the mechanisms and functions of D-chiro-inositol, laying the foundations to discuss its use in clinical practice, across dysmetabolism, obesity, and hormonal dysregulation. ***Methods:*** A narrative review of all the relevant papers known to the authors was conducted. ***Outcome:*** D-Chiro-inositol acts through a variety of mechanisms, acting as an insulin sensitizer, inhibiting the transcription of aromatase, in addition to modulating white adipose tissue/brown adipose tissue transdifferentiation. These different modes of action have potential applications in a variety of therapeutic fields, including PCOS, dysmetabolism, obesity, hypoestrogenic/hyperandrogenic disorders, and bone health. ***Conclusions:*** D-Chiro-inositol mode of action has been studied in detail in recent years, resulting in a clear differentiation between D-chiro-inositol and its isomer myo-inositol. The insulin-sensitizing activities of D-chiro-inositol are well understood; however, its potential applications in other fields, in particular obesity and hyperestrogenic/hypoandrogenic disorders in men and women, represent promising avenues of research that require further clinical study.

Introduction, D-Chiro-Inositol: Back to the Basics

Since its discovery in 1850, inositol has generated interest among researchers and clinicians due to its role in a series of metabolic and gynecological functions [1]. Inositol exists as nine potential isomers with six occurring in nature, among which myo-inositol (myo-Ins) and D-chiro-inositol (D-chiro-Ins) are the most prevalent within the human body and food [2]. Though myo-Ins was and is the object of much literature, here it suffices to mention its effectiveness as an insulin sensitizer that led to its use treating polycystic ovary syndrome (PCOS), infertility, gestational diabetes mellitus, and the prevention of neural tube defects [3–5]. Under insulin stimulation, tissue-specific NADPH-dependent epimerases convert myo-Ins to D-chiro-Ins [6]. These enzymes facilitate this unidirectional reaction, which allows for proper balance between the two isomers. This process is tissue-specific; therefore, it varies depending on the needs of the particular organ. Upon synthesis of D-chiro-Ins, it proceeds to facilitate glycogen storage [7].

Unlike myo-Ins, which is readily available through the diet, primarily in corns, beans, fruits, and nuts [8], it is near impossible to consume sufficient D-chiro-Ins as only a few foods (e.g., buck wheat, soy-lecithin, carob, and lentils) contain significantly high levels of this molecule. Therefore, D-chiro-Ins is mainly synthesized in the body from myo-Ins [6]. The epimerization process is inhibited in cases of acquired insulin resistance (IR), causing an increased ratio between myo-Ins and D-chiro-Ins, such that an elevated myo-Ins/D-chiro-Ins ratio may be considered as a hallmark of IR [9]. Myo-Ins controls D-chiro-Ins levels within the cell,

due to the aforementioned conversion process; therefore, the synthesis of myo-Ins is important to consider when contemplating the effect of D-chiro-Ins within the body [10]. The ratio between myo-Ins and D-chiro-Ins is of great physiological importance and varies depending on the tissue type. In the plasma, the ratio between myo-Ins and D-chiro-Ins is 40:1, while in energy-dependent tissues, such as fat or liver, this drops to 2:1 [10]. In contrast, tissue types that have a high glucose consumption rate, such as the brain, display a ratio of 200:1. Interestingly, in follicular fluid from healthy women the myo-Ins/D-chiro-Ins ratio is 100:1, while it drops to 0.2:1 in women with IR [11]. Inositol treatment typically aims either to restore a specific ratio, such the physiological ratio of 40:1 [12], or to specifically alter this ratio in order to yield a desired physiological effect.

D-Chiro-Ins exhibits a two-faceted mechanism, playing a role in both insulin signaling and aromatase-facilitated conversion of androgens to estrogens [13, 14]. Accordingly, it is vital to understand how D-chiro-Ins treatment can affect both processes in order to tailor therapy to the needs of the individual patient. Furthermore, several key factors must be considered when prescribing a treatment regimen, such as the insulin-resistance status of the patient, the administered dose of D-chiro-Ins, and the duration of treatment. However, given the proper posology, D-chiro-Ins treatment has great potential to treat numerous conditions [15]. In this perspective, we discuss the various biological effects of D-chiro-Ins and how this knowledge can be applied to treatment, with a hope to build a more complete clinical picture of this fascinating molecule.

Exploring the Metabolic and Hormonal Functions of D-Chiro-Ins

Insulin Signaling

D-Chiro-Ins plays an important role in insulin signaling by decreasing systemic insulin levels by facilitating glycogen storage [16]. Upon synthesis of D-chiro-Ins, glycogen synthase is activated; thus, tissues involved in glucose storage such as liver and skeletal muscle are more reliant on the activities of D-chiro-Ins [10]. Furthermore, other D-chiro-Ins-dependent biochemical processes, such as the activation of several insulin-affecting proteins (insulin receptor substrate 2 – IRS2, phosphoinositide 3-kinase – PI3K, and protein kinase B/AKT) at both mRNA and total protein levels, and the downregulation of glycogen synthase kinase 3 beta promote the glycogen storage process. The glucose cross-membrane equilibrium is also altered, favoring glucose uptake into muscle and adipose cells through mobilization of GLUT4 transporters [14]. The combined actions of the above mechanisms result in a reduced insulin requirement, thus decreasing systemic insulin levels [17].

Insulin affects various hormone signaling processes, as evidenced by the connection between PCOS and IR, where IR has been closely linked with hyperandrogenism [18]. Insulin signals upregulate the tissue specific epimerases that convert myo-Ins to D-chiro-Ins [19]. In pathologies such as type II diabetes mellitus, IR can increase over time, reducing epimerase activity and leading to lower systemic levels of D-chiro-Ins [6]. However, IR does not occur in the ovaries due to the so-called ovarian paradox, whereby ovarian tissue remains insulin sensitive and remains susceptive to the higher amounts of circulating insulin [20], which are present as a result of systemic IR. Hyperinsulinism causes overstimulation of the ovaries resulting in an increase in specific epimerases and subsequently increased ovarian tissue levels of D-chiro-Ins. The elevated ratio between D-chiro-Ins and myo-Ins is thought to contribute toward PCOS pathogenesis, with myo-Ins depletion causing a drop in follicle-stimulating hormone signaling and oocyte quality [11].

Aromatase Modulation

In the late 1990s, Nestler reported the metabolic effects of D-chiro-Ins and its insulin-independent activity on androgen biosynthesis [21]. It was reported that D-chiro-Ins can enhance testosterone biosynthesis in a similar manner to insulin. This was discovered using human thecal cells, where the administration of both insulin and a D-chiro-Ins-based synthetic phosphoglycan (INS-2) resulted in increased testosterone levels [22]. Additionally, it has recently been discovered that D-chiro-Ins is able to inhibit the transcription of aromatase also known as CYP19A1, an enzyme of the cytochrome P-450 family, which catalyzes the conversion of androgens to estrogens through oxidation [23]. An in vitro study in primary human granulosa cells demonstrated a significant dose-dependent reduction of aromatase mRNA after a 24-h treatment with D-chiro-Ins [24]. Subsequently, Bevilacqua et al. [25] confirmed in vivo that mice treated with increasing doses of D-chiro-Ins, at 250 mg/day, had significantly lowered aromatase levels than the control mice. D-Chiro-Ins exhibits a different mechanism with respect to known

aromatase inhibitors, which tend to directly inhibit estrogen biosynthesis, potentially leading to the development of a hypoestrogenic state and potential side effects such as reduced bone density [22, 26].

D-Chiro-Ins-induced disruption of aromatase expression was speculated as a possible explanation for the restoration of ovulatory function in anovulatory non-PCOS, non-IR women, as reported by Bezerra Espinola et al. [13] in 2 case studies. In these patients, ovulation occurred after treatment with D-chiro-Ins. As the patients were normoinsulinemic, insulin regulation was not considered to be the mechanism responsible for restoring the menstrual cycle. Therefore, the authors speculated that another D-chiro-Ins-dependent mechanism, the downmodulation of aromatase, could explain the restoration of normal ovarian function.

D-Chiro-Ins has been further hypothesized to act as a luteinizing hormone (LH) sensitizer, reducing LH synthesis and improving its signaling [27]. The mechanism behind this is not fully understood; however, it is thought that inositols and inositol phosphates could be involved. LH, in a similar manner to insulin, blocks aromatase inhibition, so it is possible that D-chiro-Ins mimics this and reduces the requirements for systemic LH [17].

Transdifferentiation of WAT to BAT

Myo-Ins and D-chiro-Ins have been investigated in relation to the differentiation of white adipose tissue (WAT) to brown adipose tissue (BAT), a process which could be harnessed to treat obesity, a major issue of the 21st century [28]. In humans, there are three types of adipose tissue white, brown, and beige (sometimes also referred to as "brite") [29]. WAT is primarily used for fat storage and energy stockpiling and is dispersed around the body, as either subcutaneous or visceral fat deposits [30]. White adipocytes are used to store lipids, typically in one large vacuole which occupies approximately 90% of the cell volume and contains relatively few mitochondria [31]. BAT on the other hand is used to disperse energy as heat to maintain body temperature homeostasis [32]. In contrast to white adipocytes, brown adipocytes are multilocular and contain large amounts of mitochondria, which are responsible for the characteristic brown color of this tissue type [33]. In addition, BAT contains a characteristic unique protein known as uncoupling protein 1 (UCP-1), which is involved in the uncoupling of mitochondria and is detected also in beige adipose tissue [34]. Beige adipose tissue displays similar characteristics to BAT; however, it is detectable in WAT regions such as subcutaneous regions of the body, where BAT is typically not present [35]. Usually, beige adipose tissue manifests as a response to cold, or under hormonal or nervous stimulation.

In a study conducted by Monastra et al. [36], UCP-1 was considered a biomarker for differentiation between WAT and BAT. While D-chiro-Ins stimulation caused an overexpression of UCP-1 mRNA in both SGBS and Lisa-2 cell models, the treatment with myo-Ins showed a significant modulation only in Lisa-2 cells. Thus, the authors concluded that both stereoisomers can induce WAT/BAT transdifferentiation through UCP-1 activation. This effect was confirmed by an increased number of mitochondria as well as oxygen consumption ratio, indicating an increased percentage of BAT as a sign of the activation of cellular metabolism. However, it should be noted that this was only significant for treatment with D-chiro-Ins. Furthermore, it was observed that treatment with myo-Ins or D-chiro-Ins induced an upregulation of estrogen receptors (ER) mRNAs. This finding is in agreement with the preexisting literature that stated the importance of estrogen signaling in adipose tissue and obesity [37]. Stimulation of ER is at odds with the increased levels of androgens sometimes associated with D-chiro-Ins supplementation [38]. It has been suggested that the increased expression of ER in this study was due to a local effect seen in the adipose tissue that is not reflected systemically. Clearly more work is required to fully unravel this complex mechanism.

In the same study, the expression of PPAR-γ, a notable target in the field of metabolic disease, was measured [39]. PPAR-γ exists as two isoforms, v1 and v2, with v1 being ubiquitously expressed and v2 limited to adipose tissue. The activation of both isoforms induces the transdifferentiation of brown adipocytes. This study provided the first report that myo-Ins and D-chiro-Ins can upregulate both isoforms of PPAR-γ, further lending credence to the notion that inositols in general are involved in BAT/WAT differentiation.

From Mechanisms of Action to Targeted Therapies

Insulin Resistance and PCOS

As outlined earlier, inositol supplementation has shown great success in counteracting IR in a variety of conditions, particularly PCOS. In this regard, inositol therapy has offered a safe natural molecule-based alternative to other insulin sensitizers such as metformin in managing the syndrome, especially for addressing metabolic symptoms and their associated hyperandrogenism.

In fact, in a meta-analysis by Facchinetti et al. [40], no difference was observed between metformin and myo-Ins treatment, thus increasing its favorability over metformin due to a better tolerance. Furthermore, in another recent meta-analysis, Fatima et al. [41] compared the efficacy of metformin and myo-Ins in improving hormonal and metabolic parameters of PCOS, concluding that both molecules are equally beneficial, although myo-Ins was more tolerated.

D-Chiro-Ins has also been used as a monotherapy to treat IR, due to its role in insulin signaling. This has shown success in both lean and obese women reducing IR-related metabolic factors [42, 43]. Some concerns have been raised about prolonged use of D-chiro-Ins in treating IR, as preclinical and clinical studies have indicated that this may result in damage to reproductive health. Indeed, in mice high doses of D-chiro-Ins lead to the development of distinct morphological features similar to those reported in human PCOS women with elevated levels of testosterone and the presence of cystic follicles [25]. In addition, Nordio et al. [44] demonstrated that long-term treatment with high dosages of D-chiro-Ins can predispose women to hormonal and menstrual abnormalities. They classified a high dose as >1,200 mg/day and recommended a treatment regimen of <30 days, compared to a standard dose of 600–1,200 mg/day for a recommended 3 months. Furthermore, the accumulation of D-chiro-Ins following such a treatment regimen may lead to detrimental effects in nonreproductive tissues, as revealed by the increase in asprosin levels.

The simultaneous administration of myo-Ins and D-chiro-Ins has gained much interest within the scientific community. As myo-Ins and D-chiro-Ins use different mechanisms to counteract IR, it is of vital importance to understand which combination of these inositols is appropriate for the patient in question [45]. Several formulations exist within the literature, with some drawing criticism with the ratios lacking scientific evidence to support their use [12]. Scientific consensus has settled upon the physiological ratio seen in humans which is 40:1 myo-Ins/D-chiro-Ins [46–48]. This combination offers a treatment option that can utilize the insulin-sensitizing action of D-chiro-Ins, without using excessive dosages and receiving any of the pro-hyperandrogenic affects, while still providing the positive effect of myo-Ins. The 40:1 myo-Ins/D-chiro-Ins ratio has exhibited better clinical outcomes than myo-Ins alone and has thus been indicated for the restoration of metabolic alterations and ovulation in PCOS patients, specifically overweight or obese patients with BMI ≥25 [49]. The perceived long-term risk to ovarian health due to prolonged D-chiro-Ins use is offset by its action in aiding metabolic signaling, with IR thought to be a major driver of hyperandrogenic PCOS in these patients [49].

Dysmetabolism and Obesity

The success of D-chiro-Ins in treating IR in PCOS has prompted the investigation of its supplementation to counteract dysmetabolism in general, with a focus on obesity care. Obesity has become a worldwide health problem with an estimated 15% of the global female population presenting a BMI >30, representing a 300% increase since 1975 [50]. Furthermore, obesity is a major risk factor in many various malignancies including endometrial cancer. As highlighted earlier in the text, higher WAT accretion can contribute to hyperestrogenism, as reflected in the endometrial cancer patient populations where 41% have been reported to be obese [51]. Diet and exercise represent the typical clinical recommendation for dysmetabolic and/or obese patients; however, in severe cases medications are often co-prescribed. Pharmaceutical inventions that tackle obesity have an infamous past, with several drugs being withdrawn from the market due to serious adverse side effects including cardiovascular events, suicide, cancer, and risk of abuse and dependence [52]. More recently, promising results have been observed with GLP-1R agonists, with two drugs, semaglutide and liraglutide, being approved by the FDA for the treatment of obesity, and demonstrate relatively tolerable side effects [53, 54]. However, safe therapies are still required, with D-chiro-Ins representing a promising avenue of research in the pursuit of alternative or adjunct therapies to pharmaceutical intervention.

Various studies have investigated the use of D-chiro-Ins in regulating dysmetabolism and obesity. In 2017, D-chiro-Ins supplementation in combination with folic acid was investigated as an adjuvant treatment in overweight and obese patients with type I diabetes [55]. Obesity rates have increased causing the required doses for insulin to be increased in these patients, resulting in the development of a hybrid phenotype, referred to as "double diabetes" [56]. This insulin-resistant phenotype is responsive to insulin sensitizers such as metformin [57]; however, given the known side effects of metformin, the use of D-chiro-Ins was considered. Following 3 months of the administration of D-chiro-Ins and folic acid, HbA1c was reduced from 8.1% to 7.5%, and these levels were maintained following a further 3-month treatment [55].

In addition, a recent meta-analysis investigated 15 studies comparing the effects of inositol supplementation on typical obesity measures such as BMI and waist-hip ratio. Zarezadeh et al. [58] reported a significant reduction in BMI in patients

under 30 years of age and PCOS women, suggesting that inositol may have potential as an adjunct therapy to obesity care. Similar results have been reported in obese PCOS patients where D-chiro-Ins treatment over a 12-month period resulted in a significant improvement in insulin sensitivity, androgen levels, and BMI [27]. Furthermore, in a 2019 study comparing weight loss in obese PCOS myo-Ins in combination with D-chiro-Ins, treatment accelerates weight loss over diet alone [59].

Finally, a recent study investigated the use of a 40:1 ratio of myo-Ins/D-chiro-ins, in combination with α-lactalbumin and *Gymnema sylvestre* in obese and dysmetabolic patients [60]. Basciani et al. [60] divided 37 patients into two groups: a control group following a hypocaloric Mediterranean diet and the study group who underwent combined inositol treatment. While both arms of the study observed a reduction across all assessed parameters, a greater improvement was seen in the study group, with respect to the control in terms of Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) index, triglyceride levels, BMI, body weight, and waist circumference. This work, despite its relatively small size, indicates that the combined supplementation of the myo-Ins, D-chiro-Ins, α-lactalbumin, and *G. sylvestre* may offer a safe dietary aid to obese patients, warranting further study.

Hyperestrogenism and Hypoandrogenism Disorders in Men

The modulation of aromatase expression by D-chiro-Ins has opened the door for its use in treating male and female disorders that are associated with an unbalance in androgen/estrogen levels. As highlighted above, D-chiro-Ins can inhibit the transcription of aromatase, which is responsible for converting androgens to estrogens, thus maintaining androgen levels, while decreasing estrogens levels. Testosterone levels are commonly reduced with age in men, typically decreasing approximately 1% per year after the age of 30 years [61–63]. This reduction is often defined as male hypogonadism, which is a pathological condition characterized by decreased testosterone production in the testis, in addition to reduced testicular function, leading to problems including infertility and erectile dysfunction. Also, there is growing evidence, suggesting that low testosterone concentration may originate from disorders of adipose tissue metabolism [64]. Indeed, excessive aromatase activity in adipose tissue leads to increased conversion of androgens into estrogens, eventually resulting in a reduction of testosterone levels that is the underlying reason for obesity-related hypogonadisms and infertility [65, 66]. While more common in women, hyperestrogenism in men has been demonstrated to interfere with sexual function, leading to reduced erectile function and sexual desire and has been touted as a possible risk factor for heart attacks, strokes, and prostate cancer. Furthermore, increased levels of estradiol have been linked to further progression of obesity and metabolic disease creating a vicious cycle between hyperestrogenism and obesity [67].

In clear cases of hypogonadism with specific testicular or pituitary disease, testosterone replacement is the treatment of choice having special care to avoid adverse effect on prostate and cardiovascular health [68]. However, there are unclear cases of hypoandrogenism, i.e., late-onset hypogonadism or functional hypoandrogenism. In these cases, a block of aromatase could represent a solution and for this purpose D-chiro-Ins, having good safety profile, has been investigated [69].

Nordio et al. [69] demonstrated that the supplementation of D-chiro-Ins over 30 days improved the levels of testosterone and androstenedione in patients over 65 with basal low testosterone levels. Furthermore, this was associated with a reduction in estrone, estradiol, and LH levels. In addition, D-chiro-Ins positively affected the metabolic profile of these men, causing reductions in HOMA-IR, plasma insulin, glycemia coupled with a drop in BMI, and waist circumference. Moreover, phenotypic signs of increased testosterone levels were present, including increased muscle mass and self-reported erectile function. In a similar vein, Monastra et al. [38] supplemented a sample of primarily overweight male volunteers between the ages of 30 and 55 with D-chiro-Ins over a 30-day period, reporting an overall 23.4% increase in testosterone. Further minor increases in dehydroepiandrosterone sulfate levels were observed with an average of 13.8% increase that was paired with a notable reduction in estradiol (E2) in most volunteers. Both studies, albeit with small study groups, clearly suggest that D-chiro-Ins may be suitable as a therapeutic intervention for hypoandrogenism/hyperestrogenism in men, particularly in overweight and obese patients.

Hyperestrogenism Disorders in Women

In women, the phenomenon of "unopposed estrogens" is defined as the presentation of unusually high estrogen levels, or normal estrogen levels with low progesterone levels, together with an imbalance in estrogen and progesterone receptors [70]. Localized elevated estrogen can cause physiological issues such as thickening of the endometrial lining, with various biochemical processes including IR and chronic hyperinsulinemia, leading to increased cell proliferation and decreased apoptosis [71]. Risk factors for unopposed estrogens include aging, obesity,

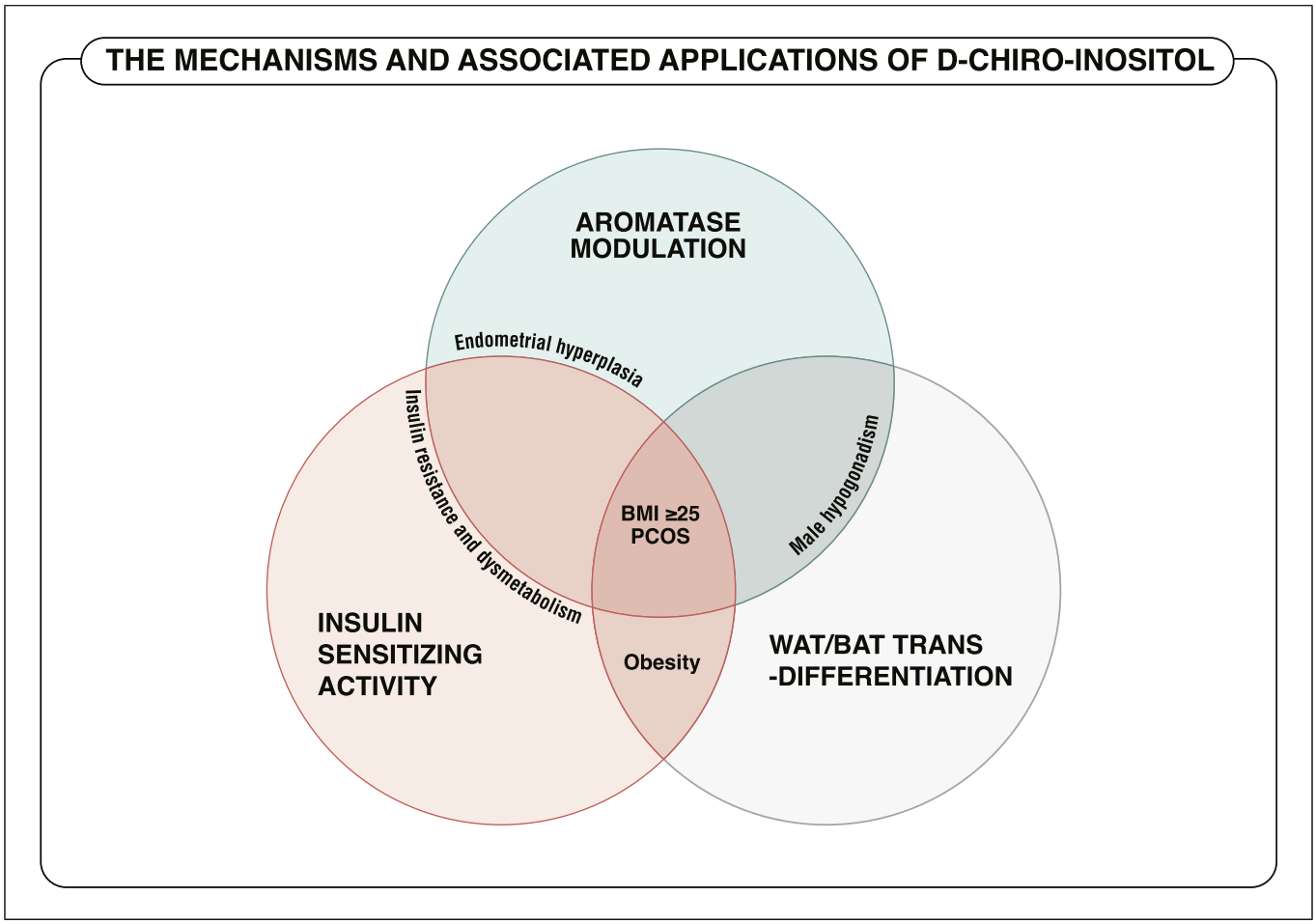

Fig. 1. A Venn diagram comparing the known mechanisms of D-chiro-inositol and the pathologies discussed within the review. The placement of each pathology represents to which mechanism a potential treatment with D-chiro-inositol could take effect.

genetic factors, in addition to various medicines such as breast cancer medications, such as tamoxifen [72–74]. Unopposed estrogens have been linked to a range of conditions including endometrial polyps, endometriosis, adenomyosis, and notably endometrial hyperplasia. Progestogens represent the first-line treatment for most of these estrogen-dependent disorders; however, recurrence rates are high and, furthermore, those who relapse are 10% more likely to be diagnosed with endometrial cancer [75]. Interestingly, aromatase has been observed to be significantly overexpressed in estrogen-dependent tissues, raising the local production of these hormones and causing a proliferative effect that can hasten the growth of lesions [76]. It has therefore been theorized that D-chiro-Ins may represent an adjunct therapy to progestogen treatment in patients with endometrial hyperplasia, in a similar manner to other insulin sensitizers, as seen in the case of metformin. Unfer et al. [77] proposed that, especially in obese patients where hyperestrogenism may be complicated by metabolic alterations, such as IR, D-chiro-Ins may have clinic use due to its antiaromatase and estrogen-reducing effects.

The first pilot study to test this hypothesis was recently published by Porcaro et al. [78], who investigated the use of D-chiro-Ins in 13 premenopausal women diagnosed with simple endometrial hyperplasia presenting an endometrial thickness >8 mm on the 10th day of the menstrual cycle, and at least one disease-associated symptom. The cohort received 600 mg/day of D-chiro-Ins over a 6-month period, with follow-ups at 3 and 6 months. The group responded well to the treatment, with an average 25.1% reduction in endometrial thickness after 3 months, followed by a further 13.8% by the end of the study, likely explainable by D-chiro-Ins

Table 1. A summary of the notable in vitro studies, preclinical, and clinical studies exploring the use of D-chiro-Ins

Reference	Pathology	Action	Model	Dosage	Time
[21] Nestler	PCOS	Insulin signaling	Ex vivo theca cells	From 1 to 100 μM	16 h
[42] Iuorno	PCOS		Lean women with PCOS	600 mg per day	6–8 weeks
[44] Nordio	Insulin resistance		Insulin-resistant women	1,200 mg per day	6 months
[49] Nordio	PCOS		Women with PCOS	2 g per day 40:1 (MI:DCI)	3 months
[13] Bezerra	Anovulation	Aromatase modulation	Healthy women	400 μg folic acid and 1,200 mg DCI per day	6 months
[24] Sacchi	Infertility		Ex vivo granulosa cells	From 0 to 20 nM	24 h
[25] Bevilacqua	---		Mouse	5, 10, and 20 mg per day	21 days
[38] Monastra	---		Healthy men	1 g per day	30 days
[44] Nordio	---		Healthy women	1,200 mg per day	30 days
[69] Nordio	Hypogonadism		Hypogonadal men	1,200 mg per day	30 days
[78] Porcaro	Endometrial hyperplasia		Women with unopposed estrogen symptoms	600 mg per day	6 months
[14] Montt-Guevara	---	Transdifferentiation WAT/BAT	In vitro adipocyte model (SGBS cells)	10 nM	24 h
[36] Monastra	---		In vitro adipocyte model (SGBS and LiSa-2 cells)	60 μM	72 h
[60] Basciani	Obesity		Obese people with fasting glucose >100 mg/dL	1,950 mg MI, 50 mg DCI, 50 mg α-lactalbumin, 250 mg *Gymnema sylvestre*, and 7.5 mg zinc	6 months
[86] Liu	Loss of bone mass	Bone metabolism	Ovariectomized mice	100 mg/kg body weight once per day	7 weeks
[87] Liu	Loss of bone mass		Diabetic osteoporotic mice	50 or 100 mg/kg body weight once per day	5 weeks

interfering with the enhanced proliferation associated with unopposed estrogens. The average length of menstruation was reduced from 8.85 ± 0.99 to 6.39 ± 0.77 days. Lastly, patients had fewer days of heavy menstrual bleeding by the end of the study, decreasing from 5.54 ± 1.11 to 1.38 ± 0.87 days. In total, all symptomatic hallmarks of endometrial hyperplasia were reduced; however, it should be noted that these preliminary findings were performed on a limited patient population, and larger more robust studies are required to evaluate whether the effect was due to D-chiro-Ins supplementation or intraobserver variability.

Practical Considerations (a Focus on Posology, Timeline of Treatment, and Patients)

Inositols exhibit an excellent safety profile compared to other insulin sensitizers such as metformin, which is typified by gastrointestinal issues, with myo-Ins demonstrating mild effects only at high concentrations, typically over 12 g/day [79]. Despite this, high doses of D-chiro-Ins as previously mentioned are thought to worsen the gynecological condition, especially in hyperinsulinemic and hyperandrogenic women, such as PCOS patients. Furthermore, prolonged treatment of D-chiro-Ins in healthy women has been associated with an increase in total testosterone and asprosin levels [44]. These risks must therefore be considered when tailoring treatment regimens to the individual patient. The first pharmacokinetic study in the inositol field was conducted with myo-Ins, which found that two 2 g administrations of myo-Ins are required to maintain therapeutic levels in plasma, as opposed to one 4 g dosage [80]. These findings were compared with a study on 10 healthy male volunteers who received a 1 g dose of D-chiro-Ins with serum peak occurring after 240 min and plateauing for around an hour [38]. Interestingly, it was observed that the D-chiro-Ins peak was shifted to the right, suggesting a longer half-life of D-chiro-Ins in serum than myo-Ins (serum peak at 180 min).

Low intestinal absorption of both pharmaceuticals and dietary supplements alike can derail otherwise promising clinical candidates. In a minority of patients, myo-Ins has been observed to have poor bioavailability and efficacy, leading to the phenomenon of "inositol resistance," which

is associated with patients who are unresponsive to myo-Ins supplementation. However, the use of α-lactalbumin peptides in combination with myo-Ins improved response rates from 62% to 82% [81]. In the same vein, in vitro experiments conducted by Ranaldi et al. [82] found that likewise D-chiro-Ins suffers from a low intestinal bioavailability that could be improved through the use of α-lactalbumin, which promotes the cellular uptake of D-chiro-Ins. This was demonstrated initially through the use of a Caco-2 permeability assay. While the authors conceded that the presence of the natural D-chiro-Ins carriers, SMIT1 and SMIT2, has not been fully evaluated, the results suggested a linear kinetic diffusion for D-chiro-Ins across Caco-2 cells. In the tested concentrations, D-chiro-Ins alone showed low absorption across the Caco-2 monolayer; however, the presence of α-lactalbumin resulted in an almost 10-fold increase in passage across the cellular monolayer. Importantly, this change in permeability did not result from permanent damage to the epithelial monolayer, but rather a reversible modulation of paracellular permeability, likely involving transient F-actin rearrangement.

Future Perspectives: Inositols in Bone Health

Recent data have brought the use of inositols as a potential adjunct therapy for improving bone health into light. Various animal studies have demonstrated the key role of inositol in bone health, with a lack of inositol causing a reduction of bone mineralization and the supplementation of inositols, positively modulating the balance between bone-producing and bone-removing cells [83, 84].

López-Gonzalez et al. [85] investigated the clinical importance of inositols and analyzed the content of urinary myo-inositol hexaphosphate (phytate) in postmenopausal women, whereby they highlighted a correlation between low urinary clearance of phytate and a reduced bone density, leading to an increase in fracture risk.

Recent literature has investigated the effectiveness of D-chiro-inositol in recovering a near-physiological phenotype in ovariectomized mice. Estrogen production is of vital importance to female bone health; thus, an ovariectomy may lead to bone issues. Liu et al. [86, 87] administered estrogen or pinitol, the 3-O-methyl-ether of D-chiro-Ins, to ovariectomized mice with the results compared between the two groups. Both estrogen and pinitol treatment increase the calcium and phosphorus blood levels, with respect to ovariectomized mice, thereby indicating a possible relatively unexplored application for D-chiro-Ins.

Conclusions

The amount of evidence regarding the use of D-chiro-Ins has recently grown considerably so that it should no longer be considered only the "active form" of myo-Ins, with the two isomers having been clearly differentiated. In total, D-chiro-Ins has demonstrated potential as a therapy for a myriad of different conditions in part due to its involvement in three distinct biological mechanisms. Understanding how these processes interplay with current clinical needs is vital to unlock the therapeutic promise of D-chiro-Ins. To aid this, the relationship between the clinical applications and mechanisms of D-chiro-Ins discussed herein is highlighted in the Venn diagram presented in Figure 1. Furthermore, the notable studies described in this paper are summarized in Table 1.

The action of D-chiro-Ins, as an insulin mimetic molecule, a modulator of WAT/BAT differentiation, and an aromatase downregulator, has made it a captivating molecule since these mechanisms were discovered at the turn of the century. The inherent interconnectivity between insulin signaling and hormone signaling positions D-chiro-Ins ideally for the treatment of a variety of interconnected conditions such as obesity, and pathologies associated with hypoandrogenism and/or hyperestrogenism. The current knowledge of the effect D-chiro-Ins has on hormone-dependent conditions is still in its infancy; however, several studies are currently ongoing. Overall, we hope this paper can act as a source of inspiration for the field, to trigger larger studies which this versatile molecule so desperately needs.

Conflict of Interest Statement

S.D. and V.U. are employees of Lo.Li Pharma s.r.l. All other authors have no conflicts of interest to declare.

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Author Contributions

S.D. and V.U.: conceptualization, investigation, writing – original draft, and writing –review and editing. C.O.S., M.I.M.Y.-G., A.B., S.B., D.B., A.W., M.N., D.D., M.A., C.A., M.S.B.E., M.B., P.C., A.C., R.D., M.H.V.-L., I.H.M., Z.K., A.S.L., G.M., M.M.O., A.C.O., B.P., G.P., O.P., L.P., N.P., S.R., S.S., A.S., M.T., V.I.U., I.V., and F.F.: writing – original draft and writing – review and editing.

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