Estudio:

Efectos de la suplementación con mioinositol sobre la calidad de los ovocitos en pacientes con síndrome de ovario poliquístico (SOP): ensayo clínico con doble ciego (se anexa traducción al español).

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Efectos de la suplementación con mioinositol sobre la calidad de los ovocitos en pacientes con síndrome de ovario poliquístico (SOP): ensayo clínico con doble ciego

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Resumen.-*Antecedentes:* el síndrome de ovario poliquístico es la causa más frecuente de infertilidad por anovulación crónica en mujeres en edad reproductiva y se caracteriza por la producción excesiva de andrógenos y estrógenos. La administración a mujeres del factor vitamínico del complejo B D-chiro-inositol, se asoció con reducción de los niveles de testosterona en suero y a mejoría significativa en la función ovárica gracias a su capacidad para aumentar la sensibilidad a la insulina. Además, la suplementación con inositol mejora la calidad y aumenta el número de ovocitos recolectados después de la estimulación ovárica en pacientes sometidas a fertilización *in vitro* (FIV).

***Objetivo:* determinar los efectos de mioinositol sobre la calidad de los ovocitos en una muestra de mujeres con síndrome de ovario poliquístico.**

***Métodos y materiales:* las pacientes se dividieron en dos grupos; las pacientes en el grupo A recibieron 2 g de mioinositol + 200 µg de ácido fólico (Inofolic®, LO.LI. Pharma, Roma, Italia) y las pacientes en el grupo B recibieron 200 µg de ácido fólico solo; ambos grupos recibieron el tratamiento dos veces al día de forma continua durante 3 meses.**

***Resultados:* al finalizar el tratamiento, se encontró que el número de folículos de diámetro > 15 mm visibles en la ecografía durante la estimulación y el número de ovocitos disponibles al momento de la recuperación fueron significativamente mayores en el grupo tratado con mioinositol, y, por lo tanto, también lo fue el número de embriones promedio transferidos y de grado 1. La media de ovocitos inmaduros también se redujo significativamente (ovocitos en estadio de vesícula germinal y degenerados).**

***Conclusiones:* estos datos sugieren que mioinositol puede ser útil en el tratamiento de pacientes con SOP sometidas a inducción de ovulación para mejorar la sensibilidad a la insulina que desempeña un papel importante en la maduración de los ovocitos.**

Palabras clave:

Inositol, calidad de ovocitos, síndrome de ovario poliquístico, infertilidad, fertilización *in vitro*.

Introducción

El síndrome de ovario poliquístico (SOP) es una enfermedad compleja caracterizada por varias alteraciones endocrinas que pueden ser la causa de anovulación e hiperandrogenismo.

Este síndrome de carácter heterogéneo afecta entre 5 y 10% de la población femenina en edad reproductiva y puede considerarse la enfermedad endocrina que afecta con mayor frecuencia a las mujeres durante su vida reproductiva[1].

El SOP no puede considerarse únicamente como una disfunción ovárica localizada o un defecto hipotálamo-hipófiso-ovárico sino como la manifestación de una alteración funcional compleja de todo el sistema reproductor.

En relación con el aspecto hormonal, los ovarios micropoliquísticos se caracterizan por la producción excesiva de andrógenos y estrógenos y disociación de niveles de gonadotropinas en suero: elevación del nivel de hormona luteinizante (HL), nivel bajo o normal de hormona foliculoestimulante (HFE) y proporción entre HL y HFE > 2,5, en las formas típicas.

Los niveles de testosterona (T), androstenediona (AS), dehidroepiandrosterona (DHEA), sDHEA (sulfato), 17-hidroxiprogesterona (17-HPG) y estrona en sangre en pacientes con SOP fueron altos. Sin embargo, los niveles de globulina fijadora a hormonas sexuales (GFHS) circulante fueron bajos.

El aumento de la conversión de androstenediona a estrona resulta en hiperandrogenismo relativamente moderado.

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Los niveles de GFHS disminuyeron en 50%, aproximadamente, debido a los niveles más altos de insulina[2-4].

Aún se desconoce la etiología del síndrome, pero es probable que involucre varios factores, entre otros, la excesiva producción de E_1, alteración de la regulación hipotalámica primaria o de la esteroidogénesis ovárica o suprarrenal.

El diagnóstico de SOP se basa en patrones clínicos, hormonales y ecográficos. De acuerdo con los criterios de Rotterdam, establecidos en 2003, sólo se puede diagnosticar SOP después de excluir otras posibles causas de hiperandrogenismo y amenorrea, y siempre y cuando se cumplan al menos 2 de los siguientes criterios:

- Oligovulación o anovulación con irregularidades en la menstruación
- Niveles elevados de andrógenos circulantes o manifestaciones clínicas de hiperandrogenismo
- Evidencia de ovarios micropoliquísticos en la ecografía transvaginal.

Debido a la pulsatilidad de la HL un solo parámetro en sangre no es suficiente para diagnosticar SOP y no existe un consenso unánime sobre qué nivel de andrógenos en sangre debe tenerse en cuenta para un diagnóstico preciso (testosterona total o libre, proporción entre testosterona y GFHS o androstenediona).

Por lo general es posible excluir el diagnóstico de SOP si se presentan niveles altos de DHEA o 17HPG solos.

Al alcanzar la menarquía o después de un corto periodo de tiempo, los ciclos menstruales se vuelven irregulares. En muchos casos se distancian gradualmente hasta resultar en periodos cortos o permanentes de amenorrea. La disfunción menstrual en mujeres con SOP puede manifestarse de diferentes maneras, pero la más frecuente es la anovulación con sangrados irregulares.

El exceso de andrógenos es responsable de hirsutismo, piel grasa, acné y engrosamiento de la túnica albuginea en los ovarios. El grado de hirsutismo puede medirse con la escala de Ferriman-Gallwey.

En casos raros se pueden evidenciar patrones de virilización como aumento del tamaño del clítoris, hipertrofia muscular, voz gruesa, calvicie temporal y aspecto masculino. Sin embargo, en estos casos debe excluirse neoplasia ovárica baja o suprarrenal secretora de andrógenos.

Un patrón de sobrepeso, incluso obesidad, también puede asociarse a este síndrome.

El SOP es una de las causas endocrinas más frecuentes de infertilidad femenina; para quedar embarazada la mujer debe someterse a inducción de ovulación[5].

Por lo general, después de corregir el sobrepeso se recuperan los ciclos de ovulación o inmediatamente después de la suspensión de estrógenos y progestinas. De lo contrario, la ovulación debe inducirse farmacológicamente (asociada con administración de metformina)[6].

Uno de los medicamentos que se utiliza para este fin, el clomifeno, es un estrógeno débil que también actúa como antiestrógeno. Probablemente interactúa con los receptores hipotalámicos de estrógeno, desplaza el estradiol endógeno y crea una condición de hipoestrogenismo artificial debido a que es prácticamente inactivo en esta área. Este fenómeno activa a su vez los centros hipotalámicos, responsables de la liberación de la hormona de liberación de gonadotropina. Después de la administración de clomifeno la frecuencia de secreción pulsátil de HL y HFE aumenta mientras que la amplitud no cambia. En el SOP la ovulación es inducida en 80% de los casos y se presenta embarazo en el 20% restante.

En los casos en que no se obtuvo respuesta al tratamiento con clomifeno y metformina o se requirió fertilización *in vitro* o inyección intracitoplasmática de esperma (FIV/IIE) la ovulación se indujo administrando gonadotropinas. Las gonadotropinas que se utilizan para este fin se obtienen de la orina de mujeres posmenopáusicas (menotrofina). Recientemente se han desarrollado gonadotropinas con técnica de biosíntesis a partir de ADN recombinante (folitropina α y folitropina β). El objetivo de la terapia con gonadotropinas o con HFE es activar los folículos en su última fase de maduración que en condiciones fisiológicas normales ocurre durante las dos primeras semanas del ciclo menstrual, cuando ocurre la ovulación [5,7].

El objetivo de este estudio es determinar los efectos de mioinositol, un compuesto vitamínico

que pertenece al complejo B, sobre la calidad de los ovocitos en un grupo de pacientes con SOP que padecen anovulación crónica e infertilidad, sometidas a técnicas de reproducción asistida (TRA) como FIV e IIE.

Los estudios científicos han demostrado que, gracias a su capacidad para aumentar la sensibilidad a la insulina, D-chiro-inositol ejerce efectos benéficos sobre la ovulación y producción de andrógenos en mujeres con SOP. La administración de D-chiro-inositol se asoció con disminución del nivel de testosterona en suero[8] y aumento del nivel de GFHS. Junto con la reducción de la secreción de insulina las mujeres que recibieron D-chiro-inositol mostraron una gran mejoría en la función ovulatoria[9,10]. La evidencia científica también muestra que la suplementación con inositol ayuda a reducir la cantidad de HFE necesaria para la ovulación, mejora la calidad de los ovocitos (reduce la cantidad total de ovocitos en estadio de vesícula germinal y degenerados) y aumenta el número de ovocitos recolectados después de la estimulación ovárica en pacientes sometidas a TRA, como FIV o IIE[11-13]. No es posible definir el inositol como una vitamina, pero se considera un factor vitamínico perteneciente al complejo B. En el organismo humano se encuentra en los fosfolípidos y puede estimular la producción endógena de lecitina. También desempeña un papel específico en la actividad biológica de control del metabolismo de grasas y azúcares y la función celular del sistema nervioso. Es fundamental en el crecimiento del cabello y puede evitar la calvicie. Los estudios científicos revelan que las personas diabéticas eliminan una mayor cantidad de inositol que las personas no diabéticas[14].

En casos de resistencia a la insulina o diabetes tipo II, el inositol ayuda a mejorar todos los patrones clínicos. En estos casos, el inositol puede ser útil para evitar y corregir mecanismos fisiopatológicos subyacentes a las anomalías metabólicas y reproductivas relacionadas con el SOP.[15-18].

Métodos y materiales

Todas las pacientes se incluyeron y trataron en el Departamento de Ciencias Ginecológicas (Hospital Santo Bambino, Catania) del Centro de Estudios Clínicos de Ginecología y Endocrinología y de Fisiopatologías de la Reproducción Humana. En el doceavo mes de la fase de preselección se seleccionaron en total 34 mujeres menores de 40 años con síndrome de ovario poliquístico (SOP).

El diagnóstico de SOP se basó en la existencia de oligonomenorrea o amenorrea (seis o menos ciclos menstruales durante un año), hiperandrogenismo (hirsutismo, acné o alopecia) o hiperandrogenemia (niveles elevados de testosterona total o libre) y características ováricas típicas observadas mediante ecografía.

Se excluyeron mujeres con patologías endocrinas y metabólicas concomitantes, como hipotiroidismo, hipertiroidismo, diabetes mellitus, tumores secretores de andrógenos, hiperplasia adrenal, síndrome de Cushing.

Se recomendaron procedimientos de IIE o FIV después de evaluar la esperma del compañero.

Según la tabla de aleatorización, las pacientes se dividieron en dos grupos: las pacientes del grupo A recibieron 2 g de mioinositol + 200 µg de ácido fólico (Inofolic®, LO.LI. Pharma, Roma, Italia) y las mujeres en el grupo B recibieron 200 µg de ácido fólico solo; ambos grupos recibían las dosis dos veces al día de forma continua durante 3 meses.

Después de recolectar los ovocitos, durante el procedimiento tecnológico de reproducción asistida que eligieron las mujeres, se evaluó su calidad. Las técnicas de IIE o FIV comprenden varias etapas: estimulación ovárica, recolección de ovocitos, evaluación de calidad de ovocitos, fertilización *in vitro* de los ovocitos, cultivo y calificación de embriones, transferencia de embriones, que son fundamentales para el éxito de la técnica. El equipo médico del Centro de Fisiopatologías de Reproducción del Hospital Santo Bambino, en Catania, realizó seguimiento del proceso en la clínica de FIV con sala de cirugía anexa.

Análisis estadístico

La comparación entre los grupos A y B se realizó utilizando los siguientes métodos:

- Prueba χ^2 para datos cualitativos (positividad para CGh-β)

- Prueba t Student para datos cuantitativos distribuidos normalmente (edad, IMC, cantidad de unidades de HFE administradas, número de folículos de diámetro >15 mm).
- Prueba U para datos cuantitativos no distribuidos normalmente (días de estimulación, pico máximo de E_2, número de ovocitos recuperados).

Resultados

Como se mencionó anteriormente, durante el estudio, las pacientes se dividieron aleatoriamente en dos grupos, y el diseño del estudio fue doble ciego.

No se encontraron diferencias significativas de edad o índice de masa corporal (IMC) entre los dos grupos.

La cantidad de unidades de HFEr administradas para estimulación ovárica fue significativamente menor en el grupo A.

Como se reporta en la literatura, los niveles máximos de E_2 al administrar gonadotropina coriónica humana (GCh) fueron más bajos en el grupo A. Sin embargo, nuestros datos no fueron estadísticamente significativos.

En el grupo A se cancelaron dos ciclos mientras que en el grupo B se cancelaron cinco ciclos debido a niveles máximos de E_2 >4000 pg/ml (riesgo de hiperestimulación).

El número de folículos con diámetro >15 mm visibles en ecografía durante la estimulación y el número de ovocitos recuperados fue significativamente mayor en el grupo tratado con mioinositol (Tabla I).

Tabla I. Ovocitos recuperados

		Percentiles	
Grupo	**Mediana**	**25**	**75**
A	12	10	16
B	8,50	6,25	10,75
	$P < 0,05$		

La media de ovocitos inmaduros (ovocitos en estadio de vesícula germinal y ovocitos degenerados) disminuyó significativamente y la tasa de ovocitos en metafase II (MII), caracterizados por tener vesícula germinal no visible y cuerpo polar primario visible (Tabla II), mostró tendencia al aumento.

No se evidenciaron diferencias estadísticamente significativas en el número de embriones fertilizados; sin embargo, en el grupo A la media de embriones transferidos fue significativamente mayor con mayores cantidades de embriones grado 1 en comparación con embriones de baja calidad (Tabla III).

De conformidad con la ley italiana ART no se transfirieron más de tres embriones. No se detectaron diferencias en el número total de embarazos bioquímicos[9,19].

Tabla II. Ovocitos en metafase II (MII), degenerados o en estadio de vesícula germinal.

	Grupo A		Grupo B		
	Frecuencia	**%**	**Frecuencia**	**%**	***P***
Ovocitos en MII	176	82,24	160	66,87	NS
Ovocitos degenerados	2	0,93	23	14,37	0,02
Ovocitos en estadio de vesícula germinal	3	1,4	15	9,37	0,02

Tabla III. Número de embriones grado I

	Grupo A		Grupo B		
	Frecuencia	%	Frecuencia	%	*P*
Embriones grado 1	30	68,1	9	29	< 0,01

Discusión

El síndrome de ovario poliquístico (SOP) es una de las alteraciones endocrinas más frecuentes entre las mujeres. La resistencia a la insulina y la hiperinsulinemia se relacionan estrechamente con el fenotipo de una gran parte de las mujeres con SOP.

Se sospecha que existe un defecto en la acción de la insulina, probablemente debido a un déficit de D-chiro-inositol, que es un componente de los inositolfosfoglicanos. Los medicamentos para reducir la insulina y en particular diferentes formas de inositol representan terapias innovadoras para la recuperación de la ovulación espontánea y es posible que tengan una acción positiva incluso en la maduración meiótica de los ovocitos. Estas terapias parecen influir directamente en la esteroidogénesis al reducir la producción de andrógenos en las células tecales. Se ha demostrado que la administración de D-chiro-inositol aumenta la acción de la insulina en pacientes con SOP, mejora la función ovulatoria[10] y reduce los niveles de testosterona en suero[8,9,19].

Actualmente existen pocos datos sobre la acción y efectos de mioinositol, un precursor del D-chiro-inositol, sobre la anovulación en mujeres en edad reproductiva u ovulación espontánea en ciclos de estimulación.

Mioinositol es un componente importante del microentorno folicular y desempeña un papel clave en el desarrollo nuclear y citoplasmático de los ovocitos.

En técnicas de reproducción asistida, la suplementación con mioinositol se asocia positivamente con la progresión meiótica de ovocitos murinos en estadio de vesícula germinal y aumento de oscilación intracelular de Ca^{2+}. Los niveles elevados de mioinositol en líquido folicular humano constituyen un marcador de ovocitos de buena calidad[21].

Nuestro estudio es uno de los pocos que se enfoca en esta molécula, que pertenece al complejo de vitamina B, y sus efectos en las pacientes con el SOP sometidas a inducción de ovulación. Los datos preliminares con los que contamos muestran que, el tratamiento con mioinositol + ácido fólico en pacientes con SOP reduce el número de ovocitos en estadio de vesícula germinal y degenerados sin afectar el número total de ovocitos recuperados, en comparación con el ácido fólico solo. Igual que en otros ensayos, estos resultados sugieren que mioinositol tiene un efecto positivo sobre el desarrollo de ovocitos maduros[22].

Se sabe que la inducción de ovulación en pacientes con SOP es un asunto fundamental, teniendo en cuenta el riesgo de síndrome de hiperestimulación ovárica[23-24]. Los niveles elevados de andrógenos en suero en la línea de base se relacionan con niveles elevados de E_2 en suero, como se detectó frecuentemente en pacientes con SOP sometidas a inducción de ovulación con gonadotropinas exógenas.

Debido a que mioinositol es un precursor de D-chiro-inositol, es posible proponer la hipótesis de que puede tener una acción de sensibilización a la insulina en el ovario con la subsecuente acción positiva sobre el perfil hormonal y reducción del nivel basal de testosterona en suero[8,9,25]. Se observó una reducción significativa en los niveles de E_2 en pacientes que recibieron mioinositol + gonadotropinas exógenas (GCh). Por lo tanto, se puede suponer que es posible adaptar este protocolo para reducir el riesgo de hiperestimulación en estas pacientes.

En conclusión, nuestras observaciones sugieren que mioinositol puede ser útil en el tratamiento de pacientes con SOP sometidas a inducción de ovulación por su actividad sensibilizadora a la insulina y el papel que desempeña en la maduración de los ovocitos.

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Effects of Myo-Inositol supplementation on oocyte's quality in PCOS patients: a double blind trial

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Abstract. – *Background:* **Polycystic ovary syndrome is the most common cause of chronic anovulation infertility in women in fertile period, and it's characterized by an increased production of androgens and estrogens. The administration of D-chiro-inositol, a B complex vitamin, was associated with a decreased of serum testosterone and simultaneously, due to its ability to increase insulin sensitivity, women who received D-chiro-inositol showed a great improvement of the ovulary function. Besides, the supplementation of inositol improves the oocytes' quality and increase the number of oocytes collected after ovarian stimulation in patients undergoing IVF (*in vitro* fertilization).**

Aim: **The aim of this study is to determine the effects of myo-inositol on oocyte's quality on a sample of women with polycystic ovary syndrome.**

Material and Methods: **The patients were divided into two groups: patients of Group A intook 2 g of myo-inositol + 200 µg of folic acid (Inofolic®, LO.LI. Pharma, Rome, Italy) while Group B only 200 µg of folic acid, both groups took the treatment twice a day, continuously for 3 months.**

Results: **At the end of treatment, the number of follicles of diameter >15 mm, visible at ultrasound during stimulation, and the number of oocytes recovered at the time of pick-ups were found to be significantly greater in the group treated with myo-inositol, so as the aver-age number of embryos transferred and embryo Score S1. Significantly reduced was the average number of immature oocytes (vesicles germ and degenerated oocytes) too.**

Conclusions: **These data suggest that myo-inositol may be useful in the treatment of PCOS patients undergoing ovulation induction, both for its insulin-sensitizing activity, and its role in oocyte maturation.**

Key Words:

Inositol, Oocyte's quality, Polycystic ovary syndrome, Infertility, *In vitro* fertilization.

Introduction

Polycystic Ovary Syndrome (PCOS) is a complex disease characterized by various endocrine disorders that can be the potential cause of anovulation and hyperandrogenism condition.

This heterogenous syndrome affects about 5-10% of female population in the reproductive age, and it can be considered as the most common endocrine disorder affecting women during the reproductive life[1].

PCOS cannot be merely considered a local ovarian dysfunction or a central hypothalamus-ovary-pituitary defect, but it is the expression of a complex functional alteration of the whole reproductive system.

Under a hormonal point of view, the micropolycystic ovary is characterized by an increased production of androgens and estrogens, and a dissociation of gonadotropins serum concentrations: elevated luteinizing hormone (LH), low or normal follicle stimulating hormone (FSH) and LH/FSH ratio that usually exceeds 2.5 in the typical forms.

In the blood of PCOS patients testosterone (T), androstenedione (AS), dehydroepiandrosterone (DHEA), DHEA-S (sulfate), 17-hydroxyprogesterone (17-OHP) and estrone resulted elevated. The circulating levels of sex hormone-binding globulin (SHBG) are instead lower.

The enhancing peripheral conversion of androstenedione to estrone leads to the modest relative hyperandrogenism.

SHBG levels are reduced of about 50% due to the increased levels of insulin[2-4].

The syndrome's etiology is still unknown, but it is probably multifactorial, due to an excessive E_1 production, or to an alteration of the primitive hypothalamic regulation and of the ovarian and/or adrenal steroidogenesis.

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The diagnosis of PCOS is based on the clinical, hormonal and ultrasound patterns. In accordance with the Rotterdam Criteria, drawn in 2003, PCOS diagnosis can be made only after the exclusion of other causes of hyperandrogenism and amenorrhea, and in the presence of at least 2 of the following criteria:

- Oligo- and/or anovulation with menstrual irregularities;
- Elevated levels of circulating androgens or clinical manifestation of hyperandrogenism;
- Transvaginal pelvic ultrasound evidence of micropolycystic ovary.

Due to the pulsatility of LH, only one blood parameter is not enough for the PCOS diagnosis, and there is no unanimous consensus on which androgen blood's level should be considered for a precise diagnosis (total or free testosterone, testosterone/SHBG ratio or androstenedione).

Usually, elevated levels of only DHEA or 17-OHP may exclude the diagnosis of PCOS.

Since menarche, or after a short period, menstrual cycles show an irregular rhythm. In many cases they gradually distance themselves from each other, up to result in short periods of amenorrhea or in permanent amenorrhea. Menstrual dysfunction in women affected by PCOS may manifest in different ways, but the probably most common way is anovulation with erratic bleedings.

Androgens excess is responsible for hirsutism, oily skin, acne and, in the ovary, for the thickening of the tunica albuginea. The degree of hirsutism can be measured with the Ferriman-Gallwey score.

In rare cases virilization patterns can be observed, with increased size of clitoris, muscle mass hypertrophy, deep voice, temporal balding and masculine aspect. In these cases, however, a lower ovarian or an adrenal androgen-secreting neoplasia must be excluded.

At the same time an overweight pattern, up to obesity can be associated to the syndrome.

PCOS is one of the most common endocrine causes of female infertility: if you want to get pregnant, ovulation should be induced[5].

Ovulatory cycles are obtained, usually, after the overweight correction, or immediately after the estrogen-progestins suspension. If it does not happen, ovulation should be induced pharmacologically (usually associated with metformin administration)[6].

Clomiphene is a drug normally used for this purpose: it is a weak estrogen that acts also as anti-estrogen. Probably, it interacts with the hypothalamic estrogen receptors, displacing the endogenous estradiol and creating a condition of artificial hypoestrogenism, due to its biological activity almost absent in this district. Hypothalamic centers, responsible for gonadotropin-releasing hormone (GnRH) release are thus stimulated to greater activity. Following the administration of clomiphene, in fact, the frequency of pulsatile secretion of LH and FSH increases, while the amplitude remains unchanged. Ovulation in PCOS is induced in 80% of cases, while pregnancy occurs in 20% of cases.

Where no response to the treatment with clomiphene and metformin was obtained, or where an *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) was necessary, ovulation induction was performed by the administration of gonadotropins. Gonadotropins used for this purpose are obtained from the urine of postmenopausal women (Menotrophin). Recently, gonadotropins obtained with biosynthetic technique from recombinant DNA have been introduced (Follicotropin α and Follicotropin β). The goal of the therapy with gonadotropins, or rather with FSH, is acting on the follicles in the last stage of their maturation process that, under physiological conditions is restricted to the first two weeks of the menstrual cycle in which ovulation occurs[5,7].

The aim of this study is to determine the effects of Myo-Inositol, a compound belonging to vitamin B complex, on oocyte's quality in a group of patients with PCOS, suffering from chronic anovulation and infertility, undergoing medically assisted reproduction techniques (ART), such as IVF and ICSI.

Scientific studies have shown that D-chiro-inositol, thanks to its ability to increase insulin sensitivity, has beneficial effects on ovulation and on the androgens production in women with PCOS. The administration of D-chiro-inositol was associated with a decreased of serum testosterone[8] and increased of SHBG concentration. Simultaneously to the reduction of insulin secretion, women who received D-chiro-inositol showed a great improvement of the ovulary function[9,10]. Scientific evidence has also shown that the supplementation with inositol contributes to reducing the amount of FSH necessary to ovulation, to improving oocyte's quality (reduction of the total amount of the germinal vesicles and the degener-

ated oocytes) and to increasing the number of oocytes collected after ovarian stimulation in patients undergoing ART techniques, as IVF or ICSI[11-13]. Inositol cannot be defined exactly as a vitamin, but it is considered a vitamin factor belonging to B complex. In the human organism it is present in the phospholipids, and it can stimulate endogenous production of lecithin. Its role also includes a specific biological activity of control on fat and sugar metabolism, and on the cellular function of the nervous system. It is also essential to hair growth and it can prevent baldness. Scientific studies revealed that diabetic subjects eliminate amounts of inositol significantly higher that no-diabetic ones[14].

In case of insulin resistance or type II diabetes, inositol helps to improve the whole clinical pattern. In these cases, inositol may be useful to prevent and to correct pathophysiological mechanisms underlying the metabolic and reproductive abnormalities related to PCOS[15-18].

Materials and Methods

All the patients were enrolled and treated in the Department of Gynecological Sciences ("Santo Bambino" Hospital, Catania), at the Gynecological Endocrinology Clinics and Human Reproduction Pathophysiology Centre. In the 12-month enrollment phase a total of 34 women, aged <40 years with polycystic ovary syndrome (PCOS) were selected.

PCOS diagnosis was indicated by oligo-amenorrhea (six or fewer menstrual cycles during a period of one year), hyperandrogenism (hirsutism, acne or alopecia) or hyperandrogenemia (elevated levels of total or free testosterone), and typical feature of ovaries at ultrasound scan.

Concomitant endocrine and metabolic pathologies, as hypothyroidism, hyperthyroidism, diabetes mellitus, androgen-secreting cancers, adrenal hyperplasia, Cushing syndrome were excluded.

The ICSI or IVF procedures were recommended after the evaluation of the sperm semen of the male partner.

According to a randomization table, patients were divided into two groups: patients of Group A intook 2 g of myo-inositol + 200 µg of folic acid (Inofolic®, LO.LI. Pharma, Rome, Italy) while Group B only 200 µg of folic acid, both groups took the treatment twice a day, continuously for 3 months.

Oocyte's quality assessment was performed after the oocyte pick-up conducted during the assisted reproductive technology procedure in which patients have been submitted. The ICSI or IVF includes several phases (ovarian stimulation, oocyte collection, oocyte quality assessment, oocytes in-vitro fertilization, embryo culture and scoring, embryo transfer), all crucial for the success of the technique. They were all followed by the medical team of the Reproduction Pathophysiology Centre of "Santo Bambino" Hospital, in Catania at the IVF clinic with the attached surgery room.

Statistical Analysis

The comparison between Group A and Group B was performed using:

- X^2 test for qualitative data (β-hCG positivity);
- Student *t* test for quantitative data normally distributed (age, BMI, total FSH units administered, number of follicles of diameter >15 mm);
- U test for quantitative data not normally distributed (days of stimulation, E_2 maximum peak, number of oocytes retrieved).

Results

During the study period, patients were randomly divided into two groups, as described before, and the investigation was performed in a double-blind design.

No significant differences were found between the two groups in mean age and body mass index (BMI).

Total r-FSH units administered for the ovarian stimulation were significantly reduced in group A.

As reported in literature, peak E_2 levels at human chorionic gonadotropin (hCG) administration were lower in group A, but our data were not statistically significant.

Two cycles were cancelled in group A, whereas in group B five cycles were suspended, because of peak E_2 >4,000 pg/mL (risk of hyperstimulation).

The number of follicles with a diameter >15 mm, visible at ultrasound scan during stimulation, and the number of oocytes retrieved at the pick-up resulted significantly higher in the myo-inositol-treated group (Table I).

Table I. Retrieved oocytes at the pick-up.

Group	Median	Percentiles 25°	Percentiles 75°
A	12	10	16
B	8.50	6.25	10.75
	$P < 0.05$		

The mean number of immature oocytes (germinal vesicles and degenerated oocytes) was significantly reduced, and there was an increasing trend of the rate of oocytes in metaphase II (MII), that are oocytes characterized by not visible germinal vesicles and visible first polar body (Table II).

No statistical significance in the number of fertilized embryos was emerged, but in group A the mean number of transferred embryos resulted significantly higher, with higher amounts of score 1 embryos in comparison with lower-quality embryos (Table III).

In compliance with the italian ART law, no more than three embryos were transferred. No differences in the total number of biochemical pregnancies were detected.

Discussion

Polycystic ovary syndrome (PCOS) is one of the most common female endocrine disorders. Insulin-resistance and hyperinsulinemia are strictly correlated with the phenotype of a large part of PCOS women.

A defect in the insulin's action has been suspected, probably because of a deficiency of D-chiro-inositol, that is a component of inositol phosphoglycans. Insulin-lowering drugs, particularly different forms of inositol, represent novel therapies in spontaneous ovulation restoration, with a potential positive action even on meiotic oocyte maturation. These therapies seem to directly influence steroidogenesis, by reducing androgen production in theca cells. In fact, the administration of D-chiro-inositol has been demonstrated to increase the insulin action in PCOS patients, improving ovulatory function[10], and reducing serum testosterone concentration[8, 9,19].

Nowadays, there are few data on the action and effects of myo-inositol, a precursor of D-chiro-inositol, on the anovulatory women in reproductive age or on the spontaneous ovulation in stimulated cycles.

Anyway, myo-inositol is an important constituent of the follicular microenvironment, playing a key role in the nuclear and cytoplasmic oocyte's development.

In the assisted reproduction techniques, in fact, the supplementation with myo-inositol is positively related to meiotic progression of mouse germinal vesicle oocytes, enhancing intracellular Ca^{2+} oscillation[20]. Furthermore, higher concentrations of myo-inositol in human follicular fluid provide a marker of good-quality oocytes[21].

Our study is one of the fews focusing on this molecule, that belongs to vitamin B complex, and on its effects in PCOS patients undergoing ovulation induction. Preliminary data in our hands show that, in PCOS patients the treatment with myo-inositol and folic acid, compared with folic acid alone, reduces the number of germinal vesicles and degenerated oocytes, without compromising the total number of retrieved oocytes. These results, as other trials', suggest that myo-inositol has a positive effect on mature oocytes development[22].

Furthermore, it is well known that ovulation induction in PCOS patients is a pivotal matter, even because of the risk of the ovarian hyperstimulation syndrome[23-24]. Elevated basal serum levels of androgens are involved in the produc-

Table II. Metaphase II (MII) oocytes, degenerated oocytes and germinal vesicles.

	Group A		Group B		
	Frequency	%	Frequency	%	*P*
MII oocytes	176	82.24	160	66.87	NS
Degenerated oocytes	2	0.93	23	14.37	0.02
Germinal vesicles	3	1.4	15	9.37	0.02

Table III. Number of score 1 embryos.

	Group A		Group B		
	Frequency	%	Frequency	%	*P*
Score 1 embryos	30	68.1	9	29	< 0.01

tion of high serum E_2 levels, as typically detected in PCOS patients undergoing ovulation induction with exogenous gonadotropins.

Because myo-inositol is a D-chiro-inositol precursor, an insulin-sensitizing action on the ovary may be similarly hypothesized, with a subsequent positive action on the hormonal profile, particularly on basal serum testosterone reduction[8,9,25]. In fact, in patients treated with myo-inositol plus exogenous gonadotropins a significant reduction in E_2 levels at hCG administration was found. As consequence, it can be supposed that this protocol could be adopted to reduce the risk of hyperstimulation in such patients.

In conclusion, these observations suggest that myo-inositol may be useful in the treatment of PCOS patients undergoing ovulation induction, both for its insulin-sensitizing activity, and its role in oocyte maturation.

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Estudio:

Efectos metabólicos y hormonales de mioinositol en mujeres con síndrome de ovario poliquístico: ensayo doble ciego (se anexa traducción al español).

Efectos metabólicos y hormonales de mioinositol en mujeres con síndrome de ovario poliquístico: ensayo doble ciego

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Resumen- **El objetivo de este estudio fue investigar los efectos del tratamiento con mioinositol (medicamento sensibilizante a la insulina) sobre la insulina circulante, la tolerancia a la glucosa, la ovulación y las concentraciones séricas de andrógenos en las mujeres con síndrome de ovario poliquístico (SOP). Cuarenta y dos mujeres con SOP fueron tratadas en un ensayo doble ciego con mioinositol más ácido fólico o ácido fólico solo como placebo. En el grupo tratado con mioinositol la testosterona sérica total disminuyó de 99,5±7 a 34,8±4,3 ng/dl (grupo de placebo: de 116,8±15 a 109±7,5 ng/dl; *P*=0,003), y la testosterona sérica libre de 0,85±0,1 a 0,24±0,33 ng/dl (grupo de placebo: de 0,89±0,12 a 0,85±0,13 ng/dl; *P*= 0,01). Los triglicéridos plasmáticos disminuyeron de 195±20 a 95±17 mg/dl (grupo de placebo: de 166±21 a 148±18 mg/dl; *P*=0,001). La presión sanguínea sistólica disminuyó de 131±2 a 127±2 mmHg (grupo de placebo: de 128±1 a 130±1 mmHg; *P*=0,002). La presión arterial diastólica disminuyó de 88±1 a 82±3 mmHg (grupo de placebo: de 86±1 a 90±1 mmHg; *P*=0,001). El área bajo la curva de insulina plasmática después de la administración oral de glucosa disminuyó desde 8,54±1,149 a 5,535±1,792 μU/ml/min (grupo de placebo: de 8,903±1,276 a 9,1±1,162 μU/ml/min; *P*=0,03). El índice de sensibilidad a la insulina compuesto en todo el cuerpo (ISIcomp) aumentó de 2,80±0,35 a 5,05±0,59 mg-2/dl-2 (grupo de placebo: de 3,23±0,48 a 2,81±0,54 mg-2/dl-2; *P*=0,002). Dieciséis de las 23 mujeres del grupo de mioinositol ovularon (4 de las 19 en el grupo de placebo). El tratamiento de las pacientes con SOP con mioinositol proporcionó una disminución de la insulina circulante y de la testosterona sérica total, así como una mejora en los factores metabólicos.**

Palabras Claves:

Síndrome de Ovario Poliquístico, SOP, mioinositol, Síndrome Metabólico.

Introducción

El síndrome de ovario poliquístico (SOP) es uno de los trastornos endocrinos más frecuentes en las mujeres en edad reproductiva[1-3]; su etiología continúa siendo desconocida[4]. Los trastornos ovulatorios representan una causa mayor de infertilidad, y la oligovulación y la anovulación con el síndrome de ovario poliquístico (SOP) son causas comunes de infertilidad, que es la endocrinopatía más frecuente de las mujeres en edad reproductiva que afecta a 6-10% de la población.

La definición actual de SOP requiere la presencia de dos de las siguientes tres condiciones: (i) oligoanovulación y/o anovulación; (ii) signos clínicos y/o bioquímicos de hiperandrogenismo que pueden estar asociados con hirsutismo; y (iii) ovarios poliquísticos – y la exclusión de otras etiologías. Otras características del SOP son acné, seborrea, obesidad, resistencia a la insulina, deterioro de la tolerancia a la glucosa y diabetes mellitus tipo 2, dislipidemia, enfermedad cardiovascular e infertilidad. Además, las alteraciones endocrinas y metabólicas evaluadas mediante las concentraciones séricas elevadas de testosterona, insulina, hormona luteinizante (LH) y prolactina son prevalentes en la población con SOP. Éstas últimas pueden tener implicaciones profundas para la salud a largo plazo de las pacientes.

En mujeres jóvenes con SOP, la resistencia a la insulina puede ocurrir con alta frecuencia. De hecho, muchos estudios revelaron que es intrínseco al síndrome y afecta 30 a 40% de las pacientes con SOP[5]. Algunos estudios mostraron que la resistencia a la insulina en el SOP puede estar vinculada a la esteroidogénesis ovárica anormal por medio de la alteración de la transducción de las señales de insulina[6,7].

La molécula de inositol fosfoglicano se conoce por tener un papel en la activación de las encimas

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que controlan el metabolismo de la glucosa[8-10]. Un defecto en la disponibilidad en el tejido o en la alteración del metabolismo de los mediadores de inositol o inositol fosfoglicano, como en las mujeres con SOP, puede contribuir a la resistencia a la insulina[11,12].

La isoforma de inositol pertenece al complejo de vitamina B. La epimerización de los seis grupos de hidroxilo de inositol lleva a la formación de hasta nueve estereoisómeros, incluido mioinositol (MI) y D-chiro-inositol (DCI). Las concentraciones elevadas de MI en el líquido folicular parecen desempeñar un papel en la madurez folicular y proporcionan un marcador de ovocitos de buena calidad[13-15]. Además, los experimentos con ovocitos de ratón mostraron que la adición de MI al cultivo aumenta la progresión meiótica de la vesícula germinal mejorando la oscilación intracelular de Ca^{2+} [16].

Las mujeres con SOP podrían responder favorablemente al tratamiento con medicamentos sensibilizadores a la insulina. Los estudios previos han demostrado que la utilización de metformina, troglitazona o mioinositol reducen los andrógenos séricos y mejoran la ovulación en las mujeres con SOP. En dichos estudios, la administración de metformina a las pacientes mostró una reducción en la circulación y disminución de las concentraciones séricas de testosterona total y libre[17].

En un estudio reciente se determinó que existe deficiencia de mioinositol en mujeres con SOP con resistencia a la insulina y permitió establecer que la administración de mioinositol reduce la insulina y testosterona séricas y mejora la ovulación[18].

El objetivo de este estudio fue investigar los efectos metabólicos y hormonales de MI en las pacientes con SOP.

Materiales y Métodos

Este estudio fue un ensayo doble ciego (pacientes e investigadores).

Cuarenta y dos pacientes de 18 a 40 años de edad fueron seleccionadas para el estudio. Eran pacientes con SOP afectado con oligomenorrea, concentraciones altas de testosterona sérica libre e hirsutismo.

Las mujeres fueron observadas mediante ultrasonografía pélvica y se determinó que padecían SOP[19].

Trece de las 42 mujeres estaban tomando algunos medicamentos (anticonceptivos orales, sensibilizadores a la insulina y otros) durante dos meses antes del estudio.

Después de la asignación aleatoria, 23 mujeres recibieron 4 g de mioinositol más 400 mcg de ácido fólico (Inofolic®) y 19 mujeres recibieron 400 mcg de ácido fólico (Fertifol®) sólo como placebo. El tratamiento se realizó durante 12-16 semanas.

Siete mujeres presentaban deterioro de la tolerancia a la glucosa (concentración plasmática de glucosa >140 mg/dl, <200 mg/dl dos horas después de la ingestión oral de 75 g de dextrosa). Cuatro de ellas fueron asignadas para recibir mioinositol (Inofolic®) y 3 fueron asignadas para recibir placebo (únicamente ácido fólico).

El estudio fue aprobado por las juntas de revisión institucional y cada mujer dio su consentimiento informado por escrito.

Cuando se inició el estudio, las pacientes estaban en la fase folicular del ciclo menstrual (concentración sérica de progesterona menor de 2,5 ng/ml). El primer día se midió la presión arterial, el peso, la estatura y el índice cintura-cadera. En la mañana, 8:30, 8:45 y 9:00 am, se obtuvieron muestras para exámenes de globulina fijadora de hormonas sexuales y esteroides séricos. A las 9:00 am se administraba 75 g de dextrosa y se medían la glucosa plasmática y la insulina después de los 30, 60, 90 y 120 minutos.

Se explicó cómo tomar los medicamentos (mioinositol o placebo vía oral una vez al día) a las pacientes y se les recomendó no cambiar sus hábitos usuales de comidas, deporte y estilo de vida.

La progesterona sérica se midió semanalmente y si los resultados relevantes estaban por encima de 8 ng/ml se suponía que estaba ocurriendo la ovulación.

Después de 6 semanas de recibir los medicamentos (día 49), las mujeres en la fase folicular (concentración sérica de progesterona <2,5 ng/ml) se repitieron todas las mediciones realizadas en la línea de base.

Análisis estadísticos

Se reportaron los resultados como media ± desviación estándar (DE).

Las áreas bajo la curva de respuesta por la regla trapezoidal se utilizaron para evaluar la glucosa plasmática y la concentración de insulina después de la administración oral de glucosa.

Para determinar la sensibilidad a la insulina se utilizó la prueba de tolerancia a la glucosa oral (PTGO) mediante el índice de sensibilidad a la insulina compuesto (ISIcomp). Esta metodología se desarrolló por Matsuda y De Fronzo[20]: ISI-comp=10.000/raíz cuadrada de ([glucosa en ayunas x insulina en ayunas] x [glucosa media x

Tabla 1. Características basales.

Variable	Mioinositol N = 23	Placebo N = 19
Edad	28,8 ± 1,5	27,1 ± 1,4
Índice cintura-cadera	0,88 ± 0,02	0,87 ± 0,02
IMC (kg/m^2)	22,8 ± 0,3	22,5 ± 0,3
Periodo menstrual/año	3 ± 1	3 ± 1
Testosterona libre (ng/dl)	0,85 ± 0,11	0,89 ± 0,12
Androstenediona (ng/dl)	267 ± 19	271 ± 21
DHEAS (µg/dl)	366 ± 47	384 ± 63
Testosterona total (ng/dl)	99,5 ± 6,9	116,8 ± 14,7
17 beta estradiol (pg/ml)	45 ± 2,5	70 ± 6,7
Globulina fijadora de hormonas sexuales (nmol/L)	144,4 ± 18,6	147 ± 14,5
Colesterol total (mg/dl)	210 ± 10,4	195 ± 7,35
Triglicéridos (mg/dl)	195 ± 20,2	166 ± 20,6
ISIcomp (mg-2/dl-2)	2,80 ± 0,35	3,23 ± 0,48
AUC de Glucosa (mg/dl/min)	12,409 ± 686	12,970 ± 802
AUC de Insulina (µU/ml/min)	8,549 ± 1,149	8,903 ± 1,276
Insulina en ayunas (µU/ml)	32,5 ± 4,1	30,8 ± 7,3
Glucosa en ayunas (mg/dl)	87,6 ± 3,5	84,9 ± 5,8
Presión arterial sistólica (mmHg)	131 ± 2,3	128 ± 1,3
Presión arterial diastólica (mmHg)	88 ± 1,0	86 ± 7,0

DHEAS= dehidroepiandrosterona; AUC= área bajo la curva durante 2 horas, prueba de tolerancia a la glucosa oral de 75 g; ISIcomp= índice de sensibilidad a la insulina compuesto en todo el cuerpo.

media durante PTGO]).

Para analizar la diferencia en las velocidades de ovulación entre las mujeres que recibieron mioinositol y las que recibieron placebo se utilizó la prueba exacta de Fisher. Los resultados de las demás variables se obtuvieron comparando los cambios desde la línea de base hasta el final del estudio en ambos grupos. La distribución de los cambios en los dos grupos fue sometida a prueba primero para normalidad con la utilización de la prueba de Wilks-Shapiro, y posteriormente las distribuciones se compararon entre sí utilizando la prueba t de Student bilateral no pareada o prueba de suma de rangos de Wilcoxon. Los valores de P <0,05 se consideraron significativos.

Resultados

Las mujeres de los dos grupos fueron similares para las características basales (edad, IMC, índice cintura-cadera, lípidos plasmáticos y otros) (Tabla I). No se registraron diferencias significativas en los dos grupos para insulina plasmática en ayunas, glucosa plasmática, área bajo la curva para insulina y glucosa durante la PTGO, y la frecuencia de la tolerancia a la glucosa.

Se observó un leve cambio en el IMC en ambos grupos del estudio (Tabla II).

No existió ninguna modificación estadísticamente significativa en el índice cintura-cadera en ambos grupos.

Existió una disminución en la presión sistólica en el grupo de mioinositol (de 131±2 a 127±2 mmHg) mientras que en el grupo de placebo se presentó aumento (de 128±1 a 130±1 mmHg; P=0,002); con la presión arterial diastólica ocurrió algo similar, se presentó disminución (de 88±1 a 82±3 mmHg) en el grupo de mioinositol y aumento (de 86±7 a 90±1 mmHg) en el grupo de placebo (P=0,001).

En el grupo de mioinositol, los triglicéridos plasmáticos disminuyeron 52% (de 195±20 a 95±17 mg/dl) y el colesterol total disminuyó significativamente (de 210±10 a 171±11 mg/dl).

Tabla II. Características antropomórficas y de lípidos

Características	Grupo de mioinositol N=23		Grupo de placebo N=19		Valor P Para comparación del cambio
	Línea de base	Después del tratamiento	Línea de base	Después del tratamiento	
Presión arterial sistólica (mmHg)	131 ± 2	127 ± 2	128 ± 1	130 ± 1	0,002
Presión arterial diastólica (mmHg)	88± 1	82 ± 3	86 ± 7	90 ± 1	0,001
Triglicéridos (mg/dl)	195 ± 20	95 ± 17	166 ± 21	148 ± 19	0,001
Colesterol total (mg/dl)	210 ± 10	171 ± 11	195 ± 7	204 ± 9	0,001
IMC (kg/m^2)	22,8 ± 0,3	22,9 ± 0,3	22,5 ± 0,3	22,4 ± 0,1	NS
Índice cintura-cadera	0,88 ± 0,02	0,87 ± 0,02	0,87 ± 0,02	0,89 ± 0,01	NS

NS: no significativa

Tabla III. Mediciones de la glucosa plasmática e índice de sensibilidad a la insulina (durante 6 a 8 semanas)

Características	Grupo de mioinositol N=23		Grupo de placebo N=19		*Valor P* Para comparación del cambio
	Línea de base	Después del tratamiento	Línea de base	Después del tratamiento	
Insulina en ayunas (µU/ml)	32 ± 4	26 ± 8	30,8 ± 7	38 ± 7	0,20
Glucosa en ayunas (µU/ml)	87,6 ± 4	81,6 ± 4	84,9 ± 6	88 ± 4	0,12
AUC de glucosa (mg/dl/min)	12,409 ± 686	10,452 ± 414	12,970 ± 802	12,992 ± 7,93	0,04
AUC de insulina (µg/ml/min)	8,54 ± 1,149	5,535 ± 1,792	8,903 ± 1,276	9,1 ± 1,162	0,03
ISIcomp (mg^{-2}/dl^{-2})	2,80 ± 0,35	5,05 ± 0,59	3,23 ± 0,48	2,81 ± 0,54	<0,002

AUC= área bajo la curva durante 2 horas, prueba de tolerancia a la glucosa oral de 75 g; ISIcomp= índice de sensibilidad a la insulina compuesto en todo el cuerpo.

La concentración plasmática en ayunas de insulina no cambió significativamente en ningún grupo de estudio (Tabla III). El área bajo la curva de insulina plasmática disminuyó 36% (de 8,54±1,149 a 5,535±1,792 µU/ml/min) aunque no ocurrió lo mismo en el grupo de placebo (de 8,903±1,276 a 9,1±1,162 µU/ml/min; *P*=0,003).

Igualmente ocurrió para la concentración plasmática en ayunas de glucosa (de 87,6±4 a 81,6±4 mg/dl). El área bajo la curva de glucosa plasmática durante la PTGO disminuyó en el grupo de mioinositol (de 12,409±686 a 10,452±414 mg/dl/min) aunque un leve incremento ocurrió en el grupo de placebo (de 12,970±802 a 12,992±793 mg/dl/min; *P*=0,004).

El índice de sensibilidad a la insulina compuesto (ISIcomp) aumentó 84% (de 2,80±35 a 5,05±0,59 mg^{-2}/dl^{-2}) en el grupo de mioinositol y no cambió en el grupo de placebo (de 3,25±0,48 a 2,81±0,54 mg^{-2}/dl^{-2}) (Tabla III). El cambio entre los dos grupos fue significativo (*P*<0,002).

Dieciséis (69,5%) y cuatro (21%) de las mujeres ovularon en el grupo de mioinositol y en el grupo de placebo, respectivamente. La diferencia es estadísticamente significativa (*P*=0,001).

El valor máximo para progesterona fue mayor en el grupo de mioinositol (15,1±2,2 ng/ml).

En el grupo de mioinositol existió una disminución de la testosterona sérica total (de 99,5±7 a 34,8±4,3 ng/dl) y las concentraciones de testosterona libre (de 0,85±0,11 a 0,24±0,03 ng/dl) (Tabla IV).

Un aumento en la globulina fijadora de hormonas sexuales sérica se observó para cada grupo (*P*=0,40).

Existió una disminución importante del sulfato dehidroepiandrosterona sérica en el grupo de mioinositol (de 366±47 a 188±24 µg/dl; *P*=0,003) aunque no fue significativo en el grupo de placebo (de 384±63 a 320±35 µg/dl; *P*=0,06).

La concentración de los otros esteroides sexuales séricos no cambio entre los dos grupos.

Discusión

Los medicamentos sensibilizantes a la insulina se han sugerido recientemente como terapia a seleccionar para el síndrome de ovario poliquístico (SOP), aunque la resistencia a la insulina y la hiperinsulinemia asociada se reconocen como factores patogénicos importantes del síndrome. De hecho, casi todas las mujeres obesas con SOP y más de la mitad de las que tienen peso normal son resistentes a la insulina, y por tanto presentan algún grado de hiperinsulinemia. Por esta razón la utilización de sensibilizadores a la insulina se ha sugerido en la mayoría de las pacientes con SOP, como tratamiento útil para la reducción de los niveles séricos de andrógenos y gonadotropinas, y para la mejora de los lípidos séricos y del inhibidor del activador plasminógeno del factor protrombótico tipo 1. Estas terapias se han asociado también con una disminución en el hirsutismo y el acné, y con una regulación de la menstruación y una mejora de la ovulación y la fertilidad.

Tabla IV. Hormona sexual en suero (por 6 a 8 semanas)

Características	Grupo de mioinositol N=23		Grupo de placebo N=19		*Valor P* Para comparación del cambio
	Línea de base	Después del tratamiento	Línea de base	Después del tratamiento	
Testosterona total (ng/dl)	99,5 ± 7	34,8 ± 4,3	116,8 ± 15	109 ± 7,5	0,003
Testosterona libre (ng/dl)	0,85 ± 0,11	0,24 ± 0,03	0,89 ± 0,12	0,85 ± 0,13	0,01
DHEAS (µg/dl)	366 ± 47	188 ± 24	384 ± 63	320 ± 35	0,06
SHBG (nmol/l)	144,4 ± 19	198 ± 24	147 ± 4	163 ± 26	0,40
Androstenediona (ng/dl)	267 ± 19	196 ± 26	271 ± 21	306 ± 41	0,09
Valor máximo de progesterona (ng/ml)*	--	15,1 ± 2,2	--	6,6 ± 1,3	0,003

DHEAS= dehidroepiandrosterona; SHBG= globulina fijadora de Hormonas Sexuales; * la concentración más alta de progesterona medida para una persona durante el estudio.

Recientemente un defecto en la vía de señalización de la insulina (mediadores de fosfoglicanos que contienen inositol) se ha descubierto como parte de la patogénesis de la resistencia a la insulina[8,12]. En consecuencia, la administración de diferentes isoformas de inositol como D-chiro-inositol (DCI) o mioinositol (MI) ha demostrado recientemente mejorar la actividad fisiológica del receptor de insulina restaurando la función ovulatoria espontánea en la mayoría de las mujeres con SOP[14,15,18,21].

El objetivo de nuestro estudio se centró en la implicación metabólica de un tratamiento crónico con MI en pacientes con SOP.

Analizamos un total de 42 pacientes tratadas con mioinositol (23) o placebo (19). El mioinositol aumentó la sensibilidad a la insulina, mejoró la tolerancia a la glucosa y disminuyó la liberación de insulina estimulada por la glucosa. En estas pacientes existió una disminución del 66% de la testosterona sérica total y 73% de disminución de las concentraciones de testosterona sérica libre. Además, existió una disminución en la presión arterial sistólica y diastólica. La concentración de triglicéridos plasmáticos y del colesterol total disminuyó.

En las mujeres con SOP, la resistencia a la insulina puede estar relacionada con la deficiencia de la acción del mediador de la insulina que contiene mioinositol y la administración de mioinositol mejora la sensibilidad a la insulina.

En conclusión, el mioinositol disminuye las concentraciones séricas de andrógenos, reduce la insulina circulante y mejora la tolerancia a la glucosa y otros valores metabólicos alterados asociados con la resistencia a la insulina en mujeres afectadas por el síndrome de ovario poliquístico.

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Metabolic and hormonal effects of myo-inositol in women with polycystic ovary syndrome: a double-blind trial

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Abstract. – To investigate the effects of treatment with Myo-inositol (an insulin sensitizing drug), on circulating insulin, glucose tolerance, ovulation and serum androgens concentrations in women with the Polycystic Ovary Syndrome (PCOS). Forty-two women with PCOS were treated in a double-blind trial with Myo-inositol plus folic acid or folic acid alone as placebo. In the group treated with Myo-inositol the serum total testosterone decreased from 99.5±7 to 34.8±4.3 ng/dl (placebo group: from 116.8±15 to 109±7.5 ng/dl; *P*=0.003), and serum free testosterone from 0.85±0.1 to 0.24±0.33 ng/dl (placebo group: from 0.89±0.12 to 0.85±0.13 ng/dl; *P*=0.01). Plasma triglycerides decreased from 195±20 to 95±17 mg/dl (placebo group: from 166±21 to 148±19 mg/dl; *P*=0.001). Systolic blood pressure decreased from 131±2 to 127±2 mmHg (placebo group: from 128±1 to 130±1 mmHg; *P*=0.002). Diastolic blood pressure decreased from 88±1 to 82±3 mmHg (placebo group: from 86±1 to 90±1 mmHg; *P*=0.001). The area under the plasma insulin curve after oral administration of glucose decreased from 8.54±1.149 to 5.535±1.792 μU/ml/min (placebo group: from 8.903±1.276 to 9.1±1.162 μU/ml/min; *P*=0.03). The index of composite whole body insulin sensitivity (ISIcomp) increased from 2.80±0.35 to 5.05±0.59 mg-2/dl-2 (placebo group: from 3.23±0.48 to 2.81±0.54 mg-2/dl-2; *P*<0.002). 16 out of 23 women of Myo-inositol group ovulated (4 out of 19 in placebo group). Treatment of PCOS patients with Myo-inositol provided a decreasing of circulating insulin and serum total testosterone as well as an improvement in metabolic factors.

Key Words:

Polycystic Ovary Syndrome, PCOS, Myo-inositol, Metabolic syndrome.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age[1-3]; its aetiology remains unknown[4]. Ovulatory disorders represent a major cause of infertility, and the oligoovulation and anovulation with polycystic ovary syndrome (PCOS) are common cause of infertility, which is the most common endocrinopathy of reproductive aged women affecting 6-10% of the population.

The current definition of PCOS requires the presence of two of the following three conditions: (i) oligo- and/or anovulation; (ii) clinical and/or biochemical signs of hyperandrogenism that may be associated with hirsutism; and (iii) polycystic ovaries – and the exclusion of other aetiologies. Other features of PCOS are acne, seborrhea, obesity, insulin resistance, impaired glucose tolerance and type 2 diabetes mellitus, dyslipidaemia, cardiovascular disease and infertility. Furthermore, endocrine and metabolic alterations as elevated serum concentrations of testosterone, insulin, luteinizing hormone (LH) and prolactin are prevalent in PCOS population. These last may have a profound implications for the long-term health of patients.

In young women with PCOS, insulin resistance may occur with high frequency. In fact many studies revealed that it is intrinsic to the syndrome and affects 30 to 40% of patients with PCOS[5]. Some studies showed that insulin resistance in the PCOS may be linked to abnormal ovarian steroidogenesis by means of altered insulin signal transduction[6,7].

Inositol phosphoglycan molecule is known to have a role in activating enzymes that control

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glucose metabolism[8-10]. A defect in tissue availability or altered metabolism of inositol or inositol phosphoglycan mediators, as in PCOS women, may contribute to insulin resistance[11,12]. Isoform of inositol belongs to the vitamin B complex. Epimerization of the six hydroxyl-groups of inositol leads to the formation of up to nine stereo isomers, including Myo-inositol (MYO) and D-chiro-inositol (DCI). Elevated concentrations of MYO in follicular fluid appear to play a role in follicular maturity and provide a marker of good quality oocytes[13-15]. Furthermore, experiments on mouse oocytes showed that an adding of MI to the culture increases the meiotic progression of germinal vesicle by enhancing the intracellular Ca^{2+} oscillation[16].

Women with the PCOS could be respond favourably to treatment with insulin-sensitizing drugs. Previous studies have shown that the use of metformin, troglitazone or Myo-inositol reduces serum androgens, and improves ovulation in women with the PCOS. In those studies, administration of metformin to patients showed a reduction in circulating and a decrement serum total and free testosterone concentrations[17].

A recent study outlines a deficiency of Myo-inositol in insulin resistance in women with the PCOS and the administration of Myo-inositol reduces serum insulin, decreases serum testosterone, and enhances ovulation[18].

The aim of this study was to investigate the metabolic and hormonal effects of MI in PCOS patients.

Material and Methods

This study was a double-blind trial (subjects and investigators).

42 patients, 18 to 40 years of age, were selected to study. They were PCOS affected with oligomenorrhea, high serum free testosterone level and/or hirsutism.

Women were observed by pelvic, ultrasonography and PCOS was found[19].

13 of the 42 women were taking some drugs (oral contraceptives, insulin-sensitizing agents and others) during two months before the study.

After randomization, 23 women received 4 gr of Myo-inositol plus 400 mcg of folic acid (Inofolic®) and 19 women received 400 mcg folic acid (Fertifol®) alone as placebo. The treatment was made for 12-16 weeks.

Seven women had impaired glucose tolerance (plasma glucose concentration >140 mg/dl, <200 mg/dl two hours after oral ingestion of 75 g of dextrose). Four of them was assigned to receive Myo-inositol (Inofolic®) and three were assigned to receive placebo (Folic acid only).

The study was approved by the Institutional Review Boards and each woman gave written informed consent.

When we started the study, the patients were in the follicular phase of the menstrual cycle (serum progesterone concentration lower than 2.5 ng/ml). On the first day blood pressure, weight, height, waist to hip ratio were measured. In the morning, 8:30, 8:45 and 9:00 am., sex hormone binding globulin and serum steroids were obtained. At 9:00 a.m., 75 g of dextrose were administered and plasma glucose and insulin were measured after 30, 60, 90, 120 minutes.

How to take drugs (Myo-inositol or placebo orally once a day) was explained to the patients as well as not to change usual habits both for food, sport and lifestyle.

The serum progesterone was measured weekly and if the relevant results were over 8 ng/ml the ovulation was supposed

After 6 weeks of drugs (day 49), women in the follicular phase (serum progesterone concentration <2.5 ng/ml) repeated all the baseline measurement.

Statistical Analysis

The results are reported as mean values ± SE.

The areas under the response curves by the trapezoidal rule were used to evaluate the plasma glucose and insulin concentration after the oral administration of glucose.

The oral glucose tolerance test (OGTT), by the use of the index of composite whole-body insulin sensitivity (ISIcomp), was used to determine the insulin sensitivity. This methodology was developed by Matsuda and De Fronzo[20]: ISIcomp=10.000/square root of ([fasting glucose × fasting insulin] × [mean glucose × mean during OGTT]).

To analyze the difference in ovulation rates between the women who received the Myo-inositol and those who received placebo the Fisher exact test was used. The results of the other variables were obtained by comparing the changes from to baseline to the end of the study in both the groups. The distribution of the changes in the two groups was first tested for normality with use of the Wilks-Shapiro test, and then the distri-

Table I. Baseline characteristics.

Variable	Myo-inositol N = 23	Placebo N = 19
Age	28.8 ± 1.5	27.1 ± 1.4
Waist to hip ratio	0.88 ± 0.02	0.87 ± 0.02
BMI (kg/m^2)	22.8 ± 0.3	22.5 ± 0.3
Menstrual period/yr	3 ± 1	3 ± 1
Free testosterone (ng/dl)	0.85 ± 0.11	0.89 ± 0.12
Androstenedione (ng/dl)	267 ± 19	271 ± 21
DHEAS (µg/dl)	366 ± 47	384 ± 63
Total testosterone (ng/dl)	99.5 ± 6.9	116.8 ± 14.7
17 beta estradiol (pg/ml)	45 ± 2.5	70 ± 6.7
Sex hormone binding globulin (nmol/L)	144.4 ± 18.6	147 ± 14.5
Total cholesterol (mg/dl)	210 ± 10.4	195 ±7.35
Triglycerides (mg/dl)	195 ± 20.2	166 ± 20.6
ISIcomp (mg-2/dl-2)	2.80 ± 0.35	3.23 ± 0.48
Glucose AUC (mg/dl/min)	12.409 ± 686	12.970 ± 802
Insulin AUC (µU/ml/min)	8.549 ± 1.149	8.903 ± 1.276
Fasting insulin (µU/ml)	32.5 ± 4.1	30.8 ± 7.3
Fasting glucose (mg/dl)	87.6 ± 3.5	84.9 ± 5.8
Systolic blood pressure (mmHg)	131 ± 2.3	128 ± 1.3
Diastolic blood pressure (mmHg)	88 ± 1.0	86 ± 7.0

DHEAS = dehydroepiandrosterone; AUC = area under the curve during 2 hours, 75 g oral glucose tolerance test; ISIcomp = index of composite whole body insulin sensitivity.

butions were compared with each other by using the Student to-tailed unpaired or the Wilcoxon rank sum test.

P values <0.05 were considered significant.

Results

The women of the two groups were similar for baseline characteristics (age, BMI, waist to hip ratio, plasma lipids, and other) (Table I). No significant differences were recorded in the two groups for fasting plasma insulin, plasma glucose, areas under the curve for insulin and glucose during the OGTT, and frequency of glucose tolerance.

There was a slight change in BMI in both study groups (Table II).

There was not a statistically significant modification of waist to hip ratio in both groups.

There was a decrement in systolic pressure in Myo-inositol group (from 131±2 to 127±2 mmHg) while an increment in placebo group (from 128±1 to 130±1 mmHg; *P*=0.002); similarly about the diastolic blood pressure, with

Table II. Anthropomorphic and lipid characteristics.

Characteristic	Myo inositol group N = 23		Placebo group N = 19		*P* value for change comparison
	Baseline	*After treatment*	*Baseline*	*After treatment*	
Systolic blood pressure (mmHg)	131 ± 2	127 ± 2	128 ± 1	130 ± 1	0.002
Diastolic blood pressure (mmHg)	88 ± 1	82 ± 3	86 ± 7	90 ± 1	0.001
Triglycerides (mg/dl)	195 ± 20	95 ± 17	166 ± 21	148 ± 19	0.001
Total cholesterol (mg/dl)	210 ± 10	171 ± 11	195 ± 7	204 ± 9	0.001
BMI (kg/m^2)	22.8 ± 0.3	22.9 ± 0.3	22.5 ± 0.3	22.4 ± 0.1	NS
Waist to hip ratio	0.88 ± 0.02	0.87 ± 0.02	0.87 ± 0.02	0.89 ± 0.01	NS

NS: not significant.

Table III. Plasma glucose and insulin sensitivity index measurements (for 6 to 8 weeks).

Characteristic	Myo inositol group N = 23		Placebo group N = 19		*P* value for change comparison
	Baseline	*After treatment*	*Baseline*	*After treatment*	
Fasting insulin (μU/ml)	32 ± 4	26 ± 8	30.8 ± 7	38 ±7	0.20
Fasting glucose (mg/dl)	87.6 ± 4	81.6 ± 4	84.9 ± 6	88 ± 4	0.12
Glucose AUC (mg/dl/min)	12.409 ± 686	10.452 ± 414	12.970 ± 802	12.992 ± 793	0.04
Insulin AUC (μg/ml/min)	8.54 ± 1.149	5.535 ± 1.792	8.903 ± 1.276	9.1 ± 1.162	0.03
ISIcomp (mg^{-2}/dl^{-2})	2.80 ± 0.35	5.05 ± 0.59	3.23 ± 0.48	2.81 ± 0.54	< 0.002

AUC = Area under the curve during 2 hours, 75 g oral glucose tolerance test; ISIcomp = index of composite whole body insulin sensitivity.

decrement (from 88±1 to 82±3 mmHg) in Myo-inositol group and increment (from 86±7 to 90±1 mmHg) in placebo group respectively (*P*=0.001).

In the Myo-inositol group plasma triglycerides decreased by 52% (from 195±20 to 95±17 mg/dl) and total cholesterol decreased significantly (from 210±10 to 171±11 mg/dl).

The fasting plasma insulin concentration did not change significantly in either study group (Table III). The area under the plasma insulin curve decreased by 36% (from 8.54±1.149 to 5.535±1.792 μU/ml/min) while the same was not in the placebo group (from 8.903±1.276 to 9.1±1.162 μU/ml/min; *P*=0.03).

Likely was for the fasting plasma glucose concentration (from 87.6±4 to 81.6±4 mg/dl). The area under the plasma glucose curve during OGTT decreased in Myo-inositol group (from 12.409±686 to 10.452±414 mg/dl/min) while a slight increment was in placebo group (from 12.970±802 to 12.992±793 mg/dl/min; *P*=0.04).

The composite whole body insulin sensitivity index (ISIcomp) increased by 84% (from 2.80±0.35 to 5.05±0.59 mg^{-2}/dl^{-2}) in the Myo-inositol group and did not change in the placebo group (from 3.23± 0.48 to 2.81±0.54 mg^{-2}/dl^{-2}) (Table III). The change between two groups was significant (*P*<0.002).

Sixteen (69,5%) and four (21%) women ovulated in the Myo-inositol group and the placebo group respectively. The different is statistically significant (*P*=0.001).

The progesterone peak value was higher in the Myo-inositol group (15.1±2.2 ng/ml).

In the Myo-inositol group there was a decrement of serum total testosterone (from 99.5±7 to 34.8±4.3 ng/dl) and free testosterone concentrations (from 0.85±0.11 to 0.24±0.03 ng/dl) (Table IV).

An increase of serum sex hormone binding globulin was revealed for each groups (*P*=0.40).

There was an important decrement of the serum dehydroepiandrosterone sulphate in the Myo-inositol group (from 366±47 to 188±24 μg/dl; *P*=0.003) while it wasn't significant in the placebo group (from 384± 63 to 320±35 μg/dl; *P*=0.06).

The other serum sex steroid concentration did not change between two groups.

Table IV. Serum sex hormone (for 6 to 8 weeks).

Characteristic	Myo inositol group N = 23		Placebo group N = 19		*P* value for change comparison
	Baseline	*After treatment*	*Baseline*	*After treatment*	
Total testosterone (ng/dl)	99.5 ± 7	34.8 ± 4.3	116.8 ± 15	109 ± 7.5	0.003
Free testosterone (ng/dl)	0.85 ± 0.11	0.24 ± 0.03	0.89 ± 0.12	0.85 ± 0.13	0.01
DHEAS (μg/dl)	366 ± 47	188 ± 24	384 ± 63	320 ± 35	0.06
SHBG (nmol/l)	144.4 ± 19	198 ± 24	147 ± 4	163 ± 26	0.40
Androstenedione (ng/dl)	267 ± 19	196 ± 26	271 ± 21	306 ± 41	0.09
Progesterone peak value (ng/ml)*	–	15.1 ± 2.2	–	6.6 ± 1.3	0.003

DHEAS= Dehydroepiandroserone; SHBG= Sex Hormone binding globulin; *the highest progesterone concentration measured for an individual subject during the study.

Discussion

Insulin-sensitizing agents have been recently suggested as the therapy of choice for polycystic ovary syndrome (PCOS), since insulin resistance and associated hyperinsulinemia are recognized as important pathogenetic factors of the syndrome. In fact, almost all obese PCOS women and more than half of those of normal weight are insulin resistant, and therefore present some degree of hyperinsulinemia. For this reason the use of insulin sensitizers had been suggested in most patients with PCOS, as a treatment useful in the reduction of serum androgen levels and gonadotropins, and in the improvement in serum lipids. and prothrombotic factor plasminogen-activator inhibitor type 1. These therapies have also been associated with a decrease in hirsutism and acne, and with a regulation of menses and an improvement of ovulation and fertility.

Recently a defect in the insulin signal pathway (inositol-containing phosphoglycan mediators) had been discovered to be implicated in the pathogenesis of insulin resistance[8,12]. As consequence, the administration of different isoforms of inositol as D-Chiro-inositol (DCI) or myo-inositol (MYO) is newly demonstrated improving the physiological insulin-receptor activity, restoring spontaneous ovulatory function in most of PCOS women[14,15,18,21].

Aim of our study was to better focus on metabolic implication of a chronic treatment with MYO in PCOS patients.

We analyzed a total of 42 patients treated by Myo-inositol (N° 23) or placebo (N° 19). Myo-inositol increased insulin sensitivity, improved glucose tolerance and decreased glucose stimulated insulin release. In these patients there was a 66% decrement of serum total testosterone and 73% decrement of serum free testosterone concentrations. In addition there was a decrement in systolic and diastolic blood pressure. Plasma triglycerides and total cholesterol concentration decreased.

In women with the PCOS, insulin resistance may be related to a deficiency in Myo-inositol containing mediator of insulin action and the administration of the Myo-inositol improves insulin sensitivity.

In conclusion, Myo-inositol decreases serum androgen concentrations, reduces circulating insulin and improves glucose tolerance and other metabolic values altered associated with insulin resistance in women affected by Polycystic ovary syndrome.

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Estudio:

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Randomized, double blind placebo-controlled trial: effects of Myo-inositol on ovarian function and metabolic factors in women with PCOS

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Abstract. – Oligomenorrhea and polycystic ovaries in women are one of the most important causes of the high incidence of ovulation failure. This is linked, perhaps, to insulin resistance and related metabolic features. A small number of reports show that myo-inositol improves ovarian function, but in these trials the quality of evidence supporting ovulation is suboptimal. Furthermore, few of them have been placebo-controlled. The aim of our study was to use a double-blind, placebo-controlled approach with detailed assessment of ovarian activity (two blood samples per week) to assess the validity of this therapeutic approach in this group of women. Of the 92 patients randomized, 47 received 400 mcg folic acid as placebo, and 45 received myo-inositol plus folic acid (4 g myo-inositol plus 400 mcg folic acid). The ovulation frequency assessed by the ratio of luteal phase weeks to observa-tion weeks was significantly ($P < 0.01$) higher in the treated group (25%) compared with the placebo (15%), and the time to first ovulation was significantly ($P < 0.05$) shorter [24.5 d; 95% confidence interval (CI), 18, 31; compared with 40.5 d; 95% CI, 27, 54]. The number of patients failing to ovulate during the placebo-treatment period was higher ($P < 0.05$) in the placebo group, and the majority of ovulations were characterized by normal progesterone concentrations in both groups. The effect of myo-inositol on follicular maturation was rapid, because the E2 circulating concentration increased over the first week of treatment only in the myo-inositol group. A significant increase in circulating high-density lipoprotein was observed only in the myo-inositol-treated group. Metabolic risk factor benefits of myo-inositol treatment were not observed in the morbidly obese subgroup of patients (body mass index > 37). After 14-wk myo-inositol or placebo therapy, no change in fasting glucose concentrations, fasting insulin, or insulin responses to glucose challenge was recorded. There was an inverse relationship between body mass and treatment efficacy. In fact a significant weight loss (and leptin reduction) ($P < 0.01$) was recorded in the myo-inositol group, whereas the placebo group actually increased weight ($P < 0.05$).

These data support a beneficial effect of myo-inositol in women with oligomenorrhea and polycystic ovaries in improving ovarian function.

Key Words:

Myo-inositol, PCOS, Ovarian function.

Introduction

Polycystic ovary syndrome (PCOS) is shared by many women like a common premenopausal disorder, characterized by hyperandrogenism and chronic anovulation[1,2]. Its etiology remains unsolved in spite of the fact that there have been no specific population-based studies, but probably only a 5-10% prevalence of this kind of disorder in women of reproductive age is a reasonable moderate value. This early is based to get the upper hand of any studies prevalency on polycystic ovaries which detected that a 20% of self-selected normal women had polycystic ovary morphology on ovarian ultrasound[3]. The most of them had a slight endocrine abnormality[3]. The lower amount is based on the reported 3% prevalence rate of secondary amenorrhea for 3 or more months[4]: an available datum shows that the 75% of women with secondary amenorrhea will fulfill diagnostic criteria for PCOS[5]. PCOS women can

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also have less profound disturbances in menstrual function[1,3,6]. Burghen et al.7 in 1980 affirmed that PCOS was in association with hyperinsulinemia, and then become clear that the syndrome has major metabolic as well as reproductive morbidities. The recognition of this association stired up the relationship between insulin and gonadal function[1,8]. Therefore, women with PCOS were undergoing a treatment with insulin sensitizing agents such as troglitazone[7], metformin[8] and myo-inositol[9-11]. A number of small randomized and non randomized study groups have shown that women with PCOS respond to this therapy increasing ovarian activity and menstrual frequency. The relationships between treatment outcome, anthropometric changes, glycemic, metabolic, and lipid profile adjustments, at any rate, are less comprehensively studied and is able to be argued about. Perhaps some differences in published results, may be in patient selection. In fact patient profiles can differ between infertility and endocrinology clinics and probably also in racial and socioeconomic training. Furthermore, some published studies employing myo-inositol are not double blind, placebo-controlled in design and the greater number having approximately 20 patients. A direct assessment of follicular development, ovulation or progesterone elevations is going too far away to be comprehensive. The latter point is relevant because a number of the ovulations in women with PCOS show subnormal progesterone concentrations[15], which may be a sign for a suboptimal follicular maturation and ovulation. The aim of this study was to search into the effects of myo-inositol on detailed ovarian function in women with oligomenorrhea and polycystic ovaries (PCOs) who were treated using a randomized, double blind placebo-controlled trial of 16-wk treatment duration.

Patients and Methods

Patients

Ninety-two women with oligomenorrhea (cycle length 41d; 8 cycles for year) or amenorrhea and PCOS, aged less than 35 years old, were recruited from gynecology, endocrine, and infertility outpatient clinics. There's not considered any patients with significant hyperprolactinemia, abnormal thyroid function tests, and congenital adrenal hyperplasia. By using transvaginal ultrasound, effected by a single observer (Z.E.H.), were undertaken to estimate ovarian appearance, and ovaries were described as polycystic (PCOs) about the criteria of Adams et al.[16]. None of the patients was taking medications likely to influence hormonal profiles. This diagnosis was used on the understanding that the great part of patients defined on this basis would show elevated androgen activity, symptoms of hyperandrogenism or both[17].

Protocol

Ovarian activity was established throughout the study, using two blood samples per week for assessment of reproductive hormone concentrations. Before randomization, all patients underwent a 4-wk period of investigation to confirm abnormal ovarian function. The same assessment schedule was maintained through a subsequent 16-wk treatment period after randomization to Inofolic® (LO.LI. Pharma, Rome, Italy) or matching folic acid as placebo. Anthropometric, endocrine, and ovarian ultrasound assessments were effected before and after 14-wk treatment (between 12-16 wk).

The last time window was used to take the measurements outside a luteal phase. The tests were performed only after confirmation that the circulating progesterone concentration was less than 6 nmol/liter.

Randomization and Study Power

Randomization was effected in a double blind fashion; patients received either Myo-inositol combined with folic acid (Inofolic®) or only folic acid as placebo, according to the code provided by computer-generated randomization. The study power was based upon predicted changes in the ovulation rate and circulating lipoprotein concentrations, using data derived from the literature[18]. The calculation was adapted to account for the fact that 70-80% of the cases would have classical PCOS, a significant dropout rate (15%), and a failure to attain normal menstrual frequency in another 15% of cases. It was estimated that 13 patients in each arm would detect changes in high-density lipoprotein (HDL) cholesterol with more than 90% power with a type 1 error (a) 0.05. It was predicted that the study required 35 cases in each arm to achieve the stated aim. Before randomization and during the ovarian function assessment, all patients were evaluated for endocrine factors while outside the luteal phase (progesterone concentration, 6 nmol/liter) when they attend the hospital after an overnight fast. Blood samples were taken for assays of E2, T, androstenedione, LH, FSH, triglycerides, choles-

terol, low-density lipoprotein (LDL) cholesterol, and HDL cholesterol. Then, a standardized 75-g oral glucose tolerance test (GTT) was undertaken with blood samples collected at 0, 60, and 120 min for determination of serum glucose and insulin concentrations. This process was repeated at the 14-wk assessment point.

Ovarian Activity Ovulation and the Luteal Ratio

Ovarian activity was monitored using serum E2 rapid (same day) measurements; where follicular activity was diagnosed (E2 > 300 pmol/liter), progesterone and LH concentrations were determined to diagnose ovulation and the luteal phase. Ovulation frequency was calculated using the ratio of luteal phase weeks to observation weeks (the luteal ratio), such that an individual with normal menstrual rhythm would show two luteal weeks in four observation weeks, yielding a ratio of 0.5, expressed as a luteal ratio of 50%. One patient conceived within a week of the end of her treatment schedule, and her data were included in the completed trial analyses, because all samples and tests had been undertaken for the treatment period.

Anthropometric and Lifestyle Parameters

Anthropometric data were collected (weight, height, waist and hip measurements) before and at the 14th week of treatment or placebo by a single trained observer (Z.E.H.) using standardized techniques[19]. The body mass index (BMI) was calculated using the standard formula. Each volunteer completed a questionnaire of medical and social history (desiring pregnancy, smoking habits), from which subjective information about menstrual patterns, skin oiliness, acne, and hirsutism were recorded. Ovarian ultrasound assessments were also effected before treatment and at 14 wk by the same observer.

Assay Methods

The reproductive hormones, E2 and progesterone, were assayed routinely using the semi automated Immulite technology (Diagnostic Products, Los Angeles, CA). The analytes T, LH, FSH, and human chorionic gonadotrophin were assayed retrospectively in batches using the same system. Inhibin-B was measured using the specific two-site immuno-assay (Serotec Ltd., Oxford, UK). Plasma total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol measurements were performed by a modification of the standard Lipid Research Clinics protocol[20]. Serum leptin concentrations were measured by a validated in-house RIA[21]. Plasma glucose was measured using the glucose oxidase method (Glucose Reagent Kit, Bayer, Newbury, UK), whereas insulin was measured using a competitive RIA (Coat-A-Count I, Diagnostic Products).

The intra- and inter-assay coefficients of variation were less than 7 and 10%, respectively, over the sample concentration range. The detection limit of the assay was 0.5 ng/ml.

Data Analyses and Statistics

Fasting and postglucose insulin [area under curve (AUC)], SHBG, waist to hip ratio (WHR), triglyceride, and the ovulatory function were compared between treatment and placebo groups. Hormone and comparative data were introduced with confidence limits at 95%. Statistical information was prepared using the SPSS for Windows software (SPSS, Inc., Chicago, IL). Hormone data were compared using *t* test after log transformation if distributions were normalized.

Ethical Approval

Ethical committee approval was obtained before the study, and written informed consent was given by each patient.

Results

Recruitment, Randomization, and Pretreatment Assessments

A total of 92 patients proceeded to randomization having either Myo-inositol combined with folic acid (Inofolic®) 2 g twice a day was administrated continuously and controls received folic acid only as placebo.

Infertility was an ailment in only about half of the patients in each group. There was no difference in the proportions of infertile women within the groups (Table I). Although patient selection was based on the more wide-ranging definition often used in Europe *(i.e.* ultrasound-diagnosed PCOS and oligomenorrhea), 90% had biochemical or clinical evidence of hyperandrogenism. Table 1 also shows that the Inofolic® and placebo groups were matched for menstrual frequency in the preceding year, age, BMI, T, SHBG, fasting glucose, hemoglobin A1c, and circulating lipid fractions before treatment. The proportions of

Table I. Characteristics of the patients randomized to receive myo-inositol or placebo treatment.

	Placebo		Inofolic®	
	Mean	*CIs*	*Mean*	*CIs*
Age (yr)	29.7	28.5-30.9	29.0	27.1-30.9
Menses per year	4.1	3.2-4.9	4.7	3.6-5.7
BMI (kg/m^2)	34.8	32.4-37.1	34.0	31.5-36.5
WHR	0.90	0.87-0.92	0.89	0.87-0.91
LH (IU/liter)	10.1	8.4-11.7	8.3	6.9-9.7
T (nmol/liter)	4.0	3.8-4.2	2.8	2.4-3.2
SHBG (nmol/liter)	27.8	23.1-32.5	29.3	24.8-33.8
Free androgen index	13.6	11.3-15.9	10.6	9.3-11.8
Fasting insulin (μU/ml)	18.4	15.0-21.8	16.3	13.2-19.3
Insulin AUC (GTT)	229	180-278	191	160-222
Fasting glucose (nmol/liter)	4.86	4.78-4.93	4.99	4.77-5.21
Leptin (ng/ml)	39.3	32.9-45.6	40.1	33.0-47.2
Inhibin-B (pg/ml)	80	65-95	99	89-109

No. of patients: placebo-treated, 47 (infertile, 19; hirsutism, 22); myo-inositol-treated, 45 (infertile, 23; hirsutism, 13). P values are NS. CIs, Confidence intervals (95%).

patients seeking fertility treatment were also similar in each group.

All women showed a classical picture of PCOS on vaginal ultrasound scan.

Conception During Treatment

There were eight conceptions in eight patients during the study, and one miscarried in the first trimester. However, only 42 of the patients declared before the study that they wished to conceive. Of these, the distribution of pregnancies was: placebo, 1 of 19 patients; and myo-inositol 4 of 23 patients.

The results are not significantly different *(P* = 0.23).

Ovarian Function: Ovulation

An intention to treat analysis revealed that 8 of 45 myo-inositol-treated patients failed to ovulate during treatment, compared with 17 of 47 placebo-treated. This difference was statistically significant (Fisher's exact test; *P* = 0.04; Odd's Ratio, 0.38).

Table II shows the data from all cases in which ovulation data (over any length of time) were available. The myo-inositol-treated group had a significantly increased frequency of ovulation compared with the placebo group, defined by the luteal ratio. The distributions show that the placebo group was dominant at low ovulation rate (zero and one ovulations), whereas the myo-inositol group was dominant in the high ovulation rate (two to four ovulations).

Table II also shows the frequency of ovulations with deficient luteal phases assessed by the maximum progesterone concentration less than 7 ng/ml.

Table II. Details of ovulations during placebo and myo-inositol treatment.

	Placebo	Inofolic®	*P*
Observation weeks	497	352	
Luteal weeks [luteal ratio (%)]	74 (15)	88 (25)	< 0.001
Luteal phases with P_{max} 7 ng/ml (%)	6 (14)	2 (9)	NS
Days to first ovulation, mean	40.5	24.5	0.02
(CIs, 95%)	(27, 54)	(18, 31)	

P_{max}, Maximum progesterone concentration.

According to these data, the concentrations of progesterone recorded during monitoring of ovarian function indicated that most of the ovulations showed normal endocrine profiles during both myo-inositol and placebo treatment. All patients started treatment outside the luteal phase, and the delay to the first ovulation after starting the program (Table II) was significantly shorter in the myo-inositol-treated group.

Initial Responses to Treatment: Follicular Development

Inhibin-B is a marker of early follicular granulosa cell activity, and circulating E2 represents follicular maturation. Table III shows the E2, inhibin-B, and T concentrations on the first and eighth days of treatment, showing that the Myo-inositol-treated group had a significant ($P = 0.03$, paired data) increase in mean E2, whereas the control group showed no change. There was no change in the circulating inhibin-B or T concentrations. These profiles suggest that although improved follicular maturation was detected, there appeared to be no change in the remainder of the ovarian metabolism (total immature granulosa cell activity and stromal androgen biosynthesis).

Metabolic and Anthropometric Assessments

Table IV shows that after 14-wk treatment, the BMI decreased significantly in the myo-inositol group, whereas it increased in the placebo group. There was no change seen in the WHR in either group. The circulating leptin concentration declined in the myo-inositol-treated group, in contrast to the control group, but there was no change recorded in the fasting glucose, fasting insulin, or insulin AUC in response to the glucose challenge in either group. Circulating very LDL (VLDL) showed little change during the treatment period, but the LDL showed a trend toward reduction, and HDL increased significantly in the myo-inositol-group. It is possible that the reduction in HDL was related to the weight loss achieved in the myo-inositol-treated patients, although the ANOVA ($r > 0.34$; $P > 0.07$) did not reach conventional levels of significance.

Subgroup Analyses Characteristics of the Group That Responded to Myo-Inositol With Normal Ovulation Frequency

A total of 12 patients who responded to myo-inositol by establishing normal ovulation frequency (n = 6) and/or pregnancy (n = 6) were compared with those patients who did not respond with establishment of normal ovarian function (less than three ovulations in 16 wk; n = 9). The two groups showed similar BMI, WHR, and circulating E2 and inhibin-B concentrations. However, responders to myo-inositol treatment showed significantly lower T (2.3 nmol/liter *vs.* 3.4 nmol/liter; 95% CI = 0.07 and 2.1, respectively; $P > 0.04$), higher SHBG (35.9 nmol/liter *vs.* 25.8 nmol/liter; 95% CI, 20.6 and 0.13; $P < 0.05$), and thus lower free androgen index (6.9 *vs.* 11.6; 95% CI, 1.2 and 8.1; $P = 0.01$). Fasting insulin or glucose concentrations or responses to the GTT were not significantly different.

Table III. The reproductive hormone changes over the first week of myo-inositol treatment.

	Day 1		Day 8		*P*
	Mean	*CIs*	*Mean*	*CIs*	
Placebo					
E2 (pmol/liter)	159	108-209	177	119-235	NS
Inhibin-B (pg/ml)	82	69-95	88	72-103	NS
T (nmol/liter)	4.2	3.6-4.7	4.1	3.4-4.8	NS
Myo-inositol					
E2 (pmol/liter)	141	122-159	224	147-300	< 0.03
Inhibin-B (pg/ml)	99	89-109	96	87-105	NS
T (nmol/liter)	2.9	2.3-3.5	3.3	2.5-4.0	NS

Table IV. Changes in metabolic parameters during placebo or myo-inositol treatment.

	Placebo			Inofolic®		
	Pretreatment	*14 wk*	*P*	*Pretreatment*	*14 wk*	*P*
BMI (SD)	35.2	35.5	0.04	35.0	34.4	0.03
WHR	0.90	0.90	NS	0.89	0.89	NS
Leptin (ng/ml) (SD)	40.5	39.0	NS	41.3	37.5	0.05
Fasting insulin (µU/ml)	18.1	17.3	NS	16.6	16.8	NS
GTT insulin AUC	218	220	NS	190	202	NS
Fasting glucose (nmol/liter)	4.9	5.0	NS	5.0	5.1	NS
Total cholesterol (nmol/liter)	4.85	4.92	NS	4.53	4.42	NS
Triglycerides (mmol/liter)	1.39	1.43	NS	1.59	1.60	NS
VLDL cholesterol (mmol/liter)	0.40	0.52	NS	0.50	0.55	NS
LDL cholesterol (mmol/liter)	3.25	3.32	NS	3.05	2.89	0.09
HDL cholesterol (mmol/liter)	1.15	1.15	NS	1.10	1.16	0.03

Statistical probability by t test for paired data.

Metabolic Responses and Obesity

It was observed that morbidly obese women (BMI > 37; n = 10) showed a similar number of ovulations (mean, 1.5) during 16-wk myo-inositol treatment to the leaner women (mean, 2.2), but they showed no indication of changes in either BMI (pretreatment, 42.6 kg/m^2; week 14, 42.4 kg/m^2) or HDL cholesterol (pretreatment, 0.94 mmol/liter; week 14, 0.94 mmol/liter). The leaner women (BMI < 37 kg/m^2) showed distinct changes during treatment as follows: BMI, pretreatment, 29.2 kg/m^2; week 14, 28.3 kg/m^2 *(P* = 0.01); or HDL cholesterol, pretreatment, 1.19 mmol/liter; week 14, 1.30 mmol/liter *(P* = 0.02).

Discussion

This study is the first to give a comprehensive, detailed endocrinological assessment of ovarian function in the context of a large randomized placebo-controlled trial of myo-inositol in women with abnormal ovarian function. Our data show clear beneficial effect of myo-inositol treatment upon ovarian function, anthropometric measures, and lipid profiles in women with oligomenorrhea and PCOS. We observed that more than 70% of the patients established normal ovarian rhythm (three or more ovulations) through the 16-wk treatment period. This contrasted with 13% for the placebo group. The luteal phases had normal progesterone concentration profiles in a high frequency of the cycles, showing that these were fertile cycles. The mean time until the first ovulation was significantly shorter in the myo-inositol-treated group (25 d) than in the placebo-treated group (41 d).

This suggests a relatively rapid effect of treatment upon ovarian function, which is further supported by the significant increase in E2 concentrations during the first week of treatment.

At week 14 assessment, the myo-inositol patients showed significant reductions in weight, in contrast to patients in the placebo group who actually increased their BMI. Associated with the weight loss were significant reductions in circulating leptin and increased HDL cholesterol concentrations in the myo-inositol-treated group. LDL cholesterol showed a trend toward reduction, and overall the LDL cholesterol to HDL cholesterol ratio improved significantly in the myo-inositol group.

For all increased ovulation frequency, there were no changes in circulating androgen concentrations, glycemic indices, basal or provoked insulin levels, or circulating VLDL cholesterol concentrations. Our data on HDL cholesterol are important, because no previous study has addressed this important issue.

Subgroup analyses comparing those patients who showed a high ovulation rate during myo-in-ositol treatment with those who were resistant to it, indicated that the least androgenic patients were more likely to respond with establishment of normal menstrual rhythm. Furthermore, the morbidly obese patients (BMI > 37) showed no cardiovascular risk factor (BMI and HDL cholesterol) benefit.

Taken together, these data suggest that either higher doses of myo-inositol may prove to be more beneficial in the morbidly obese patient or such patients may be resistant to this form of therapy. These assertions remain to be tested in future studies. A number of reports have indicated that insulin sensitizing agents improve ovulation rates in women with PCOS, and they have shown conflicting results with respect to changes in ovulation rate and also changes in endocrinology during myo-inositol treatment

On the other hand, a number of studies have shown decreases in hyperandrogenism and markers of insulin resistance with myo-inositol in PCOS[9-14]. A recent comprehensive multicenter, multidose study using the peroxisome proliferator-activated receptor (PPAR) agonist troglitazone[7] showed improvements in hyperan drogenism, mediated through circulating free androgens rather than total androgen concentrations, and also in glycemic indices. These changes were dose-related, as were improvements in ovulation rates. It is possible that patient selection criteria may have an impact on the potential for beneficial effects of myo-inositol on surrogate markers of insulin resistance and hyperandrogenism.

The principal inclusion criteria in our study was disturbances of ovarian function, whereas in other studies the emphasis may have been on more profound metabolic derangements, including clinical manifestations of hyperandrogenism. It is considerable that the higher doses of troglitazone treatment (300 and 600 mg) were associated with weight increase in women who were generally overweight at the time of starting[7]. Weight loss achieved in the myo-inositol-treated patients would be considered a beneficial effect of treatment. The increase in ovulation rate seen in the myo-inositol-treated patients appeared to take place rapidly, as evidenced by significant increases in circulating E2 concentrations, representing follicular maturation, within the first 8 d of treatment and also the shorter mean time to first ovulation. This effect is likely to have taken place before significant weight loss or changes in the lipid profiles, and also in the absence of changes in glycemic indices. This leads to the possibility of direct gonadal effects of myo-inositol as has been demonstrated for the PPAR agonist troglitazone[29,30].

These should be dose-determining and aimed to define patient characteristics that best predict beneficial response to myo-inositol treatment.

Furthermore, we also suggest that the problems of maternal obesity be carefully considered with such treatment, and that weight loss may be the better approach[31] in many circumstances.

Finally, the high dropout rate in the myo-inositol arm (more than 30%) is notable. Clinically, this observation is important and indicates that significant side effects on the dosage regime we used are common. Most of the discontinuation cases occurred at the early part of treatment, suggesting that women prescribed myo-inositol should be adequately counseled and perhaps actively supported through this stage.

In conclusion, using a comprehensive, detailed endocrinological assessment of ovarian function, we have shown that myo-inositol treatment increases ovulation rates by a significant degree in women with oligomenorrhea and PCOS. Continued treatment also resulted in significant weight loss (and leptin reduction) and an associated change in HDL cholesterol even if many different factors may contribute to the metabolic syndrome in PCOS patients. These beneficial effects of myo-inositol support a future therapeutic role in women with PCOS.

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Estudio:

Myo-inositol rather than D-chiro-inositol is able to improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial.

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Myo-inositol rather than D-*chiro*-inositol is able to improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial

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Abstract. – *Objective:* **Polycystic ovary syndrome (PCOS) is the most common cause of infertility due to menstrual dysfunction, and the most promising treatments for this disease are insulin sensitising agents. *Myo*-inositol and D-*chiro*-inositol are insulin sensitizing agents used in PCOS treatment.**

In the present paper, we aimed to compare the effects *myo*-inositol and D-*chiro*-inositol on oocyte quality in euglycemic PCOS patients.

Materials and Methods: **Eighty-four euglycemic PCOS patients, undergoing ovulation induction for ICSI, were recruited for this study. Forty-three participants received *Myo*-Inositol 2 g twice a day and forty-one patients received D-*chiro* inositol 0.6 g twice a day.**

Results: **The results of our study showed that the total number of oocytes retrieved did not differ in the two treatments groups. However, the number of mature oocytes was significantly increased in the *myo*-inositol group compared to D-*chiro*-inositol. Concurrently, the number of immature oocytes decreased in *myo*-inositol treated patients. Furthermore, the myo-inositol-treated group showed an increase in the mean number of top quality embryos and in the total number of pregnancies compared to the D-*chiro*-inositol-treated group.**

Conclusions: **Our data show that, in PCOS patients having a normal insulin response, *myo*-inositol treatment rather than D-*chiro*-inositol is able to improve oocyte and embryo quality during ovarian stimulation protocols.**

Key Words:

Myo-inositol, D-*chiro*-inositol, Oocyte quality, Embryo quality, Ovarian stimulation, ICSI cycles.

Introduction

Polycystic ovary syndrome (PCOS) is the most common cause of infertility due to menstrual dysfunction and it affects about 10% of women in childbearing age[1]. Its diagnosis is rather complex, and indeed diagnostic criteria changed over time. The most recent revision was performed during a consensus meeting sponsored by the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) held in Rotterdam in 2003[2,3]. The outcome of the meeting defined that PCOS diagnosis should be based on three different factors: (1) oligo-anovulation, (2) hyperandrogenism (clinical or biochemical) and (3) the presence of 12 or more follicles in each ovary measuring 2±9 mm in diameter, and/or increased ovarian volume (>10 ml)[2,3].

Recently, many studies focused on both the impaired glucose tolerance, that affects 30 to 40% of patients with PCOS[4], and the insulin resistance, that affects 50 to 80%[5] of women with PCOS. Insulin plays a direct role in the pathogenesis of hyperandrogenemia: it acts synergistically with luteinizing hormone (LH) to enhance the androgen production of theca cells[6]. Furthermore, insulin is able to reduce circulating levels of sex hormone binding globulin (SHBG). Therefore, lower SHBG levels result in a relative increase of free testosterone[7]. Since the report by Burghen et al[8] that described the association between PCOS and hyperinsulinemia, it has become clear that this syndrome has major metabolic as well as reproductive morbidities. How-

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ever, PCOS pathogenesis is still unknown and so far the most promising treatments are insulin sensitizing agents[1,9].

Inositol is a polyalcohol classified as insulin sensitizer, and it is the second messenger of the insulin signalling[10]. Two different stereoisomers are currently used in the treatment of PCOS: *myo*-inositol (MI) and D-*chiro*-inositol (DCI)[11-15]. Both stereoisomers have an insulin-like action *in vivo* and they exert their function of insulin mediators as Inositolphosphoglycan (IPG)[16]. MI is the most abundant form of inositol in nature, while DCI is synthetized by an epimerase that convert MI to DCI. In particular, this reaction is insulin dependent[16], and insulin sensitive tissue such as liver and muscle have shown to be the main conversion sites[17]. Additional data also showed that in such tissues there is a correlation between insulin resistance and a reduced MI to DCI epimerization rate[18]. On the basis of these data, Authors suggest that a defect in tissue availability or utilisation of MI and/or DCI or mediators may contribute to insulin resistance[16,19].

A recent review clearly summarized the complex role the MI has in human reproduction[20]. Indeed, besides the positive effects on reducing insulin resistance[12,13,21] it has been shown that MI concentration in the follicular fluid directly correlates with oocytes quality[22]. Additional studies also showed that MI supplementation improves oocytes quality in PCOS patients[23].

In the present paper, we aim to compare the effect of MI and DCI supplementation in euglycemic PCOS patients undergoing ovulation induction for intracytoplasmic sperm injection (ICSI) procedure.

Materials and Methods

Patients

All patients treated in our *in vitro* fertilization (IVF) Department of infertility for a period of more than 12 months were asked to participate to the study.

A total of 84 women, aged <40 years, diagnosed with PCOS according to Rotterdam criteria[2,3] were included in this study. We excluded from the study patients that showed insulin resistance and/or hyperglycaemia.

ICSI procedures were suggested after evaluation of two different sperm samples of the male partner.

Patients were randomly assigned to receive either MI 2 g twice a day (43 subjects, group A) or DCI 0.6 g always twice a day (41 subjects, group B).

Both treatments were performed for 8 weeks before follicle stimulating hormone (rFSH) administration.

The Institutional Ethical Committee approved the protocol, and all patients gave a written informed consent before entering the study.

Controlled Ovarian Hyperstimulation

All patients underwent pituitary desensitization by subcutaneous (s.c.) administration of a gonadotropin releasing hormone (GnRH) agonist (Decapeptyl; Ipsen, Paris, France) from midluteal phase until the intramuscular (i.m.) administration of 10,000 IU human chorionic gonadotropin (hCG). Then, controlled ovarian hyperstimulation was performed in all patients by administration of rFSH (Gonal-F; Merck-Serono, Geneva, Switzerland). Starting dose was 150 IU per day. Patients were monitored by measuring the plasma concentration of 17β-Estradiol 2 17β-E_2 and the size of follicles on day 5 of the stimulation. The amount of gonadotropin administered was adjusted according to the individual response. The 10,000 IU hCG was injected i.m. in all patients when serum 17β-E_2 exceeded 200 pg per follicle and there were at least three follicles with a minimum diameter of 18 mm. Cycles were canceled if E_2 levels were >4,000 pg/mL, due to increased risk of ovarian hyperstimulation syndrome (OHSS).

ICSI Procedure

Since March 10, 2004 the Italian IVF law state that a maximum of three oocytes per patient could be injected, while spare mature oocytes were cryopreserved according to protocols described in previous studies[24]. Oocyte and sperm preparation for conventional ICSI procedure have been thoroughly described elsewhere[25]. Concerning ICSI, cumulus and corona radiata cells were immediately removed after retrieval by a short exposure to HEPES-buffered medium (Quinn's Advantage Hepes Medium; Sage IVF, Trumbull, CT, USA) containing 20 IU/mL hyaluronidase (Sage IVF) and gentle aspiration in and out of a Pasteur pipette and mechanically cleaned from the remaining surrounding cumulus cells by aspiration using a denuding pipette (Denuding Flexi-Pet; Cook, Brisbane, Australia) with a 170-130 mm diameter. The denuded oocytes were then assessed for their meiotic maturation status. In preparation for ICSI, oocytes with an extruded first polar body presumably at the

metaphase II stage (MII) were selected (in a maximum of three) for the fresh cycle and spare MII oocytes were cryopreserved, if required[26].

Luteal Phase

Intramuscular administration of 50 mg daily progesterone in-oil was started on the day of ovum pick-up, and treatment was performed daily until either a serum pregnancy test result was negative or an embryonic heart beat was sonographically confirmed.

Determination of Pregnancy States

A biochemical pregnancy was defined as a small and transitory increase in β-hCG levels. A clinical pregnancy was determined by the visualization of an embryo with cardiac activity at 6-7 weeks of gestation. Spontaneous abortion was classified as the loss of the pregnancy between the fifth and twelfth weeks of gestation.

Statistical Analysis

The statistical package SPSS Kit SigmaStat for Windows V2.03S (SPPS, Chicago, IL, USA) was used for data analysis. Baseline characteristics and ovulation induction (Table I) were analyzed using the unpaired Student' *t*-test. Ovum pick-up outcomes were analyzed using Wilcoxon' test; pregnancy rates were compared using Fisher exact test. Results with $p<0.05$ were considered to be significant.

Results

During the study period, 84 patients matching the inclusion criteria were randomized into two groups as previously described.

Group A (MI) consisted in 43 patients and Group B (DCI) consisted in 41. No differences were found between the two groups in mean age, Body Mass Index (BMI), and duration of infertility (Table I).

Total r-FSH units (1953.6 ± 397.5 *vs.* 2360.5± 301.9; $p<0.01$) and number of days of stimulation (11.1 ± 0.8 *vs.* 12.7 ± 1.1; $p<0.01$) were significantly reduced in the MI group. Furthermore, peak estradiol levels (2261.2 ± 456.6 *vs.* 2740.0 ± 396.7 pg/ml; $p<0.01$) at hCG administration were significantly lower in MI-treated versus DCI-treated patients (Table I).

No cycle was cancelled in group A, while in group B four cycles were cancelled due to estradiol peak >4000 pg/ml ($p=0.05$ Table I).

The total number of oocytes retrieved did not differ between the two groups, while the number of mature oocytes significantly differed, being 8.21± 2.39 in the MI group *vs.* 7.08 ± 2.67 in the DCI group ($p<0.05$, Table II). Concurrently, the number of immature oocytes retrieved was significantly lower in the MI group compared to DCI group (0.69 ± 0.64 *vs.* 2.23 ± 0.85; $p<0.01$, Table II)

Noteworthy, the number of grade 1 embryos was significantly increased by MI supplementation (1.64 ± 0.88 *vs.* 0.76 ± 0.43; $p<0.01$, Table II).

In compliance with the Italian IVF law, at maximum three oocytes per patients were injected. A total of 32 pregnancies were obtained (22 in group A *vs.* 10 in group B; $p<0.05$, Table III).

Discussion

In the present paper, we show that MI rather than DCI is able to improve oocyte and embryo quality in euglycemic PCOS patients. This is in

Table I. Characteristics and outcome of treated patients (Mean ± SD).

	MI	DCI	*P* value
No. of patients	43	41	
Age (yrs)	35.5 ± 3.2	36.5 ± 2.5	NS
duration of infertility (months)	50.3 ± 10.3	45 ± 15.6	NS
BMI (kg/m^2)	24.6 ± 8.4	25.3 ± 7.8	NS
PRL (ng/ml)	18.1 ± 2.9	19.3 ±2.4	NS
TSH (mIU/L)	1.7 ± 1.1	1.8 ±0.9	NS
Stimulation (days)	11,1 ± 0,8	12,7 ± 1,1	< 0.01
FSH IU administrated	1953.6 ± 397.5	2360.5 ± 301.9	< 0.01
17b-E2 levels on hGC administration (pg/ml)	2261.2 ± 456.6	2740 ± 396.67	< 0.01
No. Of cancelled cycles (E_2 > 4000 pg/ml)	0	4	0.05

Table II. Oocyte and embryo quality (Mean ± SD).

	MI	DCI	*P* value
No. of retrived oocytes	8.90 ± 2.84	9.32 ± 3.15	NS
No. of MII oocytes	8.21 ± 2.39	7.08 ± 2.67	< 0.05
No. of immature oocytes	0.69 ± 0.64	2.23 ± 0.85	< 0.01
Embryo grade 1	1.64 ± 0.88	0.76 ± 0.43	< 0.01

Table III. Pregnancy outcome.

	MI	DCI	*P* value
No. of pregnancies (%[a])	22 (51)	10 (24)	< 0.05
No. of biochemical pregnancies (%[b])	3 (14)	2 (9)	NS
No. of clinical pregnacies (%[b])	15 (68)	5 (22)	NS
No. of spontaneous abortion (%[b])	4 (18)	3 (14)	NS

a = versus patients number; b = versus total pregnancies.

line with previous findings by Papaleo et al, who showed that MI supplementation significantly reduced ovarian stimulation days and the IU of rFSH administrated compared to placebo[23].

The most common cause of IVF-ET failure is the reduced embryo quality and several factors, such as social-environmental, aging and/or pathological factors, can negatively affect it[27-30].

Ooocyte retrieved from PCOS patients are indeed characterized by poor oocyte quality[27,28] and, therefore, any treatment able to improve oocyte quality could be considered the "holy grail" for IVF procedures.

Recently, it has been shown that two molecules, normally produced by our body, MI and Melatonin are efficient predictors for oocyte quality and IVF outcomes: indeed, high concentration of both molecules positively correlates with high oocyte quality[22,31,32]. In particular, several clinical trials have shown that supplementation with MI, alone or in association with melatonin, is a practical approach able to improve oocyte quality and IVF outcomes in both PCOS patients and normal subjects[23,30,33].

Menstrual dysfunctions in PCOS patients are mainly caused by hyperandrogenism. In these patients, insulin sensitizer compounds have been shown to be able to normalize androgen levels in both obese and lean women[11-13,15,34]. In particular, both MI and DCI have been of interest for the scientific community[11-13,15,34].

Several studies aiming at identifying the cause of insulin resistance pointed out that the relative amount of both molecules is regulated by insulin. Indeed, insulin regulates the epimerization of MI into DCI in a dose dependent fashion. Furthermore, it was found that insulin resistance impairs MI to DCI epimerization altering the ratio between MI/DCI, resulting in higher MI levels[35,36].

These studies were performed on insulin sensitive tissues such as hepatic and muscular tissue, and no data are reported on ovarian tissue, that never becomes insulin resistant. Therefore, we could speculate that PCOS patients suffering of hyperinsulinemia likely present an enhanced MI to DCI epimerization in the ovary, leading to an alteration of MI/DCI ratio and probably to a MI depletion in the ovary. This MI depletion could eventually be responsible of the poor oocyte quality observed in these patients.

However, in order to prove this hypothesis and to fully understand the role of MI and DCI on ovarian function of PCOS patients, further studies need to be specifically performed on ovarian tissue.

In conclusion, in the present paper we were able to demonstrate that MI rather than DCI supplementation has a direct positive effect on oocyte and embryo quality.

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Estudio:

Pretreatment with myo-inositol in non polycystic ovary syndrome patients undergoing multiple follicular stimulation for IVF: a pilot study.

REPRODUCTIVE BIOLOGY AND ENDOCRINOLOGY

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Pretreatment with myo-inositol in non polycystic ovary syndrome patients undergoing multiple follicular stimulation for IVF: a pilot study

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Abstract

Background: Aim of this pilot study is to examine the effects of myo-inositol administration on ovarian response and oocytes and embryos quality in non PolyCystic Ovary Syndrome (PCOS) patients undergoing multiple follicular stimulation and *in vitro* insemination by conventional *in vitro* fertilization or by intracytoplasmic sperm injection.

Methods: One hundred non-PCOS women aged <40 years and with basal FSH <10 mUI/ml were down-regulated with triptorelin acetate from the mid-luteal phase for 2 weeks, before starting the stimulation protocol for oocytes recovery. All patients received rFSH, at a starting dose of 150 IU for 6 days. The dose was subsequently adjusted according to individual response. Group B (n = 50) received myo-inositol and folic acid for 3 months before the stimulation period and then during the stimulation itself. Group A (n-50) received only folic acid as additional treatment in the 3 months before and through treatment.

Results: Total length of the stimulation was similar between the two groups. Nevertheless, total amount of gonadotropins used to reach follicular maturation was found significantly lower in group B. In addition, the number of oocytes retrieved was significantly reduced in the group pretreated with myo-inositol. Clinical pregnancy and implantation rate were not significantly different in the two groups.

Conclusions: Our findings suggest that the addition of myo-inositol to folic acid in non PCOS-patients undergoing multiple follicular stimulation for in-vitro fertilization may reduce the numbers of mature oocytes and the dosage of rFSH whilst maintaining clinical pregnancy rate. Further, a trend in favor of increased incidence of implantation in the group pretreated with myo-inositol was apparent in this study. Further investigations are warranted to clarify this pharmacological approach, and the benefit it may hold for patients.

Keywords: Myo-inositol, Inositol, Follicle, Stimulation, IVF, ICSI, Oocytes, Embryos

Background

Myo-inositol is an isomer of a C6 sugar alcohol that belongs to the vitamin B complex group [1]. Some studies suggested that myo-inositol could play an important role in cellular morphogenesis and cytogenesis, in the synthesis of lipids, in the creation of cell membranes and in cell growth [2,3]. It is also a precursor of phospholipids, which are responsible for the generation of important intracellular signals in mammalian oocytes and in the resumption of meiotic maturation [4-6]. The presence of myo-inositol in human body fluids and its effect on the *in vitro* maturation of oocytes in rats have led some authors to state that myo-inositol concentration in follicular fluid is significantly higher in follicles containing good quality oocytes than in follicles containing poor quality oocytes [7].

Myo-inositol also regulates, via signal transduction pathways, the secretion of some exocrine glands such as pancreas and other organs, including the ovaries. In the oocytes these intracellular pathways are involved in the release of cortical granules, in the inhibition of polyspermy, in the completion of meiosis and in the activation of the cell cycle that subsequently results in

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embryonic development [8]. It has been hypothesized that intrafollicular myo-inositol concentration and oocyte quality might be connected because inositol phospholipids (of which myo-inositol is a precursor) are held responsible for important intracellular signals essential for oocyte development, and because myo-inositol itself seems to improve oocytes *in vitro* maturation [8,9].

Recently, the role of myo-inositol has powerfully emerged in the pathogenesis of polycystic ovary syndrome (PCOS), in particular linked with insulin resistance. In fact, some of the actions of insulin are mediated by putative inositol-containing phosphoglycan (IPG) mediators, also known as putative insulin mediators or second messengers. These mediators are generated by hydrolysis of glycosylphosphatidylinositol lipids and/or proteinated species located in the outer leaflet of the cell membrane. Two different IPG have been identified: (i) the D-chiro-IPG mediator, which activates pyruvate dehydrogenase phosphatase, and (ii) the MYO-IPG which inhibits cyclic AMP-dependent protein kinase [10,11]. A positive role of myo-inositol in insulin-resistant women with PCOS could depend on defects in the insulin IPG-mediated signaling pathway, that seems to be primarily implicated in the pathogenesis of insulin resistance in this clinical setting [12,13]. Accordingly, myo-inositol has been classified as an insulin sensitizing agent and it is commonly used in PCOS treatment [14-16]. By rescuing the ovarian response to endogenous gonadotropins, myo-inositol reduces hyperandrogenemia and re-establishes menstrual cyclicity and ovulation, increasing the chance of a spontaneous pregnancy [17,18]. Its use in human is safe and only the highest dose (12 g/day) induced mild gastrointestinal side effects such as nausea, flatus and diarrhea [19].

The aim of this paper was to illustrate the progress recently made in the use of myo-inositol in fertility treatment and in particular to discuss its effects on ovarian response and oocyte quality in non-PCOS patients undergoing multiple follicular stimulations and *in vitro* inseminations by conventional *in vitro* fertilization (IVF) or by intracytoplasmic sperm injection (ICSI).

Methods

This prospective, randomized, open-label, multicenter pilot study compared patients treated with 400 μg of folic acid for the 3 months before and during rFSH administration, following the long protocol (Group A, n = 50) with patients that received a daily dose of 4,000 mg of myo-inositol into two administrations/day in addition to 400 μg of folic acid for the 3 months before and during rFSH administration, following the long protocol (Group B, n = 50). The trial adhered to the Helsinki Declaration and the protocol was approved by the Institutional Review Boards. All patients signed a written informed consent before entering the study. Inclusion criteria were age < 40 years old and basal FSH level <10 mUI/ml. Patients presenting diagnostic criteria for PCOS [14] or other concomitant endocrine and metabolic diseases such as hypothyroidism, hyperthyroidism, diabetes mellitus, androgen-secreting tumors, adrenal hyperplasia, Cushing's syndrome, hyper-prolactinemia, and all patients that underwent hormonal treatment in the previous 3 months were excluded from the study. Day 2 FSH, LH, 17β-estradiol (E2) and prolactin (PRL) levels were measured in the previous 6 months. Patients' BMI was between 18 and 28 and obese women (BMI greater than 30 kg/m^2) were excluded from the study: Six patients of the initial cohort had a BMI greater than 30 kg/m2 (6/198; 3%). All patients began treatment during a set period —January 2011 to January 2012 ("treatment run")— and were allocated to the treatment groups using block randomization in a computer generated sequence: computer generated numbers 1-10000 into two columns – Column A was rFSH and folic acid using the 'even integers'; Column B was rFSH and folic acid with addition of myo-inositol using odd integers (Figure 1). Allocation to group A or group B was decided on the day of first consultation and was not known to doctors who performed the monitoring of follicular development, changes in the amount of daily International Units of rFSH, egg retrieval, *in vitro* fertilization, embryo transfer and decided luteal support.

Patients enrolled in the study were recommended ICSI or IVF after evaluation of their partner's semen. Patients were down-regulated with triptorelin acetate (0.1 mg, SC; Ferring, Italy) from the mid-luteal phase (day 21 of the previous cycle) for 2 weeks, before starting the stimulation protocol for oocyte recovery. In all patients, ovarian suppression was confirmed by ultrasound scan (absence of ovarian activity, ovarian cyst formation and endometrial proliferation) and serum dosage of E2 levels (≤40 pg/ml) before starting exogenous gonadotropins administration. If ovarian suppression was not met, down-regulation was extended for a further week. Group A received folic acid at a dose of 400 μg per day for 3 months before and during treatment with gonadotropins and received rFSH (Gonal F, Merck) at a starting dose of 150 IU for 6 days. The dose was subsequently adjusted according to individual response. Group B received the same gonadotropins treatment and the stimulation was preceded by the administration of 2,000 mg of myo-inositol twice a day and 400 μg per day of folic acid for 3 months before starting follicular stimulation and continued during the stimulation itself. When conventional criteria for human chorionic gonadotropin (hCG) administration were met (at least three follicles with a mean diameter >17 mm), recombinant human hCG, (250 μg SC; Ovitrelle, Merck

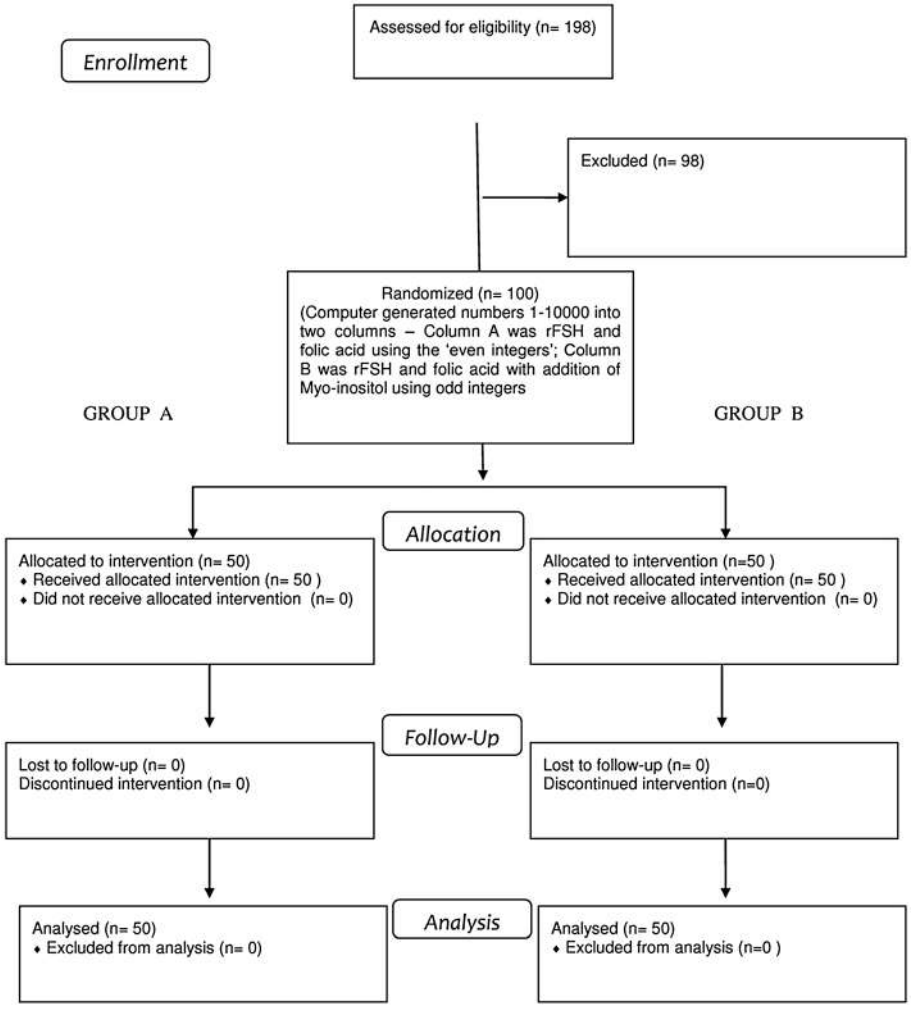

Figure 1 Flow chart showing the progress through the phases of the trial.

Serono) was given (at least 24 hours after the last rFSH administration) to induce final oocyte maturation. Transvaginal oocyte retrieval was scheduled 35-37 hours after the trigger injection. IVF or ICSI, depending on semen parameters (data not shown), were then performed. Oocytes retrieval, IVF or ICSI and embryos transfer were carried out according to our usual clinical practice [20]. Embryo quality was assessed morphologically, 2 days after fertilization using a grading system [21]. Grade 1 and 2 embryos have no or very few fragments in the cytoplasm with equal size blastomeres and therefore are considered the best embryos. Grade 3 and 4 embryos have significant or severe fragmentation; little cytoplasmic fragmentation with blastomeres of distinctively unequal size [21]. The luteal phase was supported by 800 mg/day transvaginal-micronized progesterone (Progeffik, Effik Italia S.p.A, Milan, Italy) and treatment was continued until either a serum pregnancy test result was negative or an embryonic heart beat was sonographically confirmed. Serum hCG level was measured 14 days after oocyte retrieval. A slight and transitory increase in β-hCG level was defined as a biochemical pregnancy. A gestational sac with fetal heartbeat movement seen on transvaginal ultrasound scan 4 weeks after embryo transfer, confirmed clinical pregnancy.

Statistical analysis

The number of treated patients and controls was computed with respect to a two-tailed Student *t* test for independent groups, considering a (i) difference in gonadotropins dosage required to reach follicular maturation to be detected between patients and controls $|\delta| \geq 15\%$, (ii) type I error probability $\alpha = 0.05$ and power $1 - \beta = 0.72$; this resulted in $n = 50$ for group. Sample size calculation was performed using the software nQuery Advisor, version 5.0 (Statistical Solutions, Saugus, Massachusetts). Analysis was performed according to an

intention to treat principle. Student's *t*-test for independent samples was used to evaluate statistical differences between groups for continuous variables. Comparisons between proportions were conducted using the Pearson's chi-square test.

Data are presented as mean ± SD. Probability values < 0.05 were regarded as statistically significant. All calculations were made with the computer programme STATISTICA 7 (StatSoft, Tulsa, OK, USA).

Results and discussion

Patients' age was similar in the two groups as well as BMI, FSH, LH and E2 basal levels. After the down-regulation treatment, LH level was significantly higher in patients pre-treated with myo-inositol [Group A: 1.6 ± 0.9 mUI/ml; Group B: 2.7 ± 1.1mUI/ml, $p < 0.01$], while basal FSH and basal E2 were similar (Table 1). The total length of the stimulation was similar between the two groups [Group A: 11.7 ± 1.8 days; Group B: 11.8 ± 1.5 days; $p = 0.64$]. The total amount of gonadotropins used to reach follicular maturation was significantly reduced in group B [Group A: 2,479 ± 979 IU; Group B: 2,084 ± 648 IU; $p < 0.05$]. E2 peak level on the day of hCG administration was found to be lower, but not statistically significant, in group A [Group A: 1.312 ± 629; Group B 1.516 ± 942 pg/ml, $p = 0.12$] and the number of oocytes per patient retrieved resulted significantly higher in group A [Group A: 7.6 ± 3.8 per patient (Tot. 380); Group B: 5,9 ± 2,4 oocytes per patient (Tot. 297), $p < 0.01$] (Table 2). In addition the number (mean ± SD) of Metaphase II eggs, number of inseminated eggs, number of 2PN oocytes and number of embryos were significantly higher in group A compared with group B (Table 2). Fertilization rate, cleavage rate, percentage of grade I and II embryos, number of patients receiving embryos and number of embryos received were similar in both groups (Table 2).

Table 1 Baseline and stimulation characteristics of patients in group without (Group A) and with (Group B) pretreatment with Myo-inositol

	No myo-inositol	Yes myo-inositol	p
	Group A	Group B	
	N = 50	N = 50	
No. of patients	50	50	
Age (year)	33.3 ± 2.8	34.4 ± 3.4	0.09
BMI	22.9 ± 3.3	22.7 ± 2.6	0.88
FSH (mIU/ml), basal	7.3 ± 1.5	7.2 ± 2	0.35
LH (mIU/ml), basal	4.7 ± 2	4.9 ± 2	0.87
17β-estradiol (E2) (pg/ml), basal	47.2 ± 17.8	43.6 ± 12.7	0.20
FSH (mIU/ml), down regulation	4.7 ± 1.5	4.6 ± 2.2	0.87
LH (mIU/ml), down regulation	1.6 ± 0.9	2.7 ± 1.1	<0.01
17β-estradiol (E2) (pg/ml), down regulation	26.4 ± 20	24.4 ± 14.5	0.31
rFSH treatment days	11.7 ± 1.8	11.8 ± 1.5	0.64
Total rFSH dose (IU)	2,479 ± 979	2,084 ± 648	<0.05
E2 (pg/ml), day of hCG	1,312 ± 629	1,516 ± 640	0.12
Total length of stimulation	11.7 ±1.8	11.8 ± 1.5	0.64

The number of clinical pregnancies was also similar [Group A: 12/47 (25,5%); Group B: 14/47 (29,8%), $p = 0.52$]. Finally, even the implantation rate (number of gestational sac with fetal heartbeat/total number of embryos transferred) was similar in the two groups of patients [Group A: 13,3%; Group B: 18,7%, $p = 0.08$] (Table 2).

PCOS affects 5%-10% of women in reproductive age, and is the most common cause of infertility due to anovulation. Insulin resistance is common in PCOS women, regardless of their body mass index. The importance of insulin resistance in PCOS is also suggested by the fact that insulin-sensitizing compounds have been proposed as putative treatments to solve hyperinsulinemia-induced dysfunction of ovarian response to endogenous gonadotropins. Rescuing ovarian response to endogenous gonadotropins reduces hyperandrogenemia and re-establishes menstrual cyclicity and ovulation, increasing the chance of a spontaneous pregnancy. Among insulin-sensitizing compounds, myo-inositol has been shown to be able to restore spontaneous ovarian activity, and consequently fertility, in most patients with PCOS [18,22,23]. Myo-inositol may be a useful tool in the treatment of PCOS patients undergoing ovulation induction for its insulin-sensitizing activity. Myo-inositol has also been proposed as an adjuvant in multiple follicular stimulation for IVF in patients known to suffer from PCOS : the pretreatment with myo-inositol and folic acid was shown to reduce germinal vesicles and degenerated oocytes at ovum pick-up, without compromising the total number of retrieved oocytes [8,24].

However, the intent of this study was to assess the role of myo-inositol in cellular morphogenesis and cytogenesis. At present not many data are available regarding the action and effects of myo-inositol in non-PCOS women in childbearing age, in spontaneous ovulation and in stimulation cycles. Increasing evidence supports the physiological and therapeutic role of myo-inositol in human reproduction and in particularly in oogenesis playing an important role in cell morphogenesis and cytogenesis, lipid synthesis, structure of cell membranes and cell growth [2,3]. Some studies have shown that myo-inositol is incorporated into phosphoinositides and inositol phosphates in rabbit embryos [25] and can

Table 2 Study outcomes in the two treatment groups

	No myo-inositol	Yes myo-inositol	p
	Group A	Group B	
	N = 50	N = 50	
No. oocytes retrieved/patient Mean ± SD	7.6 ± 3.8	5.9 ± 2.4	<0.01
No. metaphase II/patient Mean ± SD	6.3 ± 2.9	4.8 ± 2.2	<0.05
No. inseminated eggs/patient Mean ± SD	6 ± 2.7	4.8 ± 2.2 (240)	<0.05
No. 2PN oocytes/patient Mean ± SD	4.3 ± 2.3	3.3 ± 1.8/163	<0.01
Fertilization rate (2PN/inseminated oocytes) (%) ± SD	70.8 ± 20.4	68.4 ± 19.2	0.7
No. Embryos/patient Mean ± SD	3.58 ± 2.1	2.5 ± 1 .1	<0.001
Cleavage rate (embryos/2PN oocytes) (%) ± SD	82.2 ± 29.9	84.5 ± 28.7	0.7
No. embryos Grade I and II : number and percentage of total	173 (96.6%)	117 (93.6%)	0.2
No. of patients receiving embryos (%)	47 (94%)	47 (94%)	1
No. of embryos transferred per starting patients Mean ± SD	2.4 ± 1	2.2 ± 0.8	0.39
No. Clinical pregnancies	12/47 (25.5%)	14/47 (29.8%)	0.52
No. of fetal hearts (implantation rate)	16 (13.3%)	21 (18.7%)	0.08

enhance bovine blastocyst development from *in vitro* culture with medium supplemented with myo-inositol [26]. Results from these studies support the notion that myo-inositol serves as a precursor for the synthesis of phosphoinositides. This constitutes the phosphatidylinositol (PtdIns) signal transduction system known to be involved in the regulation of diverse cellular functions including cell proliferation [27]. During ovulation induction for an IVF cycle two important parameters need to be monitored: E2 concentration and follicles size/number. An increase in these two factors has been correlated with a higher level of myo-inositol in the follicular fluid [7]. In 1992 Chiu and Tam [28] demonstrated that serum myo-inositol could be a trophic factor responsible for promoting *in vitro* development of preimplantation embryos.

Myo-inositol is an important element of the follicular microenvironment that plays a crucial role in oocyte maturation. In fact, in Assisted Reproduction, supplementation of myo-inositol is positively related to oocyte meiotic progression of germinal vesicles in rats, increasing the intracellular calcium oscillations [4,28,29]. In patients treated with exogenous gonadotropins plus myo-inositol there was a significant reduction in the number of oocytes retrieved and in the number of follicles recruited. For this reason we can assume that this approach could be adopted to reduce the risk of hyperstimulation. Overall, these results provide a further support to the hypothesis that myo-inositol may promote the meiotic maturation acting on intracellular signal transduction in calcium pathways [29-31]. A complete meiotic maturation requires intracellular changes associated with both nuclear and cytoplasmic components [30]. At present, although most of the morphological and biochemical changes during maturation are well documented, a complete identification of specific factors that directs these changes is lacking [32]. The fertilizability of oocytes, their ability to initiate embryo splitting, and the subsequent preimplantation development are now considered a fundamental part of a proper assessment of cytoplasmic maturation [33]. It is interesting to highlight how LH level during down regulation

was higher in the group pretreated with myo-inositol [Group A: 1.6 ± 0,9 mIU/ml; Group B: 2.7 ± 1.1 mIU/ml, $p < 0.01$]. Although the meaning of this finding is not clear, we can speculate that the increased rate of circulating LH is responsible for the higher level of E2 on the day of HCG administration (although not significant in our group of patients) and for the lower number of follicles recruited, suggesting that it may be a cofactor for better oocytes and embryos quality [34].

Conclusion

The addition of myo-inositol seems to reduce gonadotropin dosage and the number of MII oocytes retrieved in non-PCOS patients pretreated with myo-inositol for 3 months. However, this study is underpowered to evaluate IVF outcomes like implantation and clinical pregnancy with the mechanism of improved oocyte competence. Therefore a subsequent adequately powered RCT is underway.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All the authors participated in designing the study, patients' enrollment, analysis of results and preparation of manuscript. FL, PC, MMO, RR, RL, RP, CM, EV, DC, RM, MM participated in designing, recruiting and treating patients, in the analysis of results and the preparation of the manuscript. VR participated in the analysis of data and in the preparation of manuscript. All authors read and approved the final version of the manuscript.

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Artículo de Investigación

Mioinositol como un Enfoque Seguro y Alternativo para el Tratamiento de las Mujeres con SOP Infértiles: Estudio Observacional Alemán

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El uso de dosis de 2 × 2000 mg de mioinositol + 2 × 200 μg de ácido fólico por día es una herramienta segura y prometedora para la mejoría efectiva de los síntomas y la infertilidad en pacientes con síndrome de ovario poliquístico (SOP). Se realizó un estudio observacional a cargo de ginecólogos alemanes, utilizando un cuestionario, para recolectar datos sobre la ovulación y las tasas de embarazo en pacientes con SOP e infertilidad. En este estudio observacional, 3602 mujeres infértiles utilizaron mioinositol y ácido fólico entre 2 y 3 meses a una dosis de 2 × 2000 mg de mioinositol + 2 × 200 μg de ácido fólico por día. En un subgrupo de 32 pacientes, se analizaron los valores hormonales de testosterona, testosterona libre y progesterona antes y después de 12 semanas de tratamiento. El tiempo promedio de utilización fue 10,2 semanas. Durante este tiempo, el 70% de estas mujeres presentaron restauración de la ovulación, y se presentaron 545 embarazos. Esto significa una tasa de embarazo de 15,1% de todas las usuarias de mioinositol y ácido fólico. En 19 casos se utilizó un medicamento concomitante, clomifeno o dexametasona. Se documentó un embarazo gemelar. Los niveles de testosterona cambiaron de 96,6 ng/ml a 43,3 ng/ml y los de progesterona de 2,1 ng/ml a 12,3 ng/ml ($p < 0,05$) después de 12 semanas de tratamiento. No se presentaron efectos secundarios relevantes entre las pacientes. Este estudio pudo demostrar que existe una nueva opción de tratamiento disponible para pacientes con SOP e infertilidad. Las tasas de embarazo alcanzadas son al menos en un rango equivalentes o incluso superiores a las reportadas con el uso de metformina.

1. Introducción

El SOP es la causa más común de trastornos menstruales, insuficiencia ovárica e infertilidad en las mujeres. Los estudios observacionales postulan que hasta el 15% de las mujeres sufren esta condición durante su vida reproductiva. La patogenia del SOP no es clara, pero lo más probable es que el factor clave sea una causa genética fuerte influenciada por el entorno gestacional y el estilo de vida. Las características más comunes del SOP son hiperandrogenismo, anovulación crónica, ecografías típicas de SOP y problemas cutáneos como acné, hirsutismo y seborrea. Además, recientemente se ha encontrado que la resistencia a la insulina desempeña un papel clave en el desarrollo clínico del SOP en casi todas las mujeres. Se han descrito trastornos graves de sensibilidad a la insulina con estado hiperinsulinémico compensatorio no sólo en pacientes con SOP obesas, sino también en mujeres delgadas, de modo que esto respalda fuertemente la hipótesis de que la resistencia a la insulina es independiente del peso [1]. En particular, la hiperinsulinemia relacionada podría inducir un exceso en la producción de andrógenos en mujeres con SOP mediante dos formas diferentes: la primera es la estimulación directa de los ovarios para producir andrógenos, y la otra, es la reducción de los niveles séricos de globulina fijadora de hormonas sexuales (GFHS) [2].

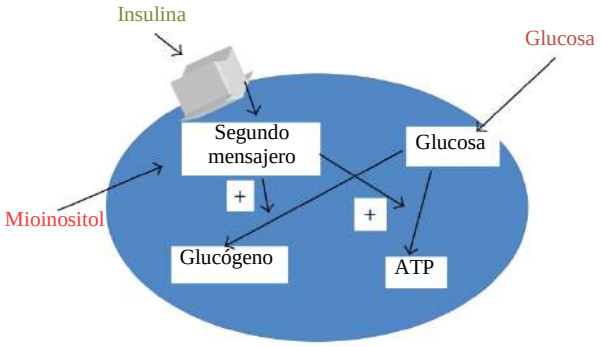

FIGURA 1: mecanismo de acción de mioinositol en la célula.

Debido al papel clave de la insulina en la patogenia del síndrome, durante muchos años, los medicamentos sensibilizadores a la insulina como metformina, pioglitazona o troglitazona, se han considerado como posibles opciones terapéuticas para el tratamiento de estos problemas. La última vez, se utilizó metformina en pacientes con estado hiperinsulinémico para mejorar la insuficiencia ovárica con anovulación consecutiva, ciclos menstruales irregulares y problemas de infertilidad [3, 4]. Sin embargo, la metformina, cuando se utilizó dentro del rango de dosis terapéuticas, demostró varios efectos secundarios graves como flatulencia, diarrea y náuseas, por esta razón muchas pacientes no pueden utilizar esta opción de tratamiento en ginecología durante un período muy largo [5, 6].

Por lo tanto, paralelamente al uso común de metformina y otros medicamentos sensibilizadores a la insulina para el tratamiento del SOP, en los últimos años, se han investigado otras alternativas terapéuticas.

Mioinositol es una de las moléculas más interesantes que se han estudiado para el tratamiento del SOP.

La sustancia inositol es un *compuesto químico* con la fórmula $C_6H_{12}O_6$ o $(\text{-CHOH-})_6$, un *alcohol* (*poliol*) de seis veces ciclohexano, con cinco grupos hidroxilo ecuatoriales y uno axial. Se encuentra ampliamente en la naturaleza. Existen nueve formas de estereoisómeros diferentes, pero mioinositol es el que se encuentra más comúnmente en la naturaleza. De hecho, el mioinositol se encuentra muy a menudo en muchas plantas y tejidos animales. La segunda forma más común es D-quiro-inositol. Es importante distinguir entre la formulación de lecitina que es biodisponible para los humanos y la formulación de fitato del maíz que no es biodisponible. Los alimentos con la mayor concentración de mioinositol son las frutas, el fríjol, el maíz y las nueces [7].

El inositol se definió en el pasado como "azúcar miometrial", pero de hecho no es una sustancia que pertenezca al grupo de carbohidratos si utilizamos las definiciones modernas. Definir el inositol como vitamina B también se ha discutido con controversia, ya que el inositol no es una sustancia esencial y puede producirse en células humanas a partir de la glucosa [8]. De hecho, varios estudios han demostrado que la molécula de inositol participa directamente en la señalización celular de la insulina.

Con respecto al SOP, varios estudios han demostrado que uno de los mecanismos de la deficiencia de insulina se origina en el mediador inositol fosfoglicano (IPG) y que la deficiencia de inositol en los inositol fosfoglicanos es responsable de la resistencia a la insulina. Se ha demostrado que la administración de D-quiro-inositol (convertido intracelularmente a partir de mioinositol) pudo reducir la resistencia a la insulina [9] (ver Figura 1).

De hecho, mioinositol, como segundo mensajero, desempeña un papel esencial en las vías de señalización de las células. En particular, la acción de mioinositol sobre la vía del SOP estaría relacionada con una mejora en la sensibilidad a la insulina y un aumento posterior en la captación de glucosa intracelular [2, 10].

Todas estas piezas de evidencia han abierto un nuevo interés clínico en el mioinositol como posible medicamento sensibilizador a la insulina para utilizarse como una opción segura y efectiva en pacientes con SOP, mediante el restablecimiento de su perfil metabólico y la consecuente inducción de la ovulación en pacientes con SOP infértiles. Los estudios también reportaron un perfil de seguridad de la molécula muy bueno, incluso cuando se administran hasta 12 gramos/día, donde únicamente se han reportado efectos secundarios gastrointestinales leves [11].

El objetivo de este estudio fue determinar las tasas de embarazo con el uso de una combinación de mioinositol y ácido fólico en pacientes con SOP en Alemania, para establecer si esta molécula se puede utilizar como una opción de tratamiento más segura para mejorar la fertilidad en esta enfermedad.

2. Pacientes y Métodos

Se creó un cuestionario estandarizado (ver Apéndice) que se presentó a 245 ginecólogos presentes en Alemania, entre junio de 2014 y marzo de 2015. Durante este tiempo, se generaron reportes de 3602 mujeres con SOP e infertilidad de acuerdo con la clasificación de Rotterdam. Las mujeres comenzaron la ingesta de mioinositol y ácido fólico a una dosis de 2 × 2000 mg de mioinositol y 2 × 200 µg de ácido fólico por día, durante al menos 2 a 3 meses. El criterio principal de valoración del estudio fue determinar la restauración de la función ovulatoria y la tasa de embarazo después del tratamiento. Los embarazos fueron documentados por los ginecólogos y se registraron en una base de datos, y estas mujeres recibieron seguimiento durante todo el embarazo. El criterio secundario de valoración fue la evaluación de los efectos secundarios reportados en las pacientes sometidas a tratamiento. En un subgrupo de pacientes, también se evaluaron los valores hormonales. Los valores investigados fueron testosterona, testosterona libre y progesterona. En este grupo de pacientes, también se verificó el desenlace del embarazo.

3. Resultados

Se evaluaron los datos de 3602 pacientes con SOP. De acuerdo con los registros obtenidos, 2520 mujeres

TABLA 1: datos hormonales antes y después del tratamiento con mioinositol

	Testosterona total (ng/dL)	Testosterona libre (ng/dL)	Progesterona (ng/mL)
Antes del tratamiento	99,6 ± 7,5	1,2 ± 0,7	2,1 ± 0,6
Después del tratamiento	43,3 ± 5,3	0,35 ± 0,1	12,3 ± 1,3

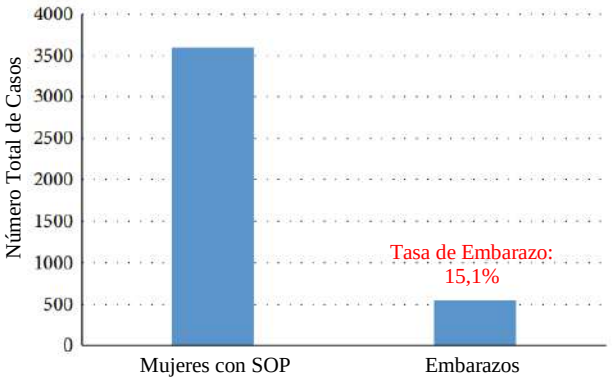

FIGURA 2: número de pacientes y tasas de embarazo.

experimentaron mejora de la ciclicidad menstrual respecto a los ciclos ovulatorios. Entre ellas, un total de 545 mujeres quedaron embarazadas. Los embarazos ocurrieron después de la ingesta de mioinositol y ácido fólico durante dos a tres meses. Esto significa que se presentó embarazó en el 15,1% de las mujeres investigadas durante este estudio observacional. No se documentaron embarazos gemelares.

No se reportaron efectos secundarios relevantes en las pacientes que tomaron mioinositol y ácido fólico.

La Figura 2 representa los datos. En el subgrupo de 32 pacientes en el que se evaluaron los valores hormonales, se observó aumento significativo de los niveles de andrógenos y de los valores de progesterona.

Esto se muestra en la Tabla 1. El Apéndice presenta el cuestionario alemán utilizado. Además, de las 32 mujeres que quedaron embarazadas, 5 experimentaron aborto, mientras que las 27 restantes dieron a luz a neonatos sanos.

4. Discusión

A pesar de las claras limitaciones del estudio observacional, existen datos confiables disponibles, ya que se puede analizar un amplio rango de pacientes. Este estudio pudo demostrar que existe una nueva opción de tratamiento disponible para pacientes con SOP e infertilidad. El 70% de las pacientes restauró la ovulación después del tratamiento. Además, las tasas de embarazo alcanzadas son al menos en un rango equivalentes o incluso superiores a las reportadas con el uso del sensibilizador a la insulina metformina. Karimzadeh y Javedani [12] describieron una tasa de embarazo de 14,4% en una cohorte de 90 mujeres, y Legro *et al.* [13], una de 12,3% en una cohorte de 75 mujeres con SOP.

Los interesantes resultados que el estudio ha presentado parecen estar relacionados con el mecanismo de acción de mioinositol. La administración de esta molécula que actúa como un mensajero directo de la señalización de la insulina y mejora la captación de glucosa por parte de los tejidos, podría mejorar el estado de resistencia a la insulina de las mujeres con SOP, y ciertamente restaurar su estado hormonal y el proceso de ovulación.

Otra evidencia importante también está relacionada con la diferencia entre mioinositol y metformina en términos del perfil de seguridad y la adherencia al tratamiento por parte de las pacientes. En las pacientes sometidas a tratamiento con metformina, se han reportado con frecuencia efectos secundarios, en particular efectos secundarios gastrointestinales leves a graves como dolor abdominal, náuseas y diarrea. Sólo en casos raros se han encontrado efectos secundarios muy graves como acidosis láctica. Por otro lado, mioinositol parece ser un enfoque seguro y bien tolerado, de algún modo capaz de producir resultados similares a los de metformina en términos de eficacia clínica.

De hecho, muchos estudios han demostrado en los últimos meses que se obtuvo una mejora en las tasas de ovulación y regularización de los ciclos menstruales mediante el uso combinado de 4 g de mioinositol y 400 μg de ácido fólico por día. Gerli *et al.* [14] pudieron demostrar en un estudio prospectivo que el grupo de pacientes que recibió mioinositol + ácido fólico experimentó ovulación en el 82% de los casos, mientras que esto sólo se observó en el 63% de los casos en el grupo de pacientes que recibieron placebo. Del mismo modo, el 70% de las pacientes del grupo de mioinositol desarrolló ciclos menstruales regulares después de 16 semanas de tratamiento, mientras que sólo el 13% de las mujeres en el grupo placebo lo hicieron.

En un estudio de Raffone *et al.* [15] que realizó una comparación entre la administración de mioinositol (2 × 2000 g + 200 μg por día) y la administración de metformina (1500 mg por día) en mujeres con SOP, se pudo demostrar que el número de embarazos fue claramente mayor en el grupo de mioinositol que en el grupo de pacientes que recibieron metformina.

Algunos estudios han demostrado la eficacia de mioinositol para aumentar la fertilidad de pacientes con SOP debido a su mejora de la resistencia a la insulina en estas mujeres [16-18].

Se han realizado muchos estudios que demuestran que el tratamiento con mioinositol + ácido fólico con la dosis clásica (2 × 2000 g de mioinositol + 200 μg de ácido fólico por día) produce cambios positivos significativos en los parámetros metabólicos y hormonales. Costantino *et al.* [19] pudieron demostrar en un estudio doble enmascarado, controlado con placebo, que mioinositol produjo mejora estadísticamente significativa en los valores de presión arterial, triglicéridos, colesterol, glucosa e insulina después de una prueba de tolerancia a la glucosa oral con 75 gramos. Estos aumentos se lograron después de un período de

tratamiento de 16 semanas. Los valores hormonales evaluados demostraron disminución significativa de los niveles séricos de testosterona total y libre, y al mismo tiempo, los niveles de progesterona, como marcador de la ovulación, aumentaron significativamente en el grupo que recibió mioinositol (ver Tabla 1). Esto pudo demostrar que mioinositol produjo no sólo cambios positivos en los parámetros metabólicos, sino también reducción de los valores androgénicos elevados y, posteriormente, mejoría de los problemas cutáneos como el acné o el hirsutismo.

Estos datos pueden ser respaldados por nuestros propios datos, ya que se pudo observar aumento en la progesterona de 2,1 ng/mL a 12,3 ng/mL. Simultáneamente, también se pudo observar reducción en los niveles de testosterona (de 96,6 ng/mL a 43,3 ng/dL) y testosterona libre (de 1,2 ng/mL a 0,35 ng/mL).

El metanálisis de Unfer *et al.* [20] pudo validar estos datos. Este metanálisis también pudo demostrar que, de acuerdo con los estudios investigados donde se utilizó la dosis de 4000 g de mioinositol + 400 mg de ácido fólico, no se observaron efectos secundarios, especialmente los que se observan cuando se utilizan otros sensibilizadores a la insulina, como la metformina, a niveles altos de 1500 mg por día.

Kamenov *et al.*, también confirmaron mejora en la inducción de la ovulación con mioinositol solo y en combinación con citrato de clomifeno en el síndrome de ovario poliquístico en pacientes con resistencia a la insulina [21]. Deberá confirmarse por más estudios si la adición de melatonina representa un beneficio, sin embargo, los primeros datos sugieren que así es [22].

Esto confirma que mioinositol no sólo es una alternativa efectiva para el tratamiento de pacientes con SOP, sino también una alternativa segura, ya que no se pudieron observar efectos secundarios con la dosis estándar. Esto, por otro lado, es relevante ya que aumenta la adherencia al medicamento, lo que produce mejores resultados en el manejo de la ovulación, el hiperandrogenismo y los parámetros metabólicos en pacientes con SOP.

Apéndice

Cuestionario Alemán

Cuestionario Número:

Fecha de nacimiento de la paciente: mes/año

Ingesta de Clavella para la Infertilidad

(1) Infertilidad: primaria – secundaria.

(2) ¿Desde cuándo sabe acerca de la infertilidad? (número de meses)

(3) Causas de infertilidad: femenina – femenina y masculina – idiopática.

(4) Infertilidad funcional:
Trastornos cíclicos (amenorrea, oligomenorrea, anovulación)
SOP
Endometriosis
Otro

(5) Infertilidad no funcional:
Trastornos de las trompas de Falopio
Trastornos inmunológicos
Adherencias
Otro

(6) Tratamiento para la infertilidad en la anamnesis
Clomifeno
Estimulación con gonadotropinas
Fertilización In Vitro / Inyección Intracitoplasmática de Esperma
Otro

(7) ¿Cuál fue la duración del tratamiento con Clavella? Meses:

(8) ¿Recibió tratamiento adicional? Sí – No

(9) Si la respuesta es afirmativa, indique el tratamiento:

(10) ¿Se presentó embarazo?

(11) Si la respuesta es afirmativa, ¿Cuánto tiempo después de iniciar el tratamiento?

Declaración de Conflictos de Interés

Pedro-Antonio Regidor es el Director Médico de la compañía Exeltis Germany GmbH y miembro del Grupo de Quimioterapia que distribuye mioinositol en Alemania.

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Research Article

Myoinositol as a Safe and Alternative Approach in the Treatment of Infertile PCOS Women: A German Observational Study

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The use of 2 × 2000 mg myoinositol + 2 × 200 μg folic acid per day is a safe and promising tool in the effective improvement of symptoms and infertility for patients with a polycystic ovary syndrome (PCOS). Using a questionnaire an observational study was performed under German gynecologists to collect data on ovulation and pregnancy rates in PCOS patients with infertility. In this observational study, 3602 infertile women used myoinositol and folic acid between 2 and 3 months in a dosage of 2 × 2000 mg myoinositol + 2×200 μg folic acid per day. In a subgroup of 32 patients, hormonal values for testosterone, free testosterone, and progesterone were analyzed before and after 12 weeks of treatment. The mean time of use was 10.2 weeks. During this time 70% of these women had a restored ovulation, and 545 pregnancies were obtained. This means a pregnancy rate of 15.1% of all the myoinositol and folic acid users. In 19 cases a concomitant medication with clomiphene or dexamethasone was used. One twin pregnancy was documented. Testosterone levels changed from 96.6 ng/ml to 43.3 ng/ml and progesterone from 2.1 ng/ml to 12.3 ng/ml ($p < 0.05$) after 12 weeks of treatment. No relevant side effects were present among the patients. This study could show that a new treatment option for patients with a PCOS and infertility is available. The achieved pregnancy rates are at least in an equivalent or even superior range than those reported by the use of metformin.

1. Introduction

The PCOS is the most common cause of menstrual disorders, ovarian dysfunction, and infertility of women. Observational studies postulate that up to 15% of women suffer under this condition during their reproductive life. PCOS etiopathology is not clear, but most probably a strong genetic cause that is influenced by gestational environment and lifestyle seems to be the key factor. The most common features of PCOS are hyperandrogenism, chronic anovulation, typical PCOS ultrasound images, and skin issues such as acne, hirsutism, and seborrhea. Furthermore, recently it has been found that insulin resistance plays a key role in the clinical development of PCOS in almost all the women. Severe disorders of the insulin sensitivity with a compensatory hyperinsulinemic state not only in obese PCOS patients but also in lean women have been described, so that the hypothesis is strongly supported that the insulin resistance is independent of the weight [1]. In particular, the related hyperinsulinemia could induce an excess of androgens production in PCOS women through two different ways: first one is direct stimulation of ovaries to produce androgens, and the other one is the reduction of sex hormone binding globulin (SHBG) serum levels [2].

Due to the key role of insulin in the syndrome etiopathology, for many years, insulin sensitizers such as metformin, pioglitazone, or troglitazone have been considered as possible therapeutic options in the management of these problems. Metformin has been used in the last time on patients with a hyperinsulinemic status for the improvement of ovarian dysfunction with consecutive anovulation, irregular menstrual cycles, and infertility problems [3, 4]. Nevertheless

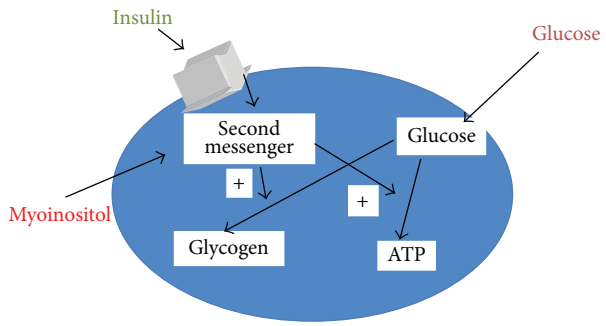

Figure 1: Mechanism of action of myoinositol in the cell.

metformin, when used in the therapeutic dose range, was shown to have several side effects such as flatulence, diarrhea, and nausea, so that many patients are unable to use this treatment option in gynecology for a longer period of time [5, 6].

Therefore, in parallel to the common use of metformin and other insulin sensitizer agents for the treatment of PCOS, in the recent years, other therapeutic alternatives have been investigated.

Myoinositol is one of the most interesting molecules that have been studied for the treatment of PCOS.

The substance inositol is a *chemical compound* with the formula $C_6H_{12}O_6$ or $(–CHOH–)_6$, a sixfold *alcohol* (*polyol*) of *cyclohexane*, with five equatorial and one axial hydroxyl group. It is widely found in nature. There exist nine different stereoisomer forms, but myoinositol is the most common one found in nature. In fact, myoinositol is very often found in many plants and in tissues of animals. The second most common form is D-chiro-inositol. It is important to distinguish between the lecithin formulation that is bioavailable for the human and the phytate formulation of corns that are not bioavailable. Foods with the highest concentration of myoinositol are fruits, beans, corns, and nuts [7].

Inositol was defined in the past as "myometrial sugar," but it is indeed not a substance belonging to the carbohydrate group if we use modern definitions. Defining inositol as a vitamin B is also being discussed with controversy as inositol is not an essential substance and it can be produced in human cells from glucose [8]. In fact, several studies have proved that the inositol molecule is directly involved in the insulin cellular signaling.

Regarding PCOS, several studies have shown that one of the mechanisms of insulin deficiency has its rise from the inositolphosphoglycan (IPG) mediator and that a deficiency of inositol in the inositolphosphoglycans is responsible for insulin resistance. It has demonstrated that the administration of D-chiro-inositol (intracellularly converted from myoinositol) could reduce the insulin resistance [9] (see Figure 1).

Indeed, myoinositol, as a second messenger, plays an essential role for the signal pathways of cells. In particular, the action of myoinositol in a PCOS pathway would be related to an improved insulin sensitivity and a sequent increased intracellular glucose uptake [2, 10].

All these pieces of evidence have opened a new clinical interest on myoinositol, as a potential insulin sensitizer agent to be used as safe and effective option in PCOS patients, through the restoration of their metabolic profile and a consequent ovulation induction in infertile PCOS patients. Studies report also a very good safety profile of the molecule, even when administered up to 12 grams/day, where only mild gastrointestinal side effects have been reported [11].

The aim of this study was to determine the pregnancy rates under the use of a combination of myoinositol and folic acid in patients with a PCOS in Germany, to establish if this molecule can be used as a safer treatment option for the fertility improvement of this disease.

2. Patients and Methods

A standardized questionnaire was created and a questionnaire (see Appendix) was presented to 245 gynecologists present in Germany, between June 2014 and March 2015. During this time reports were generated of 3602 women with a PCOS and infertility according to the Rotterdam classification. The women started with the intake of myoinositol and folic acid at a dosage of 2×2000 mg myoinositol and $2 \times 200\,\mu$g folic acid per day and used it for at least 2-3 months. The primary outcome of the study was to determine the ovulatory function restoration and the pregnancy rate after treatment. The pregnancies were documented by the gynecologists and registered in a database, and these women were followed up during the whole pregnancy. Secondary outcome was the evaluation of side effects reported in those patients undergoing treatment. In a subgroup of patients, hormonal values were also evaluated. The values investigated were testosterone, free testosterone, and progesterone. In this group of patients the pregnancies outcome has also been checked.

3. Results

The data of 3602 patients with a PCO syndrome were evaluated. According to the obtained records 2520 women experienced an improvement of their menstrual cyclicity towards ovulatory cycles. Among them, a total number of 545 women became pregnant. The pregnancies occurred after the intake of two to three months of mayoinositol and folic acid. This means a ratio of 15.1% of the investigated women becomes pregnant during this observational study. No twin pregnancies were documented.

No relevant side effects have been reported in the patients taking myoinositol and folic acid product.

Figure 2 depicts the data. In the subgroup of 32 patients where hormonal values were evaluated a significant improvement of androgen levels and a rise in the progesterone values were observed.

This is shown in Table 1. The Appendix depicts the used German questionnaire. Furthermore, out of these 32 women who became pregnant, 5 of them experienced an abortion, whereas the remaining 27 delivered healthy newborns.

Table 1: Hormonal data before and after treatment with myoinositol.

	Total testosterone (ng/dL)	Free testosterone (ng/dL)	Progesterone (ng/mL)
Before treatment	96.6 ± 7.5	1.2 ± 0.7	2.1 ± 0.6
After treatment	43.3 ± 5.3	0.35 ± 0.1	12.3 ± 1.3

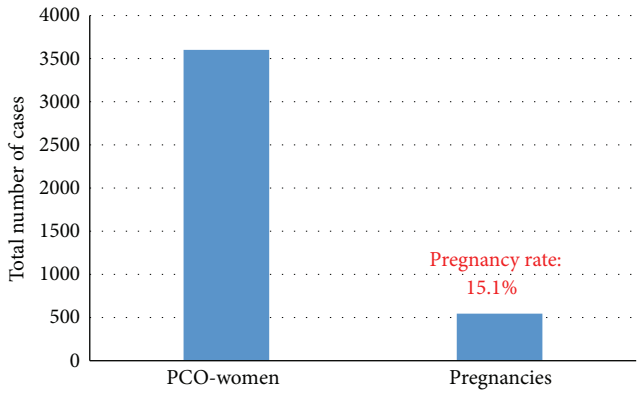

Figure 2: Number of patients and pregnancy rates.

4. Discussion

Despite the clear limitations of the observational study, there are reliable available data, since a wide range of patients can be analyzed. This study could show that a new treatment option for patients with a PCOS and infertility is available. Seventy % of the patients restored ovulation after the treatment. Furthermore, the achieved pregnancy rates are at least in a range equivalent to or even superior to those reported by the use of the insulin sensitizer metformin. Karimzadeh and Javedani [12] described a pregnancy rate of 14.4% in a cohort of 90 women and Legro et al. [13] of 12.3% in a cohort of 75 women with PCOS.

The interesting results that the study has shown seem to be related to the mechanism of action of myoinositol. The administration of this molecule, acting as a direct messenger of insulin signaling and improving the glucose tissues uptake, could improve the insulin resistance status of PCOS women, restoring indeed their hormonal status and restoring the ovulation process.

Another important evidence is also related to the difference of myoinositol and metformin in terms of safety profile and compliance for patients. In patients under metformin, side effects have been commonly reported, in particular from mild up to severe gastrointestinal side effects, such as abdominal pain, nausea, and diarrhea. Only in rare cases, very severe side effects as lactic acidosis have been found. On the other side, myoinositol seems to be a safe and well-tolerated approach, anyhow able to give similar results of metformin in terms of clinical efficacy.

In fact, many studies have demonstrated in the last months that an improvement in the rates of ovulation and regularization of menstrual cycles was obtained by the combined use of 4 g myoinositol with 400 μg folic acid per day. Gerli et al. [14] could show in a prospective study that the group of patients receiving myoinositol + folic acid experienced in 82% of the cases an ovulation, whereas this was only observed in 63% of the cases in the group of patients which received a placebo. By the same way 70% of the patients of the myoinositol group developed regular menstrual cycles after 16 weeks of treatment, whereas only 13% of the women did it in the placebo group.

In a study of Raffone et al. [15], where a comparison between the administration of myoinositol (2 × 2000 g + 200 μg per day) and the administration of metformin (1500 mg per day) in women with a PCO syndrome was performed, it could be shown that the number of pregnancies was clearly higher in the myoinositol group than in the metformin group of patients.

Some other studies upon others have shown the efficacy of myoinositol in the improvement of the fertility of PCOS patients due to its improvement of the insulin resistance of these women [16–18].

Many studies have been performed that show that the treatment with myoinositol + folic acid in the classical dosage (2 × 2000 g myoinositol + 200 μg folic acid per day) leads to significant positive changes of metabolic and hormonal parameters. Costantino et al. [19] could show in a double-blinded, placebo controlled study that myoinositol led to a statistically significant improvement of the blood pressure, triglycerides, cholesterol, glucose, and insulin values after a 75 mg oral glucose tolerance test. These improvements were achieved after a treatment period of 16 weeks. The evaluated hormonal values showed a significant decrease of the total and free testosterone serum levels and at the same time the progesterone levels, as a marker of ovulation, experienced a significant rise in the group that received myoinositol (see Table 1). This could show that myoinositol did lead not only to positive changes in metabolic parameters but also to a reduction of elevated androgenic values and subsequently to an improvement of skin problems such as acne or hirsutism.

These data can be supported by our own data as a rise of progesterone from a value of 2.1 ng/mL to a value of 12.3 ng/mL could be observed. By the same time a reduction in the levels of testosterone (from 96.6 ng/mL to 43.3 ng/dL) and free testosterone (from 1.2 ng/mL to 0.35 ng/mL) could also be observed.

A meta-analysis of Unfer et al. [20] could validate these data. This study could also show that, under the investigated studies, where the dosage of 4000 g myoinositol + 400 mg folic acid was used, no side effects were observed, especially those which are seen when other insulin sensitizers like metformin are used in high levels of 1500 mg per day.

Improvement in ovulation induction with myoinositol alone and in combination with clomiphene citrate in polycystic ovarian syndrome in patients with insulin resistance was also confirmed by Kamenov et al. [21]. Whether the addition of melatonin will represent a benefit must be confirmed by more studies but first data suggest this [22].

This confirms that myoinositol is not only an effective alternative in the treatment of PCOS patients but also a secure one as no side effects could be observed in the standard

dosage. This is on the other side relevant as the compliance of the use rises resulting in better outcomes in the management of ovulation, hyperandrogenism, and metabolic parameters on patients with a PCOS.

Appendix

German Questionnaire

Questionnaire Number

Birth date of the patient: Month/Year
Intake of Clavella because of Sterility

(1) Sterility: primary - secondary

(2) Since when is sterility known? (Number of months):

(3) Causes for sterility: female - female and male - idiopathic

(4) Functional:

Cyclical disorders (amenorrhea; oligomenorrhea; anovulation)
PCOS
Endometriosis
Other

(5) Non-functional:

Tubal disorders
Immunological disorders
Adhesions
Other

(6) Sterility treatment in the medical history

Clomiphene
Stimulation with gonadotropins
IvF/ICSI
Other

(7) How long treatment with Clavella was performed? Months:

(8) Additional treatment? Yes - No

(9) If yes: which treatment?

(10) Did a pregnancy occur?

(11) If yes; how long after starting treatment?

Competing Interests

Pedro-Antonio Regidor is medical director of the company Exeltis GmbH, Germany, a member of the Chemo Group, which distributes myoinositol in Germany.

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Estudio:

Efectos de mioinositol en mujeres con SOP: revisión sistemática de ensayos clínicos controlados aleatorizado (se anexa traducción al español).

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SOP

Efectos de mioinositol en mujeres con SOP: revisión sistemática de ensayos clínicos controlados aleatorizados

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Entre 5 y 10% de las mujeres en edad reproductiva padecen el síndrome de ovario poliquístico (SOP) y es la causa más frecuente de infertilidad debido a disfunción ovárica e irregularidad menstrual. En varios estudios se reportó que la resistencia a la insulina es frecuente en mujeres con SOP, independientemente del índice de masa corporal. La propuesta de utilizar compuestos sensibilizadores a la insulina como posibles tratamientos para resolver la disfunción de respuesta ovárica a gonadotropinas endógenas inducida por hiperinsulinemia también sugiere que la resistencia a la insulina juega un papel relevante en el SOP.
Recuperar la respuesta ovárica a gonadotropinas endógenas reduce la hiperandrogenemia y reestablece los ciclos menstruales y de ovulación, aumentando la posibilidad de un embarazo espontáneo.
Entre los compuestos sensibilizadores a la insulina se encuentra el mioinositol (MI). Estudios anteriores demostraron que MI es capaz de restaurar la ovulación espontánea y en consecuencia la fertilidad en la mayoría de pacientes con SOP. El objetivo de la presente revisión es proporcionar una perspectiva general de los resultados clínicos del uso de MI como tratamiento para mejorar la función ovárica y metabólica, y los parámetros hormonales en mujeres con SOP.

Palabras clave: infertilidad, resistencia a la insulina, FIV, mioinositol, calidad de ovocito, SOP

Introducción

El síndrome de ovario poliquístico (SOP) es la causa más frecuente de infertilidad, disfunción ovárica e irregularidad menstrual que afecta entre 5 y 10% de las mujeres en edad reproductiva [1]. La etiología y el diagnóstico del síndrome son controversiales. La Sociedad Europea de Reproducción Humana y Embriología y la Sociedad Americana de Medicina Reproductiva patrocinaron una Reunión de Consenso en Rotterdam (2003) [2, 3] para buscar un acuerdo general de la comunidad científica sobre los criterios diagnósticos para este síndrome.

Aunque en la actualidad se aceptan ampliamente los criterios establecidos en Rotterdam, estos no tienen en cuenta una condición fundamental relacionada con el SOP: la resistencia a la insulina.

En varios estudios se reportó que la resistencia a la insulina es frecuente en mujeres con SOP, independientemente del índice de masa corporal (IMC). La hiperinsulinemia debido a resistencia a la insulina afecta aproximadamente al 80% de las mujeres con SOP y obesidad central, y entre 30 y 40% de las mujeres delgadas con diagnóstico de SOP [4,5].

Se desconoce la causa exacta de la resistencia a la insulina que se evidenció en mujeres con SOP; sin embargo, se ha sugerido que un defecto postreceptor podría afectar el transporte de glucosa [6, 7]. La obesidad exacerba significativamente la resistencia a la insulina y es un factor clave en la patogénesis de la anovulación e hiperandrogenismo [5, 8]. La propuesta de utilizar compuestos sensibilizadores a la insulina, como metformina, pioglotazona y troglitazona, como tratamientos para resolver la disfunción de respuesta ovárica a gonadotropinas endógenas inducida por hiperinsulinemia también sugiere que la resistencia a la insulina juega un papel relevante en el SOP. Recuperar la respuesta ovárica a gonadotropinas endógenas reduce la hiperandrogenemia y reestablece los ciclos menstruales y de ovulación, aumentando la posibilidad de un embarazo espontáneo [9–11]. La metformina, en particular, induce reducción de los niveles de testosterona total y libre [12]. Sin embargo, los medicamentos sensibilizadores a la insulina que se utilizan con mayor frecuencia, como la metformina, pueden inducir efectos secundarios gastrointestinales [13] y resultar en una menor adherencia de las pacientes al tratamiento [13].

Estudios adicionales sugieren que el malfuncionamiento de la vía de insulina podría deberse a un defecto en el segundo mensajero de inositolfosfoglicanos (IFG) [14, 15]. Se sabe que los IFG participan en la activación de enzimas que controlan el metabolismo de la glucosa [16, 17]. En mujeres con SOP la resistencia a la insulina puede exacerbase por falta de disponibilidad tisular de inositol o mediadores de IFG, o alteraciones en su metabolismo [18].

El inositol pertenece al complejo de vitamina B. La epimerización de los seis grupos hidroxilos del inositol resulta en la formación de hasta nueve esteroisómeros, incluidos

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mioinositol (MI) y D-chiro-inositol (DCI); ambos esteroisómeros se han utilizado como medicamentos sensibilizadores a la insulina en tratamientos para SOP [19–23]. Los seres humanos adultos consumen aproximadamente 1 g de inositol (principalmente MI) al día en diferentes formas bioquímicas [18]. La mayoría de los tejidos captan el MI libre circulante mediante un cotransportador de inositol dependiente de sodio asociado a membrana; la glucosa inhibe la captación de inositol [24]. Se observó que MI es 10 veces más afín al transportador comparado con DCI [25].

A partir de los datos de otros grupos se evidenció que una epimerasa que convierte MI en DCI sintetiza el DCI; cada tejido tiene su propia tasa de conversión que probablemente depende de la necesidad específica de MI o DCI [26, 27]. Se evidenció en particular que la proporción entre DCI e índice de masa (IM) también dependía de la insulina. De hecho, en personas con diabetes tipo 2 la proporción entre DCI e IM era menor [14, 15, 27, 28] y se sintetizaba menos DCI debido a la reducción en la actividad de la epimerasa [14, 15, 27, 28].

En todos los estudios se utilizaron tejidos sensibles a insulina, como tejido muscular y del hígado. Sin embargo, a diferencia de estos tejidos, los ovarios no se vuelven resistentes a la insulina [29–31].

Además, niveles elevados de MI en líquido folicular participan en la maduración folicular y son indicadores de ovocitos de buena calidad [32,33].

Estudios previos demostraron que MI puede reestablecer la ovulación espontánea y en consecuencia la fertilidad en la mayoría de pacientes con SOP [21].

El objetivo del presente artículo es proporcionar una perspectiva general de los resultados clínicos del uso de MI como tratamiento para mejorar la función ovárica y metabólica, y los parámetros hormonales en mujeres con SOP.

Métodos

La búsqueda sistemática en literatura se realizó en diciembre de 2010 en las siguientes bases de datos electrónicas: Medline, Amed, y Cochrane Library. El parámetro de búsqueda por fecha fue desde enero de 1999 hasta noviembre de 2010 y solo se incluyeron ensayos clínicos controlados aleatorizados (ECA).

Los términos de búsqueda fueron “mioinositol,” “inositol,” “síndrome de ovario poliquístico,” “calidad de ovocito”, “estimulación ovárica”, “fertilización *in vitro*”, “función ovárica”, “resistencia a insulina”.

No se aplicaron restricciones de idioma. Se encontraron más artículos relevantes mediante búsqueda manual en las listas de referencias de revisiones sistemáticas recientes. Únicamente se incluyeron estudios con seres humanos. Se excluyeron los datos de tratamientos concomitantes con mioinositol y otros medicamentos y exámenes complementarios *in vitro* y en animales.

Obtuvimos copias físicas de todos los artículos listados de nuestra propia biblioteca universitaria o mediante préstamos interbibliotecarios. Uno de los autores (G.D.) leyó y evaluó todas las fuentes de la información obtenida y, posteriormente, los demás autores las revisaron de forma independiente (V.U.).

Resultados de la búsqueda en literatura

En la Figura 1 se ilustra el árbol de decisiones. Se identificaron 70 estudios en total de los que se excluyeron 49 porque no abordaban el tratamiento con MI en mujeres con SOP. Los 21 ensayos restantes se consideraron elegibles para la revisión. En la Tabla 1 se presentan seis ensayos [20, 22, 34–37] que correspondían a ECA (nivel de evidencia 1b) y cumplían los criterios de selección para la revisión. Cuatro ensayos [22, 34–36] evaluaron el efecto de la administración de MI sobre los niveles hormonales. Un ensayo evaluó los efectos de MI sobre la calidad de los ovocitos [20] y en el otro ensayo [37] se reportaron datos sobre mejoría de la función ovárica después de la administración de MI.

Todas las mujeres que participaron en los estudios eran pacientes con SOP. De los seis ECA, uno correspondía a ensayo clínico controlado, aleatorizado, con MI vs. ácido fólico [34], dos correspondían a ensayos clínicos controlados, aleatorizados, con doble enmascaramiento, con MI vs. ácido fólico [22, 35], otro correspondía a ensayo clínico controlado, aleatorizado, prospectivo, con MI vs. ácido fólico [20], otro correspondía a ensayo clínico controlado, aleatorizado con MI vs. metformina [36]; cuatro de los ensayos [20, 22, 35, 36] utilizaron dosis de 4 g de MI y uno [34] utilizó dosis de 2 g de MI.

El último artículo [37] listado en la Tabla 1 no se consideró en nuestra discusión porque se realizó con pacientes que recibieron tratamiento con un complejo multivitamínico.

En el estudio de Genazzani et al. [34], participaron 20 pacientes con SOP: cinco pacientes eran amenorréicas y 15 oligomenorréicas. Diez de las 20 pacientes (grupo A) se asignaron de forma aleatoria para recibir 2 g de MI + 200 μg de ácido fólico todos los días (Inofolic®, LO.LI. Pharma, Roma, Italia). Las demás pacientes (grupo B, grupo de control) recibieron únicamente 200 μg ácido fólico diarios. No se solicitó a las pacientes que realizaran cambios en su estilo de vida o dieta.

El día 7 del primer ciclo menstrual después de la semana de tratamiento 10-12 se evaluó el perfil endocrino. En el grupo A se observaron cambios consistentes y significativos. Los niveles de hormona luteinizante (HL) y prolactina (PRL) en plasma, la proporción entre HL y hormona folículoestimulante (HFE) y los niveles de insulina disminuyeron significativamente; además, el índice del modelo homeostático de evaluación (HOMA) que mide la resistencia a la insulina también disminuyó. Por otra parte, el índice de sensibilidad a la insulina y la proporción entre glucosa e insulina aumentaron significativamente. Después de una carga oral de glucosa, la respuesta a la insulina y el área bajo la curva (AUC) fueron significativamente más bajas ($p < 0,01$ y $p < 0,05$, respectivamente).

El puntaje Ferriman-Gallway bajó después de 12 semanas de tratamiento con MI, aunque la reducción no fue estadísticamente significativa (de 22,7 + 1,4 a 18 + 0,8). El volumen de los ovarios se redujo significativamente (de 12,2

+ 0,6 a 8,7 + 0,8 ml, $p < 0,05$). No se observaron cambios en el grupo B.

Mientras las pacientes recibieron tratamiento con MI, sus ciclos menstruales se normalizaron; sin embargo, las pacientes en el grupo B permanecieron oligomenorréicas durante todo el estudio.

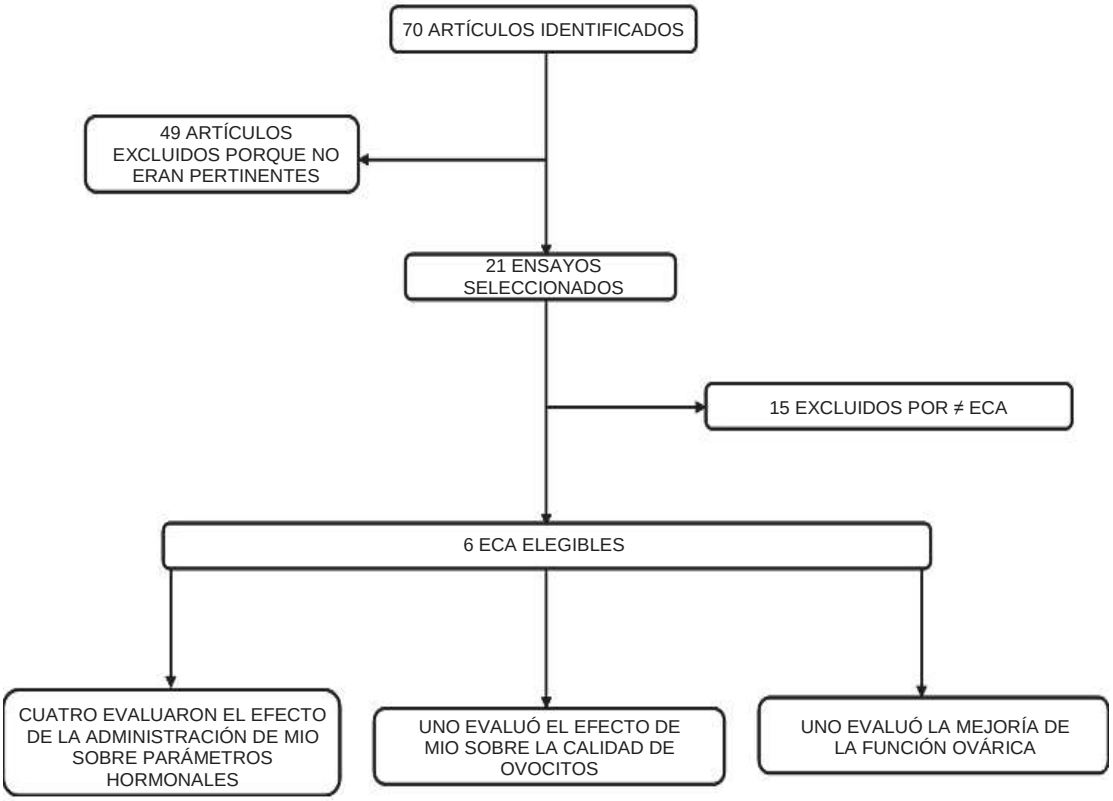

Figura 1. Diagrama de flujo de los estudios

Costantino et al. [35] seleccionaron 42 participantes; 23 se asignaron aleatoriamente para recibir 2 g de MI + 200 µg of ácido fólico (Inofolic®, LO.LI. Pharma) dos veces al día y 19 recibieron 400 µg de ácido fólico solo, como placebo. De las 42 mujeres, siete presentaron intolerancia a la glucosa; de ellas, cuatro se reasignaron al grupo con MI y tres a placebo.

El estudio inició cuando las pacientes se encontraban en la fase folicular del ciclo menstrual, no se solicitó a las pacientes que realizaran cambios en su dieta, actividades deportivas o estilo de vida.

Durante este estudio, las pacientes tratadas con MI mostraron reducción en presión sistólica y diastólica (de 131 ± 2 a 127 ± 2 mmHg y de 88 ± 1 a 82 ± 3 mmHg, respectivamente), mientras que los mismos valores aumentaron en el grupo con placebo (de 128 ± 1 a 130 ± 1 mmHg; $p = 0,002$ y de 86 ± 7 a 90 ± 1 mmHg; $p = 0,001$, respectivamente).

En el grupo tratado con MI, el nivel de triglicéridos en plasma disminuyó de 195 ± 20 a 95 ± 17 mg/dl y el colesterol total disminuyó significativamente de 210 ± 10 a 171 ± 11 mg/dl. Aunque los niveles de insulina y glucosa en ayunas no cambiaron en ninguno de los grupos, la AUC para insulina y glucosa disminuyó en el examen de tolerancia oral de glucosa (de 8,54 ± 1,149 a 5,535 ± 1,792 µU/ml/min; $p= 0,03$ y de 12,409 ± 686 a 10,452 ± 414 mg/dl/min; $p= 0,04$, respectivamente) en pacientes tratadas con MI. No se observaron cambios en el grupo con placebo.

En consecuencia, el índice de sensibilidad a insulina (ISI) compuesto, de cuerpo entero, aumentó significativamente de 2,80 ± 0,35 a 5,05 ± 0,59 mg/dl en el grupo con MI, mientras que en el grupo con placebo no cambió. Se reestableció la ovulación en 16 (69,5 %) de las mujeres en el grupo con MI y en cuatro (21%) en el grupo con placebo ($p = 0,001$). Después del tratamiento, el pico de progesterona (P) fue mayor en el grupo con MI (15,1 ± 2,2 ng/ml) comparado con placebo. Se evidenció una reducción significativa en el nivel de T total (de 99,5 ± 7 a 34,8 ± 4,3 ng/dl, $p= 0,003$) y T libre (de 0,85 ± 0,11 a 0,24 ± 0,03 ng/dl, $p = 0,01$) en suero.

La reducción de testosterona se acompañó por un aumento en el nivel de globulina de unión a hormonas sexuales en suero. Además, existió una reducción ≥ 50% en el nivel de sulfato de dehidroepiandrosterona en suero en el grupo con MI (de 366 ± 47 a 188 ± 24 µg/dl; $p= 0,003$), mientras que en el grupo con placebo la reducción no fue significativa.

Tabla 1. ECA en los que se evaluó el tratamiento con MI en pacientes con SOP

Ref.	Diseño del estudio	Duración	Tratamiento	No. de personas	Criterios de inclusión	Criterios de exclusión	Criterios de evaluación	Resultados
34	Aleatorizado, controlado con ácido fólico	12 semanas	2 g MI + 200 mg/día AF	20 Tratamiento: 10 Control: 10	Ovarios micropoliquísticos observados en ecografía; hirsutismo y/o acné leve a severo; oligomenorrea o amenorrea; sin hiperplasia suprarrenal u otras enfermedades endocrinas; niveles normales de PRL, (intervalo 5–25 ng/ml); ausencia de tratamiento hormonal al menos durante los 6 meses previos al estudio.	No descritos	HL, HFE, PRL, E2, A, 17OHP, T, insulina, cortisol, TGO* para insulina, glucosa, examen de péptido C, ecografía transvaginal, puntaje Feriman-Gallway, IMC, HOMA	Los niveles de HL, PRL, T, insulina, HL/HFE disminuyeron significativamente. La sensibilidad a la insulina mejoró significativamente. Se reestablecieron los ciclos menstruales en todas las pacientes amenorréicas y oligomenoréicas.
35	Doble enmascaramiento, aleatorizado, controlado con ácido fólico	12–16 semanas	4 g MI + 400 mg/día AF	42 Tratamiento: 23 Control: 19	Oligomenorrea, alto nivel de testosterona libre en suero y/o hirsutismo, ovarios micropoliquísticos observados en ecografía.	No descritos	Presión arterial sistólica/diastólica, triglicéridos, colesterol, IMC, índice cintura-cadera, nivel de glucosa en plasma, sensibilidad a insulina, T total/libre, DHEAT, GUHS, A, nivel pico de progesterona	MI aumentó la sensibilidad a la insulina, mejoró la tolerancia a la glucosa y disminuyó la liberación de insulina estimulada por glucosa. Se redujo el nivel de T total y libre en suero. Adicionalmente, disminuyó la presión arterial sistólica y diastólica. Los niveles de triglicéridos y colesterol total en plasma disminuyeron.
21	Prospectivo, aleatorizado, controlado con ácido fólico	Durante inducción de ovulación para IIE	4 g MI + 200 mg/día AF	60 Tratamiento: 30 Control: 30	Mujeres menores de 40 años con SOP, oligomenorrea o amenorrea, hiperandrogenismo o hiperandrogenemia y características ováricas típicas observadas en ecografía	Otras condiciones médicas que causen alteraciones de ovulación como hiperinsulinemia, hiperprolactinemia, hipotiroidismo o exceso de andrógenos, como hiperplasia suprarenal o síndrome de Cushing	Número de ovocitos morfológicamente maduros recuperados, calidad embrionaria, tasas de embarazo e implantación. Número total de días de estimulación de HFE, dosis total de gonadotropinas administrada, nivel de E2 el día de la administración de GCh, tasa de fertilización por número de ovocitos recuperados, tasa de fragmentación embrionaria, proporción entre alumbramientos vivos y abortos espontáneos, tasa de interrupción, incidencia de síndrome de hiperestimulación ovárica moderado o severo	Las unidades totales de HFEr y el número de días de estimulación disminuyeron significativamente en el grupo con mioinositol. Los niveles pico de E2 bajo administración de GCh fueron significativamente más bajos en pacientes que recibieron mioinositol. El número medio de ovocitos recuperados no fue diferente entre los dos grupos, aunque en el grupo en tratamiento concomitante con mioinositol, el número medio de vesículas germinales y ovocitos degenerados disminuyó significativamente y se evidenció tendencia a incremento porcentual de ovocitos en metafase II.
22	Doble enmascaramiento, aleatorizado, controlado con ácido fólico	16 semanas	4 g MI + 200 mg/día AF	92 Tratamiento: 45 Control: 47	Mujeres menores de 35 años con oligomenorrea, amenorrea y ovarios poliquísticos. Se tomaron los criterios de Adams et al.** para definir ovario poliquístico	Pacientes con hiperprolactinemia significativa, función anormal de tiroides, hiperplasia suprarrenal congénita.	Se realizó seguimiento de la actividad ovárica a partir de los niveles de E2, P y HL en suero. La frecuencia de ovulación se calculó a partir de la proporción entre semanas en fase lútea y semanas de observación. Inibina-b, glucosa e insulina en ayunas, AUC de insulina, LPMBD, LBD, LAD, colesterol total, triglicéridos, IMC.	Efecto benéfico del tratamiento con MI sobre función ovárica, mediciones antropométricas y perfiles lipídicos
36	Aleatorizado, controlado con metformina	Hasta finalizar el estudio o prueba de embarazo positiva	4 g MI + 400 mg/día AF	120 Tratamiento: 60 Control: 60	Mujeres menores de 35 años con diagnóstico de SOP según los criterios de Rotterdam	Otras condiciones médicas que causen disfunción de ovulación, defectos tubáricos, defectos en parámetros seminales	Recuperación de ovulación espontánea determinado a partir de niveles semanales de P en suero y ecografía transvaginal en la que se evidencie crecimiento folicular o quistes lúteos	La metformina y MI pueden considerarse tratamientos de primera línea para restaurar los ciclos menstruales en la mayoría de pacientes con SOP, aunque el tratamiento con MI parece ser más eficaz que con metformina
37	Doble enmascaramiento, aleatorizado, controlado con placebo	16 semanas	200 mg/día Inositol	283 Tratamiento: 136 Placebo: 147	Mujeres menores de 35 años con oligomenorrea, amenorrea y ovarios poliquísticos. Se tomaron los criterios de Adams et al.** para definir ovario poliquístico.	Pacientes con hiperprolactinemia significativa, función anormal de tiroides, hiperplasia suprarrenal congénita.	Se realizó seguimiento de la actividad ovárica a partir de los niveles de E2, P y HL en suero. La frecuencia de ovulación se calculó a partir de la proporción entre semanas en fase lútea y semanas de observación. Inibina-b, glucosa e insulina en ayunas, AUC de insulina, LPMBD, LBD, LAD, colesterol total, triglicéridos, IMC.	

AF= ácido fólico; IIE= inyección intracitoplasmática de espermatozoides; PRL= prolactina; E2= estradiol; A= androstenediona; 17OHP= 17-hidroxi-progesterona; T= testosterona; P= progesterona; TGO= tolerancia de glucosa por vía oral; IMC= índice de masa corporal; HL= hormona luteinizante; HFE= hormona folículoestimulante; DHEAT, dehidroepiandrosterona; GUHS= globulina de unión a hormonas sexuales; AUC= área bajo la curva de TGO; LPMBD= lipoproteína de muy baja densidad; LBD= lipoproteína de baja densidad; LAD, lipoproteína de alta densidad.

[a] Se realizaron pruebas de TGO 15 minutos antes y 30, 60, 90, 120 y 240 minutos después de la administración oral de 75 g de glucosa.

[b] Adams J, Polson JW, Franks S. Prevalencia de ovarios poliquísticos en mujeres con anovulación e hirsutismo idiopático. Br Med J 1986;293:355–359

Papaleo et al. [20] ampliaron el uso clínico de MI al evaluar su efecto sobre la calidad de los ovocitos y el protocolo de estimulación ovárica para mujeres con SOP. Sesenta mujeres participaron en el estudio, después de la asignación aleatoria, treinta mujeres recibieron 2 g de MI + 200 μg de ácido fólico (Inofolic®, LO.LI. Pharma) dos veces al día (grupo A) y 30 recibieron 400 μg de ácido fólico solo (grupo B).

En el grupo A (Inofolic®), el protocolo de estimulación fue menos intenso y más corto. La HFE recombinante total (unidades de HFEr) (1958 ± 695 vs. 2383 ± 578; p= 0,01) y el número de días de estimulación (11,4 ± 0,9 vs. 12,4 ± 1,4; p < 0,01) disminuyeron significativamente. Además, los niveles de estradiol (E2) (2232 ± 510 vs. 2713 ± 595 pg/ml; p < 0,2) después de la administración de gonadotropina coriónica humana fueron significativamente más bajos en el grupo A y resultó en un número significativamente más bajo de ciclos cancelados debido a riesgo de hiperestimulación (E2 > 4000 pg/ml). El número de ovocitos recuperados no fue diferente entre los dos grupos; sin embargo, en el grupo tratado con MI el número de ovocitos inmaduros y degenerados disminuyó significativamente (1,0 ± 0,9 vs. 1,6 ± 1,0; p= 0,01), con tendencia a incremento porcentual de ovocitos en metafase II (0,82 ± 0,11% vs. 0,75 ± 0,15%).

No se reportaron diferencias en las tasas de fertilización y fragmentación, número de embriones transferidos, número de embriones de alta calidad transferidos y tasa de embarazo.

Gerli et al. [22] incluyeron 92 pacientes para evaluar los efectos de MI sobre factores ováricos y metabólicos, 45 recibieron 2 g de MI + 200 μg de ácido fólico dos veces al día (Inofolic®, LO.LI. Pharma) y 47 recibieron 400 μg de ácido fólico como placebo.

Se presentaron ocho concepciones y un aborto espontáneo durante el primer trimestre. Sin embargo, solo 42 de las pacientes afirmaron que querían tener hijos antes del estudio. Entre ellas, la distribución de embarazos fue la siguiente: uno en el grupo con placebo (19 mujeres) y 4 en el grupo con MI (23 mujeres).

Un análisis de la intención de tratar reveló que ocho de las 45 pacientes que recibieron MI no ovularon durante el tratamiento, comparado con 17 de 47 pacientes que recibieron placebo (p= 0.04); el grupo tratado con MI presentó un aumento significativo en la frecuencia de ovulación comparado con el grupo con placebo. De acuerdo a estos datos, el nivel de P registrado durante el seguimiento de la función ovárica indica que la mayoría de las ovulaciones presentaron perfiles endocrinos normales en ambos grupos (MI y placebo). Todas las pacientes iniciaron el tratamiento sin estar en la fase lútea y el periodo hasta la primera ovulación después de iniciar el tratamiento fue significativamente más corto en el grupo con MI (24,5 vs. 40,5, p= 0,02).

El análisis de los niveles de E2, inhibina B y T en el primer y octavo día de tratamiento mostró que el grupo tratado con MI presentó un aumento significativo en el nivel de E2 (p= 0,03) mientras que no se evidenciaron cambios en el grupo de control. No se presentaron cambios en los niveles circulantes de inhibina B o de T.

El IMC disminuyó significativamente en el grupo con MI (p= 0,04). No se observaron cambios en el índice cintura-cadera en ninguno de los grupos. El nivel de leptina circulante bajó en el grupo con MI, a diferencia del grupo de control, pero no se registraron cambios en la glucosa en ayunas, insulina en ayunas o AUC de insulina como respuesta a la exposición a glucosa en ninguno de los grupos.

Se evidenciaron cambios menores en la lipoproteína de muy baja densidad durante el periodo de tratamiento, pero la lipoproteína de baja densidad (LBD) mostró tendencia a la reducción y la lipoproteína de alta densidad (LAD) aumentó significativamente en el grupo con MI.

Raffone et al. [36] realizaron un estudio con el objetivo de comparar los efectos de metformina y MI en pacientes con SOP. Se incluyeron 120 mujeres, 60 de ellas recibieron tratamiento con 1500 mg de metformina/día (Glucophage®, Merck Pharma, Roma, Italia) y 60 recibieron 4 g de MI + 400 μg de ácido fólico (Inofolic®).

Entre las pacientes tratadas con metformina, 50% recuperaron la ovulación espontánea, que ocurrió 16,7 (+2,5) días después del primer día del ciclo menstrual. Once de estas pacientes presentaron embarazos espontáneos y siete abandonaron el estudio. Las otras 42 pacientes recibieron 1500 mg de metformina + HFEr durante máximo tres ciclos. Once mujeres quedaron embarazadas; nueve de ellas eran pacientes resistentes a la metformina (n= 23) y las otras dos se encontraban en el grupo con recuperación de ovulación con metformina sola. La tasa total de embarazos fue 36,6%; cinco de los 22 embarazos (22,7%) resultaron en abortos espontáneos en la 9 semana de gestación.

Siete mujeres en el grupo con metformina abandonaron el estudio debido a la aparición de efectos secundarios y pérdida de seguimiento.

En el grupo con MI, 65% de las pacientes recuperaron la ovulación espontánea que ocurrió después de una media de 14,8 (+1,8) días desde el primer día del ciclo menstrual. Dieciocho de estas pacientes presentaron embarazos espontáneos y cuatro de ellas abandonaron el estudio. Las otras 38 mujeres recibieron tratamiento con 4 g/día de MI + 400 μg/día de ácido fólico + HFEr en dosis pequeñas (37,5 U/día durante tres ciclos). Once mujeres quedaron embarazadas; ocho de ellas eran pacientes resistentes a MI (n = 17) y tres se encontraban en el grupo que recuperó la ovulación con MI solo.

La tasa total de embarazo fue 48,4%; seis de los 29 embarazos (20,6%) resultaron en abortos espontáneos. La eficacia para reestablecer la ovulación regular se evaluó comparando el porcentaje de pacientes que respondieron al tratamiento y la duración mediana de la fase folicular en los grupos con metformina y con MI: la ovulación ocurrió después de una media de 16,7 (+2,5) días desde el primer día del ciclo menstrual en el grupo con metformina y después de una media de 14,8 (+1,8) días en el grupo con MI. La mediana entre el grupo con metformina y el grupo con MI fue significativamente diferente (p < 0,003). En la Tabla 1 se describen los criterios de inclusión, exclusión y los criterios de valoración principales de todos los estudios.

Conclusiones

El SOP es una de las alteraciones endocrinas que afecta con mayor frecuencia a las mujeres, es la causa más frecuente de infertilidad femenina y se caracteriza por la combinación de hiperandrogenismo, anovulación crónica y ciclos menstruales irregulares [38, 39]. Varias pacientes afectadas con SOP también presentan resistencia a la insulina, aunque no muestran signos de diabetes [4, 10]. La resistencia a la insulina no se relaciona con el IMC [7, 40]. La resistencia a la insulina inducida por SOP resulta en un mayor riesgo de desarrollar diabetes tipo 2, hipertensión y dislipidemia, que son elementos del síndrome metabólico [41].

MI es un componente importante del microambiente folicular y desempeña un papel determinante en el desarrollo nuclear y citoplasmático de ovocitos. El inositol 1,4,5-trisfosfato modula la liberación intracelular de Ca2+.

La señalización de calcio en ovocitos se ha estudiado de forma exhaustiva en varias especies debido al papel que aparentemente desempeña en la maduración de ovocitos y en las primeras etapas de la fertilización [42, 43]. Se ha demostrado que las oscilaciones espontáneas de calcio intracelular en vesículas germinales (VG) completamente maduras de mamíferos se asocian con una mayor incidencia de degeneración de las vesículas. Se cree que la suplementación con MI promueve la progresión meiótica de las VG. El nivel alto de MI en los líquidos foliculares humanos se asocia estrechamente con ovocitos de buena calidad [44].

A pesar del número relativamente alto de informes de estudios clínicos que evaluaban el tratamiento con MI en mujeres con SOP, solo algunos de ellos fueron diseñados como ECA (nivel de evidencia 1b).

En cuatro artículos [22, 34] se evaluaron los efectos de la administración de MI sobre parámetros hormonales y metabólicos en pacientes con SOP. Los resultados de estos estudios apoyan la hipótesis de que IFG desempeña un papel fundamental como mensajero secundario de la señal de insulina y demuestran que la administración de MI afecta significativamente el entorno hormonal de pacientes con SOP.

Estos son los efectos positivos específicos del tratamiento con MI sobre los niveles de insulina en plasma y en la respuesta insulínica a una carga oral de glucosa. MI indujo reducción en los niveles de insulina en plasma, proporción entre glucosa e insulina, índice HOMA, y otros parámetros hormonales como HL, HL/HFE, testosterona y PRL. Mioinositol tiene la capacidad de inducir normalidad de los ciclos menstruales. Estos ensayos apoyan la hipótesis de que la suplementación con MI reduce los niveles de insulina probablemente al inducir un aumento en los niveles de IFG; por lo tanto, niveles más altos de IFG podrían amplificar la señal de la insulina.

Los autores de dos estudios en particular [20, 34] sugieren que el déficit de precursores de IFG, como MI y DCI, puede ser un cofactor adicional que contribuye a la fisiopatología de la resistencia a la insulina en pacientes con SOP [18].

Todas las pacientes con SOP mostraron una mejoría significativa en los parámetros hormonales típicos después del tratamiento con MI (reducción en niveles de HL, proporción entre HL y HFE, T e índice HOMA). La AUC de insulina después de una carga de glucosa se redujo y constituye una clara evidencia de la mejoría de la sensibilidad periférica. Llama la atención que los niveles de PRL en plasma también disminuyeron significativamente con la administración de MI.

Gerli et al. evidenciaron una reducción significativa en el peso de las pacientes tratadas con MI a diferencia del grupo con placebo en el que aumentó el IMC. Junto con la pérdida de peso fue posible observar una reducción significativa en la leptina circulante y un aumento en los niveles de LAD, mientras que el nivel de LBD mostró tendencia a la reducción.

Estos datos sobre colesterol LAD fueron la primera evidencia que demostró que el tratamiento con MI podría ser útil para reducir el riesgo de enfermedades cardiovasculares en mujeres con SOP.

Varios ensayos mostraron que agentes sensibilizadores a la insulina, como la metformina y MI, son el tratamiento de primera línea para reestablecer los ciclos menstruales en mujeres con SOP [9–13], lo que sugiere que un defecto endocelular de los precursores de IFG, como MI y DCI, puede desencadenar hiperinsulinemia compensatoria en la mayoría de pacientes con SOP.

Raffone et al. evidenciaron que MI mejora levemente la tasa de embarazo comparado con metformina.

Estos hallazgos también apoyan la hipótesis de que la reducción del nivel de insulina inducida por suplementación oral con MI depende del aumento de disponibilidad del principal precursor del segundo mensajero de insulina IFG.

En un ensayo [20] se evidenció que MI es efectivo en protocolos de estimulación ovárica en mujeres con SOP.

Las mujeres con SOP presentan un mayor riesgo de síndrome de hiperestimulación [45]. Los niveles altos de andrógenos ováricos en suero se relacionan con niveles elevados de E2 en suero después de la estimulación ovárica con gonadotropinas. Las pacientes con SOP que recibieron MI + gonadotropinas mostraron una reducción significativa en los niveles de E2 después de la administración de GCh, lo que se reflejó en un menor número de ciclos de fertilización *in vitro* (FIV) cancelados debido a niveles altos de E2 (signo de síndrome de hiperestimulación [20]).

Las revisiones de literatura sugieren que MI tiene efectos positivos sobre la capacidad de desarrollo de ovocitos en etapa de maduración [46].

Un estudio clínico reciente que buscaba comparar el efecto de la suplementación con MI o DCI sobre la calidad de los ovocitos de pacientes con SOP mostró que solo MI era capaz de mejorar la calidad de los ovocitos [47], hallazgo que corresponde a la evidencia presentada anteriormente. Con base en estos datos, desarrollamos una teoría que denominamos “paradoja de DCI [48]” en la que sugerimos que los ovarios de mujeres con SOP probablemente presentan una mayor epimerización de MI a DCI, que resulta en reducción tisular de MI y que a su vez puede ser responsable de la baja calidad de los ovocitos característica en estas pacientes [49].

Es importante destacar que en todos los estudios analizados no se reportaron efectos secundarios a dosis de 2 y 4 g/día, lo que resultó en una alta adherencia de las pacientes al

tratamiento. El régimen de tratamiento de 4 g/día es útil para tratar todo el espectro de síntomas, lo que resulta en un tratamiento más completo y efectivo.

En conclusión, al analizar diferentes estudios enfocados en la suplementación con MI para mejorar varias alteraciones hormonales que se presentan en el SOP, encontramos evidencia de nivel 1a de la efectividad de MI. El mecanismo de acción de MI parece basarse principalmente en la mejoría de la sensibilidad de los tejidos diana a la insulina, lo que resulta en un efecto positivo sobre el eje reproductivo (MI reestablece la ovulación y mejora la calidad de los ovocitos) y funciones hormonales (MI reduce los parámetros clínicos y bioquímicos de hiperandrogenismo y dislipidemia) mediante la reducción de los niveles de insulina en plasma.

***Declaración de conflictos de interés*:** los autores informan que no existen conflictos de interés.

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PCOS

Effects of myo-inositol in women with PCOS: a systematic review of randomized controlled trials

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Polycystic ovary syndrome (PCOS) affects 5%–10% of women in reproductive age, and it is the most common cause of infertility due to ovarian dysfunction and menstrual irregularity. Several studies have reported that insulin resistance is common in PCOS women, regardless of the body mass index. The importance of insulin resistance in PCOS is also suggested by the fact that insulin-sensitizing compounds have been proposed as putative treatments to solve the hyperinsulinemia-induced dysfunction of ovarian response to endogenous gonadotropins. Rescuing the ovarian response to endogenous gonadotropins reduces hyperandrogenemia and re-establishes menstrual cyclicity and ovulation, increasing the chance of a spontaneous pregnancy. Among the insulin-sensitizing compounds, there is myo-inosiol (MYO). Previous studies have demonstrated that MYO is capable of restoring spontaneous ovarian activity, and consequently fertility, in most patients with PCOS. With the present review, we aim to provide an overview on the clinical outcomes of the MYO use as a treatment to improve ovarian function and metabolic and hormonal parameters in women with PCOS.

Keywords: Infertility, insulin resistance, IVF, myo-inositol, oocyte quality, PCOS

Introduction

Polycystic ovary syndrome (PCOS) is the most common cause of infertility, ovarian dysfunction and menstrual irregularity, affecting 5%–10% of women in reproductive age [1]. Both the aetiology and diagnosis of the syndrome are controversial. Indeed, the European Society of Human Reproduction and Embryology and the American Society for Reproductive Medicine sponsored a Consensus Meeting in Rotterdam (2003) [2,3], in order to reach a general agreement of the scientific community on diagnostic criteria for this syndrome.

Although nowadays the criteria established in Rotterdam are widely accepted, they leave out a crucial condition related to PCOS: insulin resistance.

Several studies have reported that insulin resistance is common in PCOS women, regardless of the body mass index (BMI). Indeed, hyperinsulinaemia due to insulin resistance occurs in approximately 80% of women with PCOS and central obesity, as well as in 30%–40% of lean women diagnosed with PCOS [4,5].

The exact cause of the insulin resistance observed in PCOS women is unknown, although a post-receptor defect, that could affect glucose transport, has been proposed [6,7]. Insulin resistance is significantly exacerbated by obesity, and it is a key factor in the pathogenesis of anovulation and hyperandrogenism [5,8]. The importance of insulin resistance in PCOS is also suggested by the fact that insulin-sensitizing compounds, such as metformin, pioglitazone and troglitazone, have been proposed as treatment to solve the hyperinsulinemia-induced dysfunction of ovarian response to endogenous gonadotropins. Rescuing the ovarian response to endogenous gonadotropins reduces hyperandrogenemia, re-establishes menstrual cyclicity and ovulation, increasing the chance of a spontaneous pregnancy [9–11]. In particular, metformin induces reduction of total and free testosterone concentrations [12]. However, commonly used insulin-sensitizing drugs, like metformin, can induce gastrointestinal side effects [13], possibly resulting in reduced patients' compliance [13].

Further studies have suggested that impairment in the insulin pathway could be due to a defect in the inositolphosphoglycans (IPGs) second messenger [14,15]. IPGs are known to have a role in activating enzymes that control glucose metabolism [16,17]. In PCOS women, a defect in tissue availability or altered metabolism of inositol or IPGs mediators may contribute to insulin resistance [18].

Inositol belongs to the vitamin B complex. Epimerization of the six hydroxyl groups of inositol leads to the formation of up to nine stereoisomers, including myo-inositol (MYO) and D-chiro-inositol (DCI); both stereoisomers were used, as insulin sensitizer drugs, in the treatment of PCOS treatments [19–23]. Human adults consume approximately 1 g of inositol (mainly MYO) per day in different biochemical forms [18]. Circulating free MYO is taken up by most tissues by a membrane-associated sodium-dependent inositol co-transporter; inositol uptake is inhibited by glucose [24]. In particular, it was shown that MYO had 10 times more affinity for the transporter compared to DCI [25].

Data from other groups have shown that DCI is synthesized by an epimerase that converts MYO into DCI, with each tissue having its own particular conversion rate, likely due to the specific needs for the two different molecules [26,27]. In particular, it was shown that the DCI to mass index (MI) ratio was itself insulin dependent. In fact, in subjects suffering from type 2 diabetes, the DCI/MI ratio was reduced [14,15,27,28], and less DCI was synthesized due to a reduction in epimerase activity [14,15,27,28].

All of these studies were performed on insulin sensitive tissues, such as muscle and liver. However, unlike tissues such as muscle and liver, ovaries do not become insulin resistant [29–31].

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Furthermore, elevated concentrations of MYO in human follicular fluid play a role in follicular maturity and provide a marker of good-quality oocytes [32,33].

Previous studies have demonstrated that MYO is capable of restoring spontaneous ovarian activity, and consequently fertility, in most patients with PCOS [21].

Here, we aim to provide an overview on the clinical outcomes of MYO as a treatment to improve ovarian function and metabolic and hormonal parameters in women with PCOS.

Methods

Systematic literature search was performed in December 2010 in the following electronic databases: Medline, Amed, and The Cochrane Library. We performed a search over the period January 1999 to November 2010 and only randomized controlled clinical trials (RCT) were included.

Search terms were as follows: "myo-inositol," "inositol," "polycystic ovary syndrome," "oocyte quality," "ovarian stimulation," "in vitro fertilization," "ovarian function," "insulin resistance."

No language restrictions were imposed. Further relevant papers were located by hand-searching the reference lists of recent systematic reviews. Only human studies were included. Data from treatments with myo-inositol in combination with other drugs as well as animal and *in vitro* investigations were excluded.

We obtained hard copies of all the papers listed through our own university library or interlibrary loans. All sources of information obtained were read and evaluated by one of us (G.D.), and successively checked independently by the other authors (V.U.).

Results of the literature search

Decision tree is reported in Figure 1. A total of 70 studies were identified; 49 out of 70 were excluded because of not involving MYO treatment in women with PCOS. The remaining 21 trials were considered eligible for this review. Six of them [20,22,34–37] were RCTs (level of evidence Ib) and met the selection criteria for this review and are presented in Table I. Four trials [22,34–36] evaluated the effect of MYO administration on hormonal levels. In one trial, it evaluated the effects of MYO on oocyte quality [20], and in another one [37] data on ovarian function improvement after MYO administration were reported.

All the subjects analysed in these studies were PCOS patients. Among the six studies that were RCTs, one trial was a randomized controlled MYO vs. folic acid [34]; two were double-blind randomized controlled trial MYO vs. folic acid [22,35]; one was a prospective randomized controlled MYO vs. folic acid [20]; one was a randomized controlled MYO vs. metformin [36] and in four of these trials [20,22,35,36] the dosage of 4 g of MYO was used; in one trial [34] 2 g of MYO were used.

The last paper [37] described in Table I is not considered in our discussion because it was performed on patients who were treated with a multivitamin complex.

In the study of Genazzani et al. [34], 20 PCOS patients were recruited, five patients were amenorrheic and 15 oligomenorrheic. Ten of the 20 patients (Group A) were randomly assigned to be treated with MYO 2 g plus folic acid 200 μg every day (Inofolic®, LO.LI. Pharma, Rome, Italy). The other patients (Group B, control group) were administered only folic acid at the daily dosage of 200 μg. No changes of life style or diet were required from the patients.

Endocrine profile was evaluated after treatment, on day 7 of the first menstrual cycle occurring after the 10–12th week of treatment. Consistent and significant changes were observed in Group A. Indeed, plasma luteinizing hormone (LH), prolactin (PRL), LH/follicle-stimulating hormone (FSH) ratio and insulin levels significantly decreased; furthermore, the homeostatic model assessment (HOMA) index that measures insulin resistance was also reduced. On the other hand, the index of insulin sensitivity glucose/insulin ratio significantly increased. Furthermore, after oral glucose load, both insulin response and the area under the curve (AUC) were significantly lower ($p < 0.01$ and $p < 0.05$, respectively).

Ferriman-Gallway score decreased after 12 weeks of MYO administration although the reduction was not statistically significant (22.7 + 1.4 to 18 + 0.8). Ovarian volumes were significantly reduced (12.2 + 0.6 to 8.7 + 0.8 ml, $p < 0.05$). No changes were observed in Group B.

Furthermore, as long as the patients were treated with MYO, the normal menstrual cycles was restored, while patients assigned to the Group B remained oligomenorrheic throughout the study.

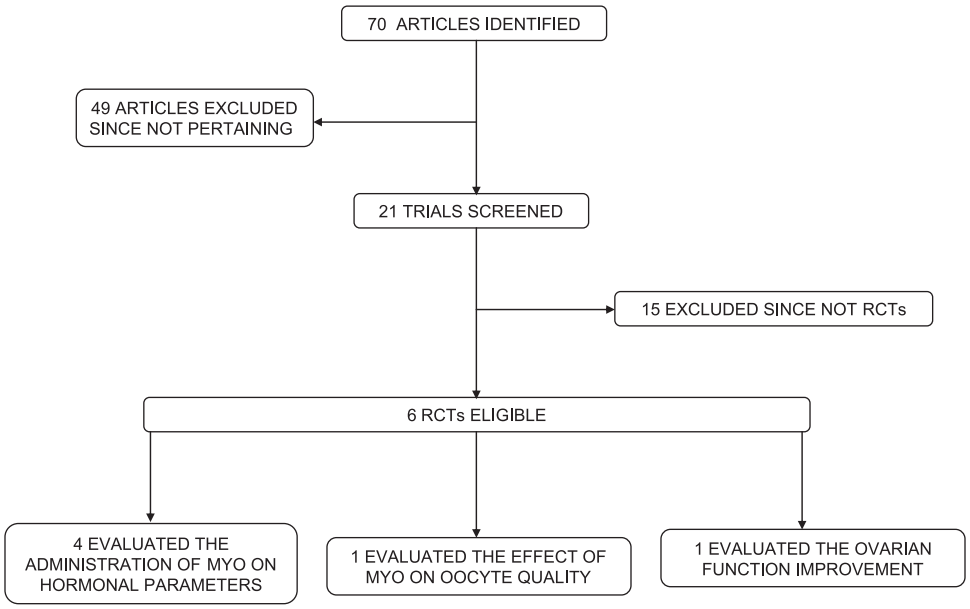

Figure 1. Flow chart of studies.

Table I. Eligible RCTs where MYO have been evaluated for the treatment of PCOS patients.

Reference	Study design	Duration	Intervention	N° of subjects	Inclusion criteria	Exclusion criteria	Assessment of the response	Results
34	Randomized, controlled vs. folic acid	12 weeks	MYO 2 g FA 200 mg/day	N° = 20 Treatment: 10 Placebo: 10	Presence of micropolycystic ovaries at ultrasound; mild-to-severe hirsutism and/or acne; oligomenorrhea or amenorrhoea; absence of enzymatic adrenal deficiency and/or other endocrine disease; normal PRL levels (range 5–25 ng/ml); no hormonal treatment for at least 6 months before the study.	Not described	LH, FSH, PRL, E2, A, 17OHP, T, insulin, cortisol, OGTT[a] for insulin, glucose, C-peptide determinations, vaginal ultrasound examination Feriman-Gallway score, BMI, HOMA	LH, PRL, T, insulin levels, LH/FSH results weresignificantly reduced. Insulin sensitivity results were significantly improved. Menstrual cyclicity was restored in all amenorrheic and oligomenorrheic subjects.
35	Double-blind, randomized, controlled vs. folic acid	12–16 weeks	MYO 4 g FA 400 mg/day	N° = 42 Treatment: 23 Placebo: 19	Presence of oligomenorrhea, high serum-free testosterone level and/or hirsutism presence of micropolycystic ovaries at ultrasound	Not described	Systolic/diastolic blood pressure, triglycerides, cholesterol, BMI, waist-to-hip ratio, plasma glucose and insulin sensitivity, total/free T, DHEAS, SHBG, A, progesterone peak value	MI increased insulin sensitivity, improved glucose tolerance and decreased glucose-stimulated insulin release. There was a decrement in serum total T and serum-free T concentrations. In addition, there was a decrement in systolic and diastolic blood pressure. Plasma triglycerides and total cholesterol concentration decreased.
21	Prospective, randomized, controlled vs. folic acid	During ovulation induction for ICSI	MYO 4 g FA 200 mg/day	N° = 60 Treatment: 30 Placebo: 30	Age: <40 years PCOS women diagnosed byoiligo amenorrhea,hyperandroge nism orhyperandrogenemia andtypical features of ovarieson ultrasound scan	Other medical conditions causing ovulatory disorders: hyperinsulinemia, hyperprolactinemia, hypothyroidism, or androgen excess, such as adrenal hyperplasia or Cushing syndrome	Number of morphologically mature oocytes retrieved, embryo quality, pregnancy and implantation rates. Total number of days of FSH stimulation, total dose of gonadotropin administered, E2 level on the day of hCG administration, fertilization rate per number of retrieved oocytes, embryo cleavage rate, live birth and miscarriage rate, cancellation rate, and incidence of moderate or severe ovarian hyperstimulation syndrome	Total r-FSH units and number of days of stimulation were significantly reduced in the myo-inositol group. Peak E2 levels at hCG administration were significantly lower in patients receiving myo-inositol. The mean number of oocytes retrieved did not differ in the two groups, whereas in the group cotreated with myo-inositol the mean number of germinal vesicles and degenerated oocytes was significantly reduced, with a trend for increased percentage of oocytes in metaphase II
22	Double-blind, randomized, controlled vs. folic acid	16 weeks	MYO 4 g FA 200 mg/day	N° = 92 Treatment: 45 Placebo: 47	Age: <35 years women with oiligoamenorrhea, amenorrhea and PCOS ovaries. Ovaries were described as polycystic (PCOs) about the criteria of Adams et al.[**]	Patients with significant hyperprolactinemia, abnormal thyroid function tests and congenital adrenal hyperplasia.	Ovarian activity was monitored using serum E2, P and LH. Ovulation frequency was calculated using the ratio of luteal phase weeks to observation weeks. Inibin-b, fasting glucose, fasting insulin, or insulin AUC, VLDL, LDL, HDL, total cholesterol, triglycerides, BMI.	Beneficial effect of MYO treatment upon ovarian function, anthropometric measures and lipid profiles
36	Randomized, controlled vs. metformin	Until the end of the study or positive pregnancy test	MYO 4 g FA 400 mg/day	N° = 120 Treatment: 60Placebo: 60	Age: <35 years Women with PCOS defined by Rotterdam Criteria	Other medical condition causing ovulatory dysfunction, tubal defects, semen parameters defects.	Restoration of spontaneous ovarian activity by weekly serum P dosage and a transvaginal ultrasound scan documenting the presence of follicular growth or luteal cyst	Both metformin and MYO can be considered as first-line treatment for restoring normal menstrual cycles in most patients with PCOS, even if MI treatment seems to be more
37	Double-blind, randomized, controlled vs. placebo	16 weeks	Inositol 200 mg/day	N° = 283 Treatment: 136 Placebo: 147	Age: <35 years Women with oligomenorrhea, amenorrhea and PCOS ovaries. Ovaries weredescribed as polycystic (PCOs) about the criteria ofAdams et al.[**]	Patients with significant hyperprolactinemia, abnormal thyroid function tests and congenital adrenal hyperplasia.	Ovarian activity was monitored using serum E2, P and LH. Ovulation frequency was calculated using the ratio of luteal phase weeks to observation weeks. Inibin-b, fasting glucose, fasting insulin, or insulin AUC, VLDL, LDL, HDL, total cholesterol, triglycerides, BMI.	Effective than metformin

FA, folic acid; PRL, prolactin; E2, oestradiol; A, androstenedione; 17OHP, 17-hydroxy-progesterone; T, testosterone; P, progesterone; OGTT, oral glucose tolerance; BMI, body mass index; LH, luteinizing hormone; FSH, follicle stimulating hormone; DHEAS, dehydroepiandrosterone; SHBG, sex hormone binding globulin; AUC, area under the curve of OGTT; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

[a]OGTT performed sampling 15 minutes before and 30, 60, 90, 120 and 240 minutes after the oral assumption of 75 g of glucose.

[b]Adams J, Polson JW, Franks S. Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. Br Med J 1986;293:355–359.

Costantino et al. [35] recruited 42 patients; after randomization, 23 received 2 g of MYO plus 200 µg of folic acid (Inofolic®, LO.LI. Pharma) twice a day and 19 received 400 µg folic acid alone as placebo. Among the 42 women, seven had impaired glucose tolerance and were assigned as follows: four of them received MYO and three received placebo.

The study started when patients were in the follicular phase of the menstrual cycle; no changes in usual habits for food, sport and lifestyle were required.

During the present study, patient treated with MYO showed a reduction in both systolic and diastolic pressure values (131 ± 2 to 127 ± 2 mmHg and 88 ± 1 to 82 ± 3 mmHg, respectively), while these values increased in the placebo group (128 ± 1 to 130 ± 1 mmHg; $p = 0.002$ and 86 ± 7 to 90 ± 1 mmHg; $p = 0.001$, respectively).

Furthermore, in the MYO treatment group, plasma triglycerides decreased from 195 ± 20 to 95 ± 17 mg/dl and total cholesterol significantly decreased from 210 ± 10 to 171 ± 11 mg/dl. Although there was no change in the fasting plasma insulin and glucose concentration in either group, AUC, for both insulin and glucose, decreased during the oral glucose tolerance test (8.54 ± 1.149 to 5.535 ± 1.792 µU/ml/min; $p = 0.03$ and 12.409 ± 686 to 10.452 ± 414 mg/dl/min; $p = 0.04$, respectively) for MYO-treated patients. No changes were observed in the placebo group.

Consequently, the composite whole body insulin sensitivity index (ISI) significantly increased from 2.80 ± 0.35 to 5.05 ± 0.59 mg/dl in the MYO group, while it did not change in the placebo group. Ovulation was restored in 16 (69.5%) women belonging to the MYO group and four (21%) belonging to the placebo group ($p = 0.001$). After treatment, the progesterone (P) peak was higher in the MYO group (15.1 ± 2.2 ng/ml) compared to placebo. Furthermore, a significant reduction in total serum T (99.5 ± 7 to 34.8 ± 4.3 ng/dl, $p = 0.003$) and in free T (0.85 ± 0.11 to 0.24 ± 0.03 ng/dl, $p = 0.01$) was observed.

Testosterone reduction was accompanied by an increase in serum sex hormone binding globulin. Furthermore, there was reduction of more than 50% in the serum dehydroepiandrosterone sulphate in the MYO group (366 ± 47 to 188 ± 24 µg/dl; $p = 0.003$), while it was not significant in the placebo group.

Papaleo et al. [20] broaden the clinical use of MYO by evaluating its effect on oocyte quality and the ovarian stimulation protocol for PCOS women. Sixty women were enrolled in the study; after randomization, 30 were assigned to receive 2 g MYO and 200 µg folic acid (Inofolic®, LO.LI. Pharma) twice a day (Group A); 30 received 400 µg folic acid only (Group B).

In the Group A (Inofolic®), the stimulation protocol was milder and shorter. Indeed, both the total recombinant FSH (r-FSH units) (1958 ± 695 vs. 2383 ± 578; $p = 0.01$) and number of stimulation days (11.4 ± 0.9 vs. 12.4 ± 1.4; $p < 0.01$) were significantly reduced. Furthermore, oestradiol (E2) levels (2232 ± 510 vs. 2713 ± 595 pg/ml; $p < 0.02$) after human chorionic gonadotropin administration were significantly lower in the Group A. These resulted in significant lower number of cancelled cycles because of hyperstimulation risk (E2 > 4000 pg/ml). The number of oocytes retrieved did not differ between the two groups, whereas in the group treated with MYO the number of immature oocytes and degenerated oocytes was significantly reduced (1.0 ± 0.9 vs. 1.6 ± 1.0; $p = 0.01$), with a trend for increased percentage of metaphase II stage oocytes (0.82 ± 0.11% vs. 0.75 ± 0.15%).

No differences were reported in fertilization and cleavage rates, the number of transferred embryos, and the number of top-quality transferred embryos and pregnancy rate.

Gerli et al. [22] enrolled 92 patients to evaluate MYO effects on ovarian and metabolic factors; 45 received 2 g MYO combined with 200 µg folic acid twice a day (Inofolic®, LO.LI. Pharma), 47 received 400 µg folic acid as placebo.

There were eight conceptions and one miscarried in the first trimester. However, only 42 of the patients declared before the study that they wished to conceive. Among these, the distribution of pregnancy was: placebo one out of 19 patients, while in the MYO group it was four out of 23 patients.

An intention to treat analysis revealed that eight of 45 MYO patients failed to ovulate during treatment, compared with 17 out of 47 placebo-treated patients ($p = 0.04$); the MYO-treated group had a significantly increased frequency of ovulation compared with the placebo group. According to these data, the concentrations of P recorded during monitoring of ovarian function indicated that most of the ovulations showed normal endocrine profiles during both MYO and placebo treatment. All patients started treatment outside the luteal phase, and the delay to the first ovulation after starting the program was significantly shorter in the MYO-treated group (24.5 vs. 40.5, $p = 0.02$).

The analysis of E2, inhibin-B, and T concentrations on the first and eighth day of treatment showed that the MYO-treated group had a significant ($p = 0.03$) increase in E2 levels, whereas the control group showed no change. There was no change in circulating levels of inhibin-B or T concentrations.

The BMI decreased significantly in the MYO group ($p = 0.04$). No change was observed in the waist-to-hip ratio in either group. Circulating leptin concentration declined in the MYO group, in contrast to the control group, but there was no change recorded in the fasting glucose, fasting insulin or insulin AUC in response to the glucose challenge in either group.

The very-low-density lipoprotein showed little change during the treatment period, but the low-density lipoprotein (LDL) showed a trend toward reduction, and the high-density lipoprotein (HDL) increased significantly in the MYO group.

Raffone et al. [36] performed a study aiming to compare the effects of metformin and MYO on PCOS patients: 120 women were recruited; 60 patients were treated with metformin 1500 mg/day (Glucophage®, Merck Pharma, Rome, Italy), while 60 received 4 g MYO plus 400 µg folic acid (Inofolic®).

Among the patients treated with metformin, 50% restored spontaneous ovulation activity; in these patients, ovulation occurred after 16.7 (+2.5) days from the day 1 of the menstrual cycle. Pregnancy occurred spontaneously in 11 of these patients; seven women dropped out. The remaining 42 patients were treated with 1500 mg of metformin plus r-FSH for a maximum of three cycles. Pregnancy occurred in 11 women, nine of these pregnancies occurred in the metformin-resistant patients (n = 23), two in the group which had ovulation restored with metformin alone. The total pregnancy rate was 36.6%, five of the 22 pregnancies (22.7%) evolved in spontaneous abortion at 9 weeks of gestation.

Seven subjects in the metformin group dropped out because of the development of side effects and loss of follow-up.

In the MYO group, 65% of patients restored spontaneous ovulation activity, ovulation occurred after a mean of 14.8 (+1.8) days from the day 1 of the menstrual cycle. Pregnancy occurred spontaneously in 18 of these patients and four women dropped out. The remaining 38 patients were treated with 4 g/day MYO, 400 µg/day folic acid and r-FSH in small doses (37.5 U/day for three cycles). Pregnancy occurred in a total of 11 women, eight of these pregnancies occurred in the MYO-resistant patients (n = 17), three in the group which had ovulation restored with MYO alone.

The total pregnancy rate was 48.4%, six of the 29 pregnancies (20.6%) evolved in spontaneous abortion. The efficacy in restoring regular ovulation was evaluated by comparing both the percentage of patients who responded to the treatment and the median length of follicular phase in metformin and in MYO group: ovulation occurred after a mean 16.7 (+2.5) days from the day 1 of the menstrual cycle in the metformin group and after a mean 14.8 (+1.8) in the MYO group. The median between metformin and MYO differed significantly ($p < 0.003$). Inclusion, exclusion criteria and the main outcome measures for all studies are described in Table I.

Conclusions

PCOS is one of the most common endocrine disorders affecting women, it is the most common cause of female infertility and it is characterized by a combination of hyperandrogenism, chronic anovulation and irregular menstrual cycle [38,39]. Several patients affected by PCOS are also affected by insulin resistance although they do not show signs of diabetes [4,10]; furthermore, insulin resistance is not linked to the BMI [7,40]. PCOS-induced insulin resistance determines a higher risk for the development of type 2 diabetes, hypertension and dyslipidemia, all elements of the metabolic syndrome [41].

MYO is an important constituent of follicular microenvironment, playing a determinant role in both nuclear and cytoplasmic oocyte development. Indeed, inositol 1,4,5-triphosphate modulates intracellular Ca^{2+} release.

Calcium signaling in oocytes has been extensively studied in various species because of its putative role in oocyte maturation and the early stages of fertilization [42,43]. It has been demonstrated that fully grown mammalian germinal vesicles (GV), that exhibit spontaneous intracellular calcium oscillations, are associated with a higher incidence of GV breakdown. MYO supplementation was suggested to promote meiotic progression of these GV. Indeed, high concentration of MI in the fluids of the human follicles strongly associates with good-quality oocytes [44].

Despite the relatively high number of reports evaluating clinical studies that used MYO as a treatment in women with PCOS, only few of them were designed as RCTs (level of evidence Ib).

Four articles [22,34–36] evaluated the effects of MYO administration on hormonal and metabolic parameters in patients with PCOS. The results of these studies support the hypothesis of a primary role of IPG as second messenger of insulin signal and demonstrate that MYO administration significantly affects the hormonal milieu in PCOS patients.

There are specific positive effects of MYO treatment on insulin plasma levels and on insulin response to oral glucose load. Indeed, MYO decreased insulin plasma levels, glucose/insulin, HOMA index as well as other hormonal parameters such as LH, LH/FSH, testosterone and PRL. Furthermore, MYO inositol is able to induce normal menstrual cycles. These trials supported the hypothesis that MYO supplementation induces the reduction of insulin levels probably by inducing an increase of IPG levels; therefore, higher IPG levels could be able to amplify insulin signal.

In particular, in two studies [20,34] the authors suggest that a deficiency in the precursors of IPG such as MYO and/or DCI might be an additional cofactor contributing to the pathophysiology of the insulin resistance of PCOS patients [18].

All the PCOS patients showed a significant improvement of typical hormonal parameters: indeed, after MYO treatment LH levels, LH/FSH ratio, T and HOMA index were decreased. Furthermore, the insulin AUC after glucose load was reduced, being a clear signal of the improved peripheral sensitivity. Interestingly, PRL plasma levels also resulted significantly lower under MYO administration.

In addition to this, Gerli et al. demonstrated a significant reduction in weight in the patients treated with MYO, in contrast to the placebo group where the BMI increased. Associated with the weight loss, it was possible to observe a significant reduction in circulating leptin and an increase in HDL concentrations, while LDL showed a trend toward reduction.

These data on HDL cholesterol were the first evidence showing that MYO treatment could be useful in reducing the risk of cardiovascular diseases in PCOS women.

Several trials showed that insulin sensitizer agents, such as metformin and MYO, are the first-line treatment to restore normal menstrual cycles in women suffering from PCOS [9–13], suggesting that an endocellular defect of the precursor of IPG such as MYO and/or DCI might trigger the compensatory hyperinsulinemia in most PCOS subjects.

Moreover, Raffone et al. showed that MYO slightly improves pregnancy rate compared to metformin.

These findings further support the hypothesis that the reduction of insulin levels induced by MYO oral supplementation depends on the increased availability of the main precursor of IPG insulin second messenger.

Furthermore, in one trial [20] it has been shown that MYO is effective in ovarian stimulation protocols in women with PCOS.

PCOS women have an increased risk of hyperstimulation syndrome [45]. Indeed, high levels of serum ovarian androgens are implicated in production of elevated serum E2 levels after gonadotropin ovarian stimulation. PCOS patients treated with MYO + gonadotropin showed a significant reduction in E2 levels after hGC administration. This was reflected on the lower number of in vitro fertilization (IVF) cycles cancelled because of high E2 levels (sign of hyperstimulation syndrome [20]).

Literature studies already suggested that MYO has positive effect on developmental competence of maturing oocytes [46].

In line with this evidence, a recent clinical trial aiming to compare the effect of MYO or DCI supplementation on oocyte quality of PCOS patients showed that only MYO rather than DCI is able to improve oocyte quality [47]. Based on these data, we developed a theory that identified a "DCI paradox [48]," where we suggest that ovaries in PCOS patients likely present an enhanced MI to DCI epimerization that leads to a MI tissue depletion; this, in turn, could eventually be responsible for the poor oocyte quality characteristic of these patients [49].

Remarkably, in all the studies analysed, no side effects were reported at the doses of both 2 and 4 g/day, thus resulting in a high patient compliance. The 4 g/day treatment regimen is useful to treat all the symptom spectrum, resulting in a more complete and effective treatment.

In conclusion, by analyzing different studies focused on MYO supplementation to improve several of the hormonal disturbances of PCOS, we provide a level Ia evidence of MYO effectiveness. MYO mechanism of action appears to be mainly based on improving insulin sensitivity of target tissues, resulting in a positive effect on the reproductive axis (MYO restores ovulation and improves oocyte quality) and hormonal functions (MYO reduces clinical and biochemical hyperandrogenism and dyslipidemia) through the reduction of insulin plasma levels.

***Declaration of Interest*:** The authors report no conflict of interest.

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Inositol transport proteins

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Review

Inositol transport proteins

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ABSTRACT

The cyclic polyol *myo*-inositol is a key molecule in many different metabolic pathways among all organisms; in addition, it is fundamental for osmotic balance in the mammalian brain. This review sums up inositol transporters from eukaryotic organisms, elucidating their vital role in regulating the intracellular distribution and uptake of inositol. They can be divided into two groups according to their transport mechanisms: (1) sodium ion coupled inositol transporters that belong to the Solute Carrier Families 5 and 6-like Superfamily and, (2) proton coupled inositol symporters that are members of the Major Facilitator Superfamily. Intriguingly members of both families offer promising targets for medical treatment of a variety of diseases.

1. Introduction

Myo-inositol is an important component of metabolic pathways. Its biosynthesis pathway is conserved among all organisms and starts with the conversion of D-glucose-6-phosphate to L-inositol-1-phosphate. This reaction is catalysed by the *myo*-inositol-phosphate-synthase. Dephosphorylation of L-inositol-1-phosphate by *myo*-inositol-monophosphatases results in *myo*-inositol. Besides *myo*-inositol, eight inositol stereoisomers exist (D-*chiro*-, L-*chiro*-, *scyllo*-, *cis*-, *neo*-, *muco*-, *epi*- and *allo*-inositol), but only seven of them occur naturally [23] (Fig. 1). However, *myo*-inositol is the most frequent inositol stereoisomer playing a crucial role in all organisms.

Myo-inositol is the structural basis of phosphatidylinositol, a membrane compound that in turn serves as educt for the synthesis of phosphorylated derivatives, the phosphoinositides. Phosphatidylinositol can be phosphorylated at position D-3, -4 and -5 of its inositol ring, giving rise to a variety of phosphoinositide stereoisomers. Phosphatidylinositol-4,5-bisphosphate is probably the most well-known due to it being the precursor for the second messenger inositol-1,4,5-trisphosphate. The spectrum of identified phosphoinositides is yet incomplete, but for all analysed isomers additional functions, besides being a structural part of membranes, were assigned so far. Phosphoinositides are, for example, involved in signal transduction, membrane trafficking, immune cell functions, chemotaxis and regulation of cytoskeletal dynamics [90,95,5].

There is a multitude of further compounds containing *myo*-inositol as an integral part, but inositol itself plays a vital role as well. It is, for example, essential for the osmoregulation in mammalian brain, liver and the renal medulla to protect cells from the negative impact of hyperosmolality [91,33,36]. Among other osmolytes, inositol serves to maintain the osmotic balance between the respective tissue and its surroundings [14].

Accordingly, *myo*-inositol and methylated inositol derivatives, e.g., pinitol, are used by some plant species as compatible solutes that prevent cell damage under salt, cold and drought stress [16,85]. A sixfold phosphorylated inositol derivative, inositol hexa*kis*phosphate, also known as phytate, is an important storage form for phosphate, inositol and also complexes bivalent cations in plant seedlings [53].

Inositol transporters are responsible for uptake and intracellular distribution of inositol and were identified in bacteria, protozoa, fungi, plants and animals. According to their transport mechanism they can be classified into two groups: sodium ion coupled and proton coupled inositol transporters, respectively. Their physiological role and importance for applied medical science are summarised.

2. Sodium ion coupled inositol transporters

In 1992 the first Na^+-coupled inositol transporter was identified in Madin–Darby canine kidney (MDCK) cells, as the transporter responsible for accumulation of *myo*-inositol in kidney cells exposed to hypertonic medium. The protein was characterised in *Xenopus* oocytes and showed strong similarity to Na^+/glucose transporters [45]. The so-called SMIT (Sodium/*Myo*-Inositol Transporter; later re-named in SMIT1 after discovery of a second Na^+-coupled inositol transporter) was soon found also in rat [104], human [7] and mouse [58]. The respective gene for SMIT1, *SLC5A3*, belongs to the Solute Carrier Family 5 (SLC5A) [7]. The

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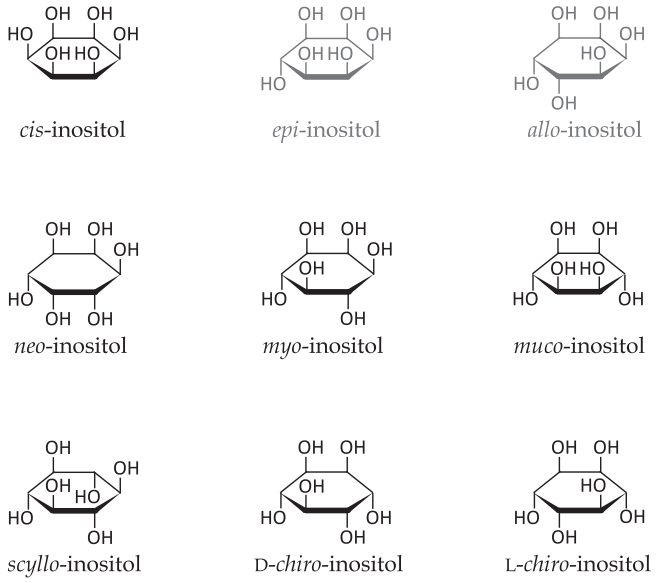

Fig. 1. Structural formulas of inositol stereoisomers. Inositols shown in grey (*epi*- and *allo*-inositol) do not occur naturally.

human *SLC5A*-family consists of eleven members, including the Na^+/glucose cotransporter SGLT1 and further Na^+-coupled transport proteins, all with 14 predicted transmembrane domains [101] (Fig. 2A).

Using primers against a conserved sequence motif, another member of the Solute Carrier Family 5, *SLC5A11*, was cloned from rabbit in 1994 and its gene product designated rkST1 [39]. It took several years until this protein was also characterised as Na^+-coupled inositol transporter and was thus renamed in SMIT2. On amino acid level, SMIT2 shows 49% and 43% sequence identity to SGLT1 and SMIT1, respectively [18]. Fig. 3 shows a phylogenetic tree for SMIT1 and SMIT2 proteins isolated from several mammals.

Both genes encoding Na^+-coupled inositol transporters show similar expression patterns. *SLC5A3*, encoding SMIT1, is expressed in kidney, brain, placenta, pancreas, heart, skeletal muscle and lung [7]. *SLC5A11*, encoding SMIT2, shows high expression in kidney, heart, skeletal muscle, liver and placenta, and is weakly expressed in the brain [76]. Since both proteins are located in the plasma membrane [27], further studies focused on investigating functional redundancy and potential differences between SMIT1 and SMIT2.

2.1. Transport characteristics of sodium ion coupled inositol transporters

SMIT1 activity was first analysed by heterologous expression of canine *SLC5A3* in *Xenopus* oocytes (see Table 1 for transport characteristics of SMIT1 and SMIT2 analysed in heterologous systems). Its preferred substrate is *myo*-inositol (K_m = 55 μM), yet *scyllo*-inositol induced similar currents [37]. SMIT2 accepts D-*chiro*-inositol (K_m = 130 μM) as well as *myo*-inositol (K_m = 120 μM) [18]. The physiological substrate of SMIT2 is supposed to be *myo*-inositol as the concentration of D-*chiro*-inositol within the plasma is rather low [18]. Both transporters show only a low affinity for glucose with a K_m value of ~50 mM for SMIT1 and ~30 mM for SMIT2 [37,18,4,49].

L-Fucose is only transported by SMIT1, but not SMIT2, and was shown to inhibit in high concentrations *myo*-inositol transport in competitor experiments [37,18,10]. D-*Chiro*-inositol competes with *myo*-inositol in SMIT2-mediated transport and has been used as a specific inhibitor of SMIT2 but not SMIT1 in non-heterologous systems. L-Fucose and D-*chiro*-inositol therefore offer a good possibility to distinguish between SMIT1 and SMIT2 activity in vivo when both transporters are present [10,4,46,11,28].

Myo-inositol transport increased after downregulation of protein kinase C activity and decreased after activation of protein kinase A in cultured human fibroblasts as well as in MDCK cells, indicating a posttranslational regulation of the inositol uptake system by phosphorylation [71,29].

Analysis of a potential impact of the serum- and glucocorticoid-inducible kinase SGK1 on SMIT1 activity revealed that phosphorylation of SMIT1 by SGK1 and closely related protein kinases activates *myo*-inositol transport by increasing the abundance of SMIT1 within the plasma membrane. As *SGK1* gene expression is

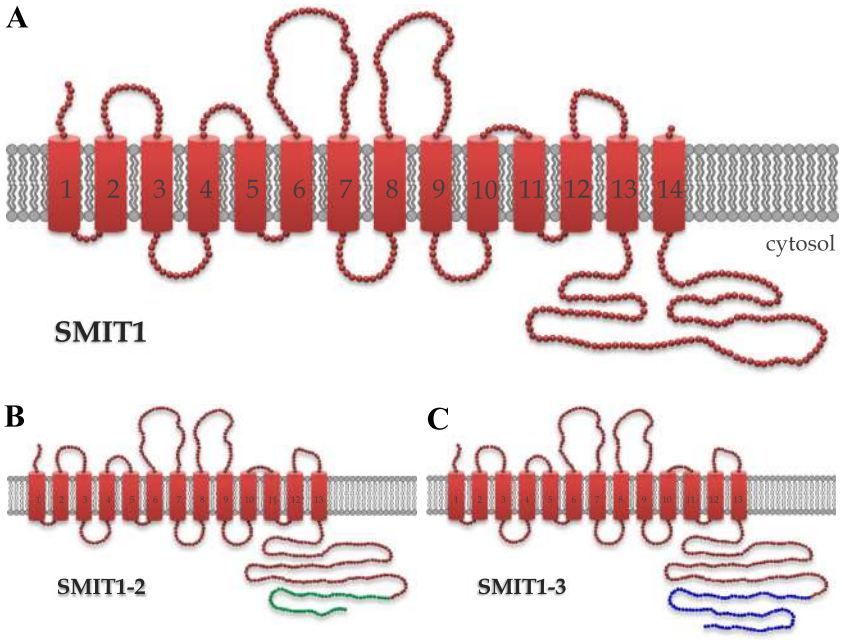

Fig. 2. Protein model for human SMIT1 and postulated splice variants. (A) SMIT1 model was generated using TMHMM Server v. 2.0 prediction for transmembrane domains (http://www.cbs.dtu.dk/services/TMHMM/) [44]. (B and C) Models for SMIT1 splice variants SMIT1-2 and SMIT1-3 are modified after [70]. Green and blue colour, respectively, was used to indicate the divergent C-termini of SMIT1-2 and SMIT1-3.

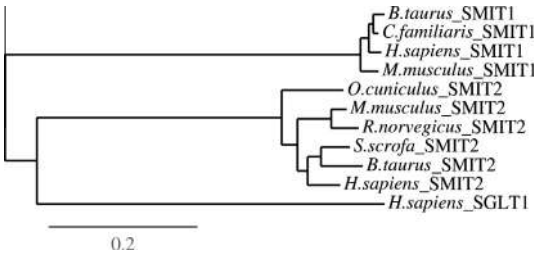

Fig. 3. Phylogenetic tree of mammalian sodium ion coupled inositol transporters (SMIT1, SMIT2) and human sodium ion coupled glucose transporter (SGLT1). Tree was created using "one click" mode from Phylogeny.fr [20]; branch length is proportional to the number of substitutions per site. Protein accessions: *Bos taurus* SMIT1: P53793, SMIT2: Q3ZC26; *Canis lupus familiaris* SMIT1: P31637; *Homo sapiens* SGLT1: P13866, SMIT1: P53794, SMIT2: Q8WWX8; *Mus musculus* SMIT1: Q9JKZ2, SMIT2: Q8K0E3; *Oryctolagus cuniculus* SMIT2: Q28728; *Rattus norvegicus* SMIT2: Q9Z1F2; *Sus scrofa* SMIT2: A8I1B9.

also induced by hypertonicity, this offers a further mechanism to regulate *myo*-inositol import during high osmolarity [43].

An additional regulation of SMIT activity at protein level by interaction with the α subunit KCNQ1 and the β subunit KCNE2 of potassium channels was shown very recently [1]. Coexpression of SMIT1 and KCNQ1–KCNE2 or a constitutively active KCNQ1 mutant in *Xenopus* oocytes inhibits *myo*-inositol uptake mediated by SMIT1 but not SMIT2, while coexpression of a constitutively active KCNQ1 mutant with SMIT1 or SMIT2 inhibits transport activity of both inositol transporters. Vice versa, channel activities of KCNQ1 and KCNQ1–KCNE2 were enhanced by SMIT1, but suppressed by SMIT2. Interactions between SMIT1/2 and potassium channel subunits provide multiple potential feedback mechanisms to control cell signalling and homeostasis [1].

A recent study on SMIT2 transport activity confirmed the earlier postulated 2:1 stoichiometry for Na^+/*myo*-inositol cotransport [18,12] and led to a very detailed five-state model including binding of Na^+ ions and a turnover rate of 21 s^{-1}. In the first state, the SMIT2 binding sites for Na^+ and *myo*-inositol are only accessible from the extracellular space. Na^+ ions bind first, initiating the next state that allows the binding of *myo*-inositol. *Myo*-inositol attachment induces a reorientation of the transporter, thereby closing the extracellular gate. The intracellular gate is opened during the third state and the substrates are released into the cell. The next two states are defined by closing the intracellular gate and opening the extracellular gate, thus restoring the starting point [80].

2.2. SMIT1: expression and gene structure

SMIT1 is a main actor in mammalian osmoregulation by accumulating *myo*-inositol in kidney and brain. The expression of the *SLC5A3* is upregulated under hypertonic stress and high osmolality [45,104,103]. Multiple tonicity-responsive enhancers are distributed within a 50 kbp 5′-flanking region of the *SMIT1* gene [75]. Hypertonicity-induced expression of *SLC5A3* is regulated by the transcription factor TonEBP (Tonicity-responsive Enhancer Binding Protein). *TonEBP* expression itself is upregulated under hypertonic conditions, resulting in an increased amount of TonEBP protein and its translocation to the nucleus [60,29,63]. Studies on *TonEBP* knockout mice showed that TonEBP is in fact the key regulator of *SMIT1* expression: no *SMIT1* mRNA was detectable in mice missing the *TonEBP* gene and furthermore, these knockout mice show the same phenotype as described for *SMIT1* knockout mice [87].

While the regulation of *SMIT1* gene expression is well established, the gene structure of *SLC5A3* is under discussion. It has been postulated that it possesses one [7,58], two [56,15] or even five exons [69,70], respectively. The five-exon model was proposed

Table 1
Substrate analysis of sodium ion coupled inositol transporters in heterologous systems.

Transporter	Origin of gene	Analysed substances	Physiological substrate	K_m values	Additionally transported	K_m values	Heterologous expression system	References
SMIT1	*C. familiaris*	*myo*-inositol, *scyllo*-inositol, α-methyl-D-glucopyranoside, D-glucose, L-glucose, D-galactose, D-fucose, L-fucose, D-xylose, L-xylose, 3-O-methyl-glucose, 2-deoxyglucose, mannitol, uridine	*myo*-inositol, *scyllo*-inositol	55 μM n.a.	L-fucose > L-xylose > L-glucose, D-glucose, α-methyl-D-glucopyranoside	~50 mM ~50 mM >50 mM >50 mM >50 mM	*Xenopus* oocytes	Hager et al. [37]
SMIT1	*C. familiaris*	*myo*-inositol, L-fucose	*myo*-inositol	n.a.	L-fucose	n.a.	*Xenopus* oocytes	Bissonnette et al. [10]
SMIT2	*O. cuniculus*	*myo*-inositol, D-*chiro*-inositol, L-*chiro*-inositol, α-methyl-glucose, D-glucose, L-glucose, D-galactose, D-fucose, L-fucose, D-xylose, L-xylose, 3-O-methyl-glucose, 2-deoxyglucose, phlorizin	*myo*-inositol	120 μM	D-*chiro*-inositol > D-glucose > D-xylose	130 μM ~30 mM n.a.	*Xenopus* oocytes	Coady et al. [18]
SMIT2	*O. cuniculus*	*myo*-inositol, L-fucose	*myo*-inositol	120 μM	–	–	*Xenopus* oocytes	Bissonnette et al. [10]
SMIT2	*O. cuniculus*	*myo*-inositol, L-fucose	*myo*-inositol	334 μM	–	–	MDCK cells	Bissonnette et al. [10]
SMIT2	*R. norvegicus*	*myo*-inositol, D-*chiro*-inositol, D-glucose, phlorizin	D-*chiro*-inositol, *myo*-inositol	270 μM 310 μM	phlorizin > D-glucose	16 μM 36 mM	*Xenopus* oocytes	Aouameur et al. [4]
SMIT2	*H. sapiens*	*myo*-inositol, D-*chiro*-inositol, pinitol, D-glucose, 3-O-methyl-glucose, 2-deoxyglucose	D-*chiro*-inositol, *myo*-inositol	110 μM 158 μM	–	–	L6 myoblasts	Lin et al. [49]

due to the discovery of differentially spliced mRNAs. One splice variant encodes the human SMIT1 protein containing 14 transmembrane helices, while two other variants, *SMIT1-2* and *SMIT1-3*, encode SMIT1 isoforms without the last transmembrane domain but with different C-Termini (Fig. 2B and C). These isoforms were analysed in *Xenopus* oocytes and displayed different transport activities for *myo*-inositol compared to SMIT1. They also possess new potential phosphorylation sites that offer additional points of regulation [70].

Recent studies revealed that the *SLC5A3* gene encoding SMIT1 is on the same gene locus as the gene coding for the mitochondrial ribosomal protein subunit 6 (MRPS6) [13]. It was shown that *SLC5A3* and *MRPS6* share parts of exon 1 and possibly of the promoter region [32,13]. Exon 2 and 3 of the *MRPS6* transcript correspond to the postulated exon 4 and 5 of the alternatively spliced *SMIT1* mRNAs, which suggests that these mRNAs might be transcripts encoding MRPS6 instead of SMIT1 isoforms [13].

2.3. The role of SMIT transporters in Down syndrome, Alzheimer's disease and bipolar disorder

Down syndrome is caused by the presence of an extra copy of genes on the long arm of chromosome 21. As *SLC5A3* is located on band q22 on chromosome 21 SMIT1 was soon suspected to be involved in Down syndrome [7,56]. Down syndrome individuals possess elevated *myo*-inositol levels in brain and cerebrospinal fluid that can already be detected in foeti during pregnancy [86,8,48,79].

The murine *SLC5A3* gene localises to the telomeric region of chromosome 16, which is syntenic to human chromosome 21q22. Murine trisomy 16 is lethal in the perinatal period, but Ts65Dn mice which are trisomic only for a segment of chromosome 16, can be used as a model system for Down syndrome. Like their human counterparts, these mice have elevated *myo*-inositol levels in the brain due to the third copy of SMIT1 [58,78].

Down syndrome individuals have a higher risk of developing Alzheimer's disease and display, when suffering from Alzheimer, even higher brain *myo*-inositol concentrations than Down syndrome individuals without Alzheimer's disease. This implicates also a potential role for SMIT1 in Alzheimer's disease [48]. Nevertheless, SMIT1 might also be helpful against Alzheimer. *Scyllo*-inositol is more and more under discussion being a potential drug against senile dementia. In the mouse model it inhibits the aggregation of amyloid β peptides, which is regarded as a key molecule in Alzheimer's disease [57,55,88] and improves learning and memory in senile mice [72]. Its import into brain cells is maintained by SMIT1, and studies showed that *SLC5A3*, encoding SMIT1, is in fact expressed in target regions for medication within the brain, at least in the mouse model [28].

The absence of *SLC5A3* gene product is lethal under normal conditions. Mice lacking SMIT1 die due to respiratory failure shortly after birth. Death can be prevented by addition of *myo*-inositol to the drinking water of mother mice during pregnancy. However, surviving *Slc5a3* knockout mice exhibit *myo*-inositol depletion in brain, kidney, skeletal muscle and liver and in addition show severe abnormalities in peripheral nerves, sciatic nerve and bones [9,15,13,19].

These pleiotropic effects underline the importance of SMIT1 activity, although the mechanism for the impact of altered *SMIT1* expression is still under discussion [13].

It was postulated that *myo*-inositol depletion might lead to altered levels of phosphoinositides, which causes impaired vesicle trafficking and disturbed phosphoinositide-dependent signalling [9,15]. However, phosphatidylinositol, five- and sixfold phosphorylated inositols and the second messenger inositol-1,4,5-trisphosphat concentrations did not vary between *Slc5a* knockout and wild type mice [6,13,19]. Nevertheless, the inositol depletion hypothesis had been well accepted for a long time as an explanation for the way lithium and other mood stabilisers work in treatment of bipolar disorder [38]. Lithium inhibits *myo*-inositol-monophosphatase and therefore recycling of phosphorylated inositols to *myo*-inositol [24]. Also SMIT1 activity is inhibited by lithium, valproate and carbamazepine, but as SMIT1 seems not to be the primary target of these mood stabilisers, the role of inositol transporters in bipolar disorder remains to be elucidated [54,96].

Na^+-coupled inositol transporters were so far only identified and characterised in mammals. In contrast to that, proton coupled inositol transporters can be found among all living organisms.

3. Proton coupled inositol transporters

All proton coupled inositol transporters identified so far belong to the Major Facilitator Superfamily. The nomenclature varies, as there are different abbreviations in use (HMIT, INT, ITR, MIT, MITR), but they all stand for H^+/*myo*-inositol transporter. Table 2 gives an overview on transport characteristics of proton coupled inositol transporters.

3.1. HMIT: a mammalian H^+/inositol transporter

The first mammalian H^+/*myo*-inositol transporters, from human and rat, were identified in 2001 via an EST screen [92]. HMIT1 is encoded by a member of the *SLC2* gene family, *SLC2A13*. *SLC2A13* is predominantly expressed in the brain and its expression is induced under hypertonic conditions [92,42]. The HMIT protein shows the same topology as other SLC2 family members: twelve transmembrane domains, an enlarged loop between the sixth and seventh transmembrane domain and N- and C-terminus located within the cytoplasm [94,62].

Rat HMIT was analysed in *Xenopus* oocytes and shown to be a H^+/*myo*-inositol symporter with a K_m of 100 μM. Competitor experiments were used to identify additional substrates. *Scyllo*-, *chiro*- and *muco*-inositol are also accepted by HMIT, while monosaccharides such as glucose or fructose are not. It was necessary to study a mutated HMIT protein for analysis in oocytes as the native HMIT protein contains a potential endoplasmic reticulum retention signal, as well as a dileucine signal for internalisation within the N-terminus and a C-terminal tyrosine-based internalisation motif. Maximal plasma membrane localisation in oocytes was achieved by mutating all three motifs [92].

The existence of these motifs and the necessity to mutate them for analysis pointed to a rather non-plasma membrane localisation of HMIT in vivo. In fact, its localisation is discussed controversially. Uldry et al. showed that HMIT is present in intracellular vesicles, but can appear on the cell surface in a P12 cell culture. This relocalisation is triggered by cell plasma membrane depolarisation, protein kinase C activation or increased intracellular Ca^{2+} concentrations [93]. In contrast to this, Di Daniel et al. observed HMIT localisation only at the Golgi apparatus and could not detect any HMIT-mediated *myo*-inositol uptake in isolated neurones [21]. On the other hand Fu et al. demonstrated that *myo*-inositol uptake in isolated astrocytes is mediated by HMIT under physiologically relevant *myo*-inositol concentrations which points to at least a partial localisation of HMIT within the plasma membrane [30]. The ambiguous results for subcellular localisation of HMIT might be due to the different cell systems used for analysis.

Independent of its subcellular localisation HMIT could play a role in Parkinson disease as studies revealed that *SLC2A13* seems to be a Parkinson related gene [31,81]. A direct impact of HMIT on Parkinson has so far not been shown.

Table 2
Substrate analysis of proton coupled inositol transporters. Red background: transporters from the animal field; green background: plant transporters; blue background: fungal transporters; yellow background: protozoan transport proteins. (See below-mentioned references for further information.)

Transporter	Origin of gene	Analysed substances	Physiological substrate	K_m values	Additionally transported	K_m values	Analysed in	Reference
HMIT[a)]	*R. norvegicus*	*myo*-inositol, *chiro*-inositol, *scyllo*-inositol, *muco*-inositol, *allo*-inositol, D-glucose, L-glucose, galactose, fructose, mannose, L-fucose, 2-deoxyglucose, glucose-6-phosphate, glucosamine, maltose	*myo*-inositol	100 μM	*scyllo*-inositol > *muco*-inositol > *chiro*-inositol	n. a.	*Xenopus* oocytes	Uldry et al. [92]
MITR1	*M. crystallinum*	*myo*-inositol, D-ononitol	*myo*-inositol	n. a.	D-ononitol	n. a.	*S. cerevisiae* cells	Chauhan et al. [16]
INT2	*A. thaliana*	*myo*-inositol, D-*chiro*-inositol, *scyllo*-inositol, *muco*-inositol, *allo*-inositol, phytic acid, pinitol, galactinol, xylitol, sorbitol, mannitol, glucose, fructose, sucrose	*myo*-inositol	950 μM	*scyllo*-inositol > D-*chiro*-inositol > *muco*-inositol > pinitol > *allo*-inositol, glucose	n. a.	*Xenopus* oocytes	Schneider et al. [84]
INT4	*A. thaliana*	*myo*-inositol, D-*chiro*-inositol, *scyllo*-inositol, *muco*-inositol, *allo*-inositol, pinitol, sorbitol, glucose, fructose, sucrose	*myo*-inositol	240 μM	D-*chiro*-inositol > *scyllo*-inositol > pinitol, *allo*-inositol, *muco*-inositol	n. a.	*Xenopus* oocytes	Schneider et al. [83] Schneider et al. [84]
ITR1	*S. cerevisiae*	*myo*-inositol	*myo*-inositol	100 μM	---	---	*S. cerevisae* cells	Nikawa et al. [67]
ITR2	*S. cerevisiae*	*myo*-inositol	*myo*-inositol	140 μM	---	---	*S. cerevisae* cells	Nikawa et al. [67]
ITR1A	*C. neoformans*	*myo*-inositol	*myo*-inositol	n. a.	---	---	*S. cerevisiae* cells	Wang et al. [98]
ITR3C	*C. neoformans*	*myo*-inositol	*myo*-inositol	n. a.	---	---	*S. cerevisiae* cells	Wang et al. [98]
ITR1	*C. albicans*	*myo*-inositol, L-*chiro*-inositol, D-*chiro*-inositol, *scyllo*-inositol, *muco*-inositol, *allo*-inositol, *neo*-inositol, *epi*-inositol, 3-fluoro-inositol, L-quebrachitol, D-pinitol, viburnitol, D-ononitol, phytic acid, D-glucose, D-galactose, D-fructose, D-mannose, D-fucose, L-fucose, D-ribose, L-ribose, D-arabinose, L-arabinose, D-xylose, L-xylose, D-lyxose , L-lyxose	*myo*-inositol	240 μM	3-fluoro-inositol > viburnitol > D-ononitol, *neo*-inositol > phytic acid > L-quebrachitol > *epi*-inositol, *muco*-inositol	n. a.	*C. albicans* cells	Jin and Seyfang [40]
MIT	*L. donovani*	*myo*-inositol, L-*chiro*-inositol, D-*chiro*-inositol, *scyllo*-inositol, *muco*-inositol, *allo*-inositol, *neo*-inositol, *epi*-inositol, 3-fluoro-inositol, L-quebrachitol, D-pinitol, viburnitol, D-ononitol, phytic acid, D-glucose, D-galactose, D-fructose, D-mannose, D-fucose, L-fucose, D-ribose, L-ribose, D-arabinose, L-arabinose, D-xylose, L-xylose, D-lyxose, L-lyxose	*myo*-inositol	84 μM	3-fluoro-inositol > viburnitol > D-ononitol > phytic acid, *epi*-inositol > L-quebrachitol, *scyllo*-inositol, D-*chiro*-inositol, *neo*-inositol	n. a.	*L. donovani* promastigotes	Mongan et al. [59]
HMIT	*T. brucei*	*myo*-inositol, *scyllo*-inositol, *myo*-inositol-2-phosphate, xylitol, D-sorbitol, D-mannitol, D-glucose, D-mannose	*myo*-inositol	n. a.	*scyllo*-inositol, *myo*-inositol-2-phosphate	n. a.	*T. brucei* procyclic forms	Gonzalez-Salgado et al. [34]

[a] A mutated form of HMIT (two putative internalisation motifs and an ER retention signal were removed) was used to achieve plasma membrane localisation.

3.2. Yeast inositol transporters

Inositol transporters ITR1 and ITR2 from the model organism *Saccharomyces cerevisiae* were identified by homologous complementation of a yeast mutant defective in *myo*-inositol transport. Both proteins are located in the plasma membrane and accept *myo*-inositol with similar affinities (ITR1: K_m = 100 μM, ITR2: K_m = 140 μM). However, mainly ITR1 appears to be responsible for inositol uptake, whereas ITR2 is only of minor importance for yeast cells. This fact is mirrored both by the high transcript abundance of the *ITR1* mRNA and by insufficient inositol transport when only ITR2 is present [67,61]. Transport activity is regulated by an inositol-dependent relocalisation of ITR1. No inositol within the medium led to a high presence of an ITR1-GFP fusion within the plasma membrane, while in an inositol containing medium the GFP fluorescence was redistributed within the yeast cells [61].

Schizosaccharomyces pombe is one of the rare organisms that are inositol auxotroph by nature [99]. Fission yeast therefore depends on inositol uptake from its environment. A *S. pombe* mutant with a defect in its *ITR2* gene is not able to grow on low inositol concentrations and growth, mating and sporulation are affected even on higher inositol concentrations. These effects can be reversed by overexpression of *ITR1*, encoding another putative inositol transporter in fission yeast. ITR1 is not sufficient to complement the loss

of ITR2 under native conditions – a similar situation as in *S. cerevisiae* with one inositol transporter of major and one of minor importance [66].

3.3. Fungal inositol transporters as targets of medical treatment

Candida albicans, normally existing commensally in its human host, can lead to life-threatening systemic bloodstream infections in patients with an impaired immune system. *C. albicans* H^+/inositol transporters aroused attention as potential targets for medical treatment as inositol is an important growth factor for fungi and the *C. albicans* inositol transporters differ kinetically and pharmacologically from the host's Na^+-coupled inositol transporters. However, deletion of the inositol transport system does not alter virulence as the need for inositol can be compensated by inositol biosynthesis. Only double mutations in inositol uptake and biosynthesis were shown to be lethal [40,17].

A different result was obtained for *Cryptococcus neoformans*. *C. neoformans* is the major cause for fungal meningitis in immunosuppressed patients. Compared with most other fungi that have one or two genes encoding inositol transporters, the *C. neoformans* genome contains an unusually large inositol transporter gene family with at least ten members. This large number of ITRs probably mirrors co-evolutionary adaption of *C. neoformans* to its host, as human brain contains inositol in abundance, and to environmental conditions [102]. Two members of the large cryptococcal ITR family, ITR1A and ITR3C, are supposed to be the major inositol transporters within this family [98].

Studies using an *itr1aΔ itr3cΔ* double mutant showed that this mutant has strongly reduced blood–brain-barrier transmission efficiency [51]. Furthermore, mice infected with the mutant *C. neoformans* strain showed a prolonged survival rate compared to those infected with wild type. This is due to a significant reduction in polysaccharide secretion in the *itr1aΔ itr3cΔ* double mutant that probably results in differences in cell surface structure and subsequently in an altered host response [52]. These findings reveal that also cryptococcal inositol transporters are attractive targets of medication.

3.4. Inositol transport in plants

First plant inositol transporters were identified in the common ice plant, *Mesembryanthemum crystallinum*. Yeast complementation tests revealed that MITR1, one of three members of *myo*-inositol transporters from common ice plant, does transport *myo*-inositol, but the transport mechanism was never analysed in detail [16]. It was hypothesised from *MITR* expression data under salt stress, that ice plant MITRs act Na^+-coupled, but this has never been proven [65,16]. Later analysis of the protein structure and the close relationship to other proton coupled inositol transporters suggested that also MITR1, MITR2 and the later discovered MITR3 are proton coupled inositol symporters [83].

The best characterised plant inositol transporters to date are those from the model plant *Arabidopsis thaliana*. The *AtINT* family consists of four members, from which only three genes, *AtINT1*, *AtINT2* and *AtINT4*, encode a functional protein. *AtINT3* was shown to be a pseudogene [84]. AtINT2 and AtINT4 are plasma membrane localised in planta and their transport characteristics were analysed in *Xenopus* oocytes. Both were shown to be H^+/inositol symporters with AtINT2 transporting *myo*-inositol > *scyllo*-inositol > *chiro*-inositol and AtINT4 prefering *chiro*-inositol > *myo*-inositol ≫ *scyllo*-inositol. K_m values for *myo*-inositol were determined and identified AtINT2 as the transport protein with lower affinity for *myo*-inositol (K_m = 0.95 mM) compared to AtINT4 (K_m = 0.24 mM) [83,84].

AtINT1 is a tonoplast localised transporter and patch clamp experiments on isolated wild type and *Atint1* knockout vacuoles revealed that AtINT1 is the only transporter releasing *myo*-inositol out of the vacuolar lumen into the cytosol. A transporter that imports inositol into the vacuole is not known so far. However, the inositol derivative phytate (inositol hexa*kis*phosphate) is transported either directly into the vacuole by AtMRP5, an ABC-transporter [64], or from the lumen of the endoplasmic reticulum via vesicle transport [35,68]. Furthermore, membrane vesicles are sequestered into the vacuole during autophagic processes and subsequently degraded [50]. Hence, vacuolar inositol probably derives from the disassembly of phytate and/or inositol containing membrane compounds [82].

While a knockout of the plasma membrane transporters AtINT2 and AtINT4 does not lead to phenotypic alterations compared to wild type, *Atint1* knockout plants show shorter roots. This can be complemented by adding *myo*-inositol to the growth medium. AtINT1 is important for maintaining the intracellular inositol distribution and affects seedling development [82]. A C-terminal acidic dileucine motif is responsible for routing AtINT1 to the tonoplast [100] (Fig. 4). The targeting of AtINT1 is mediated by adaptor protein complex AP-1 [97].

AtINT1 on the one and AtINT2 and AtINT4 on the other hand have different subcellular localisations that are also mirrored in their protein structure. AtINT2 and AtINT4 possess an enlarged extracellular loop between the ninth and tenth transmembrane domain that is missing in AtINT1 (Fig. 4). This loop contains eight conserved cysteins that form a so-called PSI (Plexin/Semaphorin/Integrin) domain [84,22]. A specific role in intercellular signalling is suggested as this PSI domain can be found in all plasma membrane H^+/inositol transporters from animals and plants analysed so far but is absent in fungal and endomembrane inositol transporters [22].

In this context the subcellular localisation of MITR1 and MITR2 from *M. crystallinum* is again in focus. Immunolocalisation in isolated vesicles with antibodies against MITR1 and MITR2 suggested a subcellular localisation in the tonoplast, but also signals within the plasma membrane fraction were found [16]. In protein sequence alignments, MITR1 and MITR2 cluster together with plasma membrane located INTs (Fig. 5), while a third inositol transporter from *Mesembryanthemum*, MITR3, is found within the clade containing AtINT1 and another recently identified tonoplastic inositol transporter, AcINT1, from *Ananas comosus* [3].

The MITR signal in the vacuolar membrane fraction might therefore derive from the later identified MITR3, while MITR1 and MITR2 are likely to be plasma membrane proteins [3].

Another recently identified plant plasma membrane inositol transporter is MfINT-like from *Medicago falcata*. MfINT-like seems to play an important role in adaptation to abiotic stress. *MfINT-like* expression is induced by cold and salt stress in *Medicago* and tobacco plants overexpressing *MfINT-like* are more tolerant to freezing temperatures and can develop more fresh weight when grown under drought or salt stress [77].

Many more genes coding for putative inositol transporters have been identified in a variety of plant species (Rice: [41]; Vine: [2]; Selaginella: [47]; Tomato: [73]) and transport characteristics as well as physiological roles of the encoded proteins are waiting to be discovered.

3.5. Inositol transporters of protozoan pathogens

Leishmaniasis is a collective term for different tropical diseases all caused by different *Leishmania* species. Drug treatment is often toxic for the human host and more and more resistances occur [105].

LdMIT, the only *Leishmania donovani myo*-inositol transporter, transports *myo*-inositol and the cytotoxic inositol analogue 3-fluoro-*myo*-inositol with similar affinities (K_m value for *myo*-

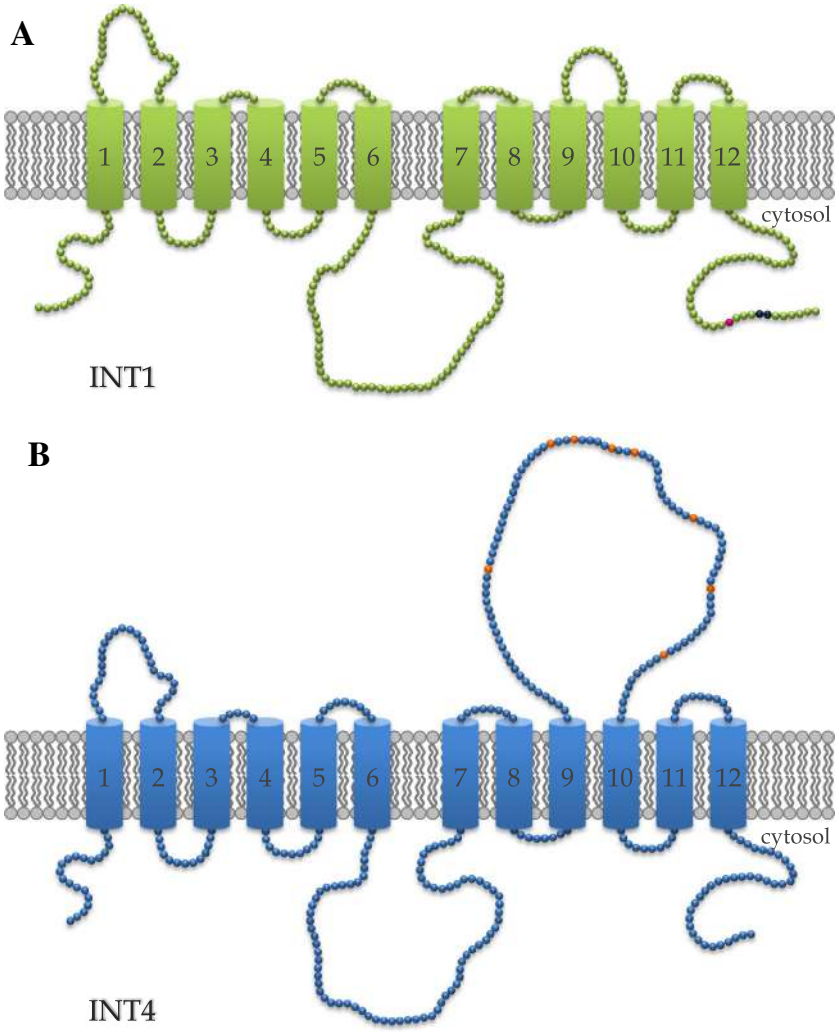

Fig. 4. Models for plant inositol transporters, based on *Arabidopsis thaliana* AtINT1 and AtINT4. (A) Model for INT1-type proteins. Red and blue coloured amino acids highlight the C-terminal [D/E]XXXL[L/I]-like acidic dileucine motif. (B) Model for INT4-type proteins. Conserved cysteins within the enlarged extracellular loop that are responsible for formation of the PSI domain are highlighted in orange. Protein structures modified after Dotzauer et al. [22].

inositol = 84 μM). Significant differences in substrate recognition and binding compared to the inositol transport system of the human host offer the chance to use the leishmanian inositol transporter as a potential gate for specific drug delivery into the pathogen [59].

In *Trypanosoma brucei*, a parasite that causes African sleeping sickness, a H^+/inositol transporter, TbHMIT, was identified and shown to be essential for the pathogen's survival in culture. RNAi silencing of *TbHMIT* led to massive reduction of *myo*-inositol import, which in turn resulted in depletion of bulk inositol lipids and death of the parasite. Since *myo*-inositol uptake is essential for *T. brucei*, TbHMIT is a promising drug target [34].

Two transporter systems for inositol were proposed for *Trypanosoma cruzi*, causative agent of Chagas' disease: one that is active at low inositol concentrations and H^+-coupled, and one that is active at higher inositol concentrations and Na^+-coupled [25]. So far, none of the proposed transport proteins was characterised on molecular level, and only one candidate gene was found in the *T. cruzi* genome [26]. From structural similarities to other H^+-coupled inositol transporters it seems likely that this gene is the candidate for a H^+-coupled transporter [34] (Fig. 5).

Search via BLAST analysis resulted in no hits for a potential *myo*-inositol-phosphate-synthase gene in the pathogens *Toxoplasma gondii* and *Cryptosporidium parvum*. Potential *myo*-inositol-phosphate-synthase genes might be too different from already identified genes to be found via BLAST, but if in fact there is no inositol biosynthesis in these pathogens, they must rely on inositol transporters as uptake from the environment is the only source for inositol. Therefore, ITRs in *T. gondii* and *C. parvum* offer good targets for medication [74].

4. Conclusions

Inositol transporters play an essential role in the uptake and intracellular distribution of inositol, an important key molecule in many metabolic pathways. Na^+/inositol transporters were so far only identified in animals, while H^+/inositol symporters were found in all eukaryotic kingdoms. Human inositol transporters, the Na^+-coupled SMITs as well as the H^+-dependent HMIT, are of medical interest due to their role in numerous diseases. Therefore, it is not surprising that these transporters and their model counterparts from the animal field have been studied extensively investigating their kinetic functions and physiological role.

More than 27 million people worldwide are affected by diseases caused by trypanosomatid parasites, mainly Leishmania or

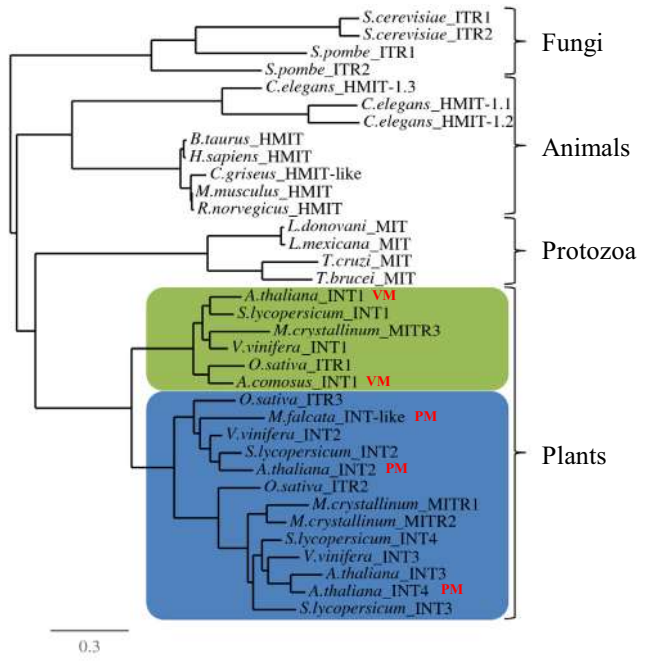

Fig. 5. Family tree of eukaryotic H^+/inositol transporters. Green background: plant transporters without PSI domain. Blue background: plant transporters containing a potential PSI domain. Subcellular localisation of plant inositol transporters analysed using GFP fusion indicated in red letters. VM = vacuolar membrane; PM = plasma membrane. Family tree was created with "one click" mode using Phylogeny.fr [20]; branch length is proportional to the number of substitutions per site. Protein accessions: *Ananas comosus* INT1: ABO21769; *Arabidopsis thaliana* INT1: CAJ00303, INT2: CAJ00304, INT3: CAJ00305, INT4: CAJ00306; *Bos taurus* HMIT: NP_001179892; *Caenorhabditis elegans* HMIT-1.1: CAA16400, HMIT-1.2: CAA16405, HMIT-1.3: CAA86519; *Cricetulus griseus* HMIT-like: ERE84815; *Homo sapiens* HMIT: Q96QE2; *Leishmania donovani* MIT: XP_003861159; *Leishmania mexicana* MIT: XP_003875880; *Medicago falcata* INT-like: AHF50208; *Mesembryanthemum crystallinum* MITR1: AAF91431, MITR2: AAF91432, MITR3: AAO74897; *Mus musculus* HMIT: Q3UHK1; *Oryza sativa* ITR1: CAD41357, ITR2: NP_001053292, ITR3: BAC79509; *Rattus norvegicus* HMIT: Q921A2; *Saccharomyces cerevisiae* ITR1: P30605, ITR2: P30606; *Schizosaccharomyces pombe* ITR1: Q10286, ITR2: P87110; *Solanum lycopersicum* INT1: K4C961, INT2: K4CKI1, INT3: K4DHS8, INT4: K4D649; *Trypanosoma brucei* MIT: Q385N3; *Trypanosoma cruzi* MIT: XP_814419; *Vitis vinifera* INT1: ADP37179, INT2: ADP37180, INT3: ADP37181.

Trypanosoma species [89]. Protozoan inositol transporters are potential targets for medication.

Analysis of plant inositol transporters indicated a new strategy for plants to adapt to abiotic stress. Furthermore, inositol transport proteins are a suitable tool to study targeting mechanisms of membrane proteins.

In the future it is undisputable that research on inositol transporters will bring further important insights in cell physiology in eukaryotic organisms.

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REVISIÓN:

Potential role and therapeutic interests of myo-inositol in metabolic diseases

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Review

Potential role and therapeutic interests of *myo*-inositol in metabolic diseases

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ABSTRACT

Several inositol isomers and in particular *myo*-inositol (MI) and D-*chiro*-inositol (DCI), were shown to possess insulin-mimetic properties and to be efficient in lowering post-prandial blood glucose. In addition, abnormalities in inositol metabolism are associated with insulin resistance and with long term microvascular complications of diabetes, supporting a role of inositol or its derivatives in glucose metabolism. The aim of this review is to focus on the potential benefits of a dietary supplement of *myo*-inositol, by far the most common inositol isomer in foodstuffs, in human disorders associated with insulin resistance (polycystic ovary syndrome, gestational diabetes mellitus or metabolic syndrome) or in prevention or treatment of some diabetic complications (neuropathy, nephropathy, cataract). The relevance of such a nutritional strategy will be discussed for each context on the basis of the clinical and/or animal studies. The dietary sources of *myo*-inositol and its metabolism from its dietary uptake to its renal excretion will be also covered in this review. Finally, the actual insights into inositol insulin-sensitizing effects will be addressed and in particular the possible role of inositol glycans as insulin second messengers.

1. Introduction

myo-Inositol is a cyclitol naturally present in animal and plant cells, either in its free form or as a bound-component of phospholipids or inositol phosphate derivatives. It plays an important role in various cellular processes, as the structural basis for secondary messengers in eukaryotic cells, and in particular as inositol triphosphates (IP_3), phosphatidylinositol phosphate lipids (PIP_2/PIP_3) and possibly inositol glycans. For this reason, *myo*-inositol is essential or important for the smooth running of a wide range of cell functions, including cell growth and survival [1], development and function of peripheral nerves [2], osteogenesis [3] and reproduction [4–9] (See Fig. 1). *myo*-Inositol and D-*chiro*-inositol, another inositol isomer, could be also implicated in glucose homeostasis since abnormalities in their metabolism were associated to insulin-resistance and long-term diabetes microvascular complications in diabetic subjects. Furthermore, given as dietary supplements, both *myo*- and D-*chiro*-inositol showed insulin-mimetic

Abbreviations: AC, adenylyl cyclase; ACC, acetyl-coenzyme A carboxylase; AGEs, advanced glycation end products; AMP, adenosine monophosphate; cAMP, cyclic AMP; AMPK, 5′ AMP-activated protein kinase; AUC, area under the curve; BMI, Body Mass Index; CDP-DAG, cytidine diphosphate-diacylglycerol; DCI, D-chiro-inositol; ECM, extracellular matrix; FA, folic acid; FSH, follicle-stimulating hormone; GFR, glomerular filtration rate; GK, Goto Kakizaki (rat); GLUT-4, glucose transporter 4; GMD, gestational diabetes mellitus; G3PAT, glycerol-3-phosphate acyltransferase; GPI, glycosyl phosphatidylinositol; GS, glycogen synthase; GSK3, glycogen synthase kinase 3; HDL, high density lipoprotein; HK, hexokinase; HMIT, H^+/*myo*-inositol transporter; HOMA-IR, homeostasis model assessment of insulin resistance; IMPase, inositol monophosphatase; INS-2, insulin second messenger with a 4-*O*-(2-amino-2-deoxy-beta-D-galactopyranosyl)-3-*O*-methyl-D-chiro-inositol structure; IPs, inositol phosphates (including in particular: Ins-P: inositol monophosphate, IP3, inositol triphosphates, IP6, inositol hexakisphosphates or phytic acid); IPG, inositol phosphoglycan; IR, insulin receptor; IRS, insulin receptor substrate(s); LD50, median lethal dose; LDL, low density lipoprotein; LH, luteinizing hormone; LysoPI, lysophosphatidylinositol; MetS, metabolic syndrome; MI, *myo*-inositol; MIPS, 1-D-*myo*-inositol-phosphate synthase; MIOX, *myo*-inositol oxygenase; MNCV, motor nerve conduction velocity; mTOR, mammalian target of rapamycin; OGTT, oral glucose tolerance test; PCOS, polycystic ovary syndrome; PDH, pyruvate dehydrogenase; PDHP, pyruvate dehydrogenase phosphatase; PDK, phosphoinositide-dependent kinase; PI, phosphatidylinositol; PI3K, phosphatidylinositol-3-kinase(s); PIPs, phosphatidylinositol phosphate lipids (including PIP_2, phosphatidylinositol 4,5-bisphosphate and PIP_3, phosphatidylinositol (3,4,5)-trisphosphate); PKA, cyclic AMP-dependent protein kinase; PKB, Protein Kinase B; PKC, protein kinase C; PLC, phospholipase C; PLD, phospholipase D; PP2Cα, phosphoprotein phosphatase 2C alpha; PP-InsPs, pyrophosphate forms of inositol phosphates; RCT, randomized controlled trial; SHR, spontaneously hypertensive rat; SHBG, Sex Hormon Binding Globulin; SMIT1/2, sodium-dependant *myo*-inositol transporter 1/2; STZ, streptozotocin.

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effects in several animal models of insulin resistance [10–12] and in women with polycystic ovary syndrome [13], a metabolic and endocrine disorder associated with insulin resistance.

The aim of this review is to compile and discuss the results of the randomized controlled trials that tested the potential benefit of a dietary *myo*-inositol supplement in contexts of insulin-resistance or long-term diabetic complications. As an introduction and to further discuss the therapeutic interest of a *myo*-inositol supplement in those contexts, the dietary sources of *myo*-inositol, its metabolism from its oral intake to its catabolism by the kidney, and the abnormalities in inositol metabolism associated with insulin-resistance will be addressed. Finally, the putative and actually unclearly defined mechanisms of action of inositol derivatives as insulin sensitizers will be discussed on the basis of animal and clinical studies.

2. Biological forms and dietary sources

Inositol or cyclohexane-1,2,3,4,5,6-hexol is a polyol existing under nine stereoisomeric forms depending on the spatial orientation of its six hydroxyl groups (Fig. 2). *myo*-Inositol, or *cis*-1,2,3,5-*trans*-4,6-cyclohexanehexol, is the predominant isomeric form of inositol that we can find in nature and in our food. *myo*-Inositol was once considered to belong to the vitamin B family, however, because it is produced in sufficient amount by the human body from D-glucose, it is no more regarded as an essential nutrient. Human diet from animal and plant sources can contain *myo*-inositol in its free form, as inositol-containing phospholipid (phosphoinositides) or as phytic acid (inositol hexaphosphate or IP_6) [14]. Indeed, all living cells (animal, plant, bacteria, fungi) contain inositol phospholipids in their membranes, and phytic acid is the principal storage form of phosphorus in many plant tissues, especially bran and seed. Hence, the greatest amounts of *myo*-inositol in common foods are found in fresh fruits and vegetables, and in all foods containing seeds (beans, grains and nuts). Especially high phytic acid contents are found in almonds, walnuts and Brazil nuts (9.4, 6.7 and 6.3% of dry weight, respectively) [15] and oats and bran contain more *myo*-inositol than cereals derived from other grains. Among the vegetables, the highest contents are observed in the beans and peas, leafy vegetables being the poorest vegetable sources. Among the fruits, cantaloupe and citrus fruits (with the exception of lemons) have extraordinarily high contents of *myo*-inositol: for example, a portion of grapefruit juice (120 g) contains about 470 mg of *myo*-inositol [16]. The amount of

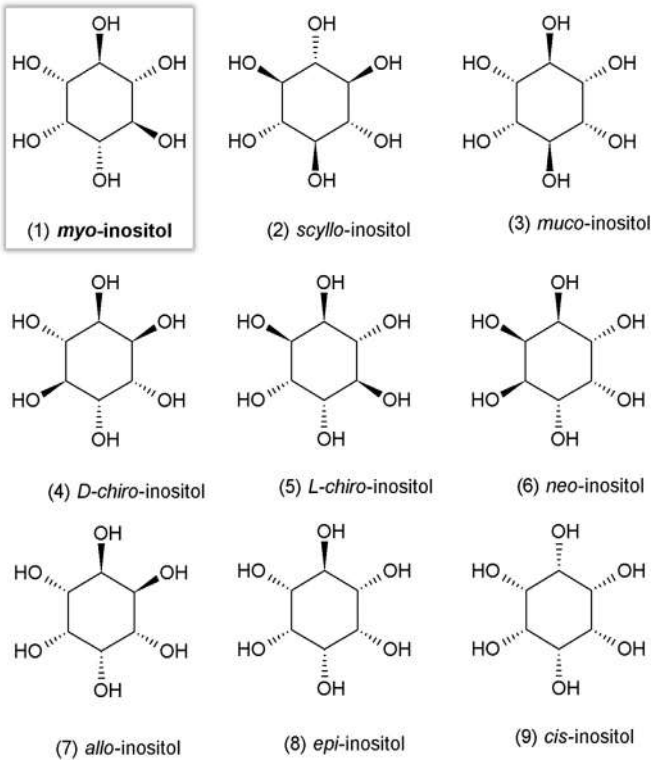

Fig. 2. Structures of the nine stereoisomers of inositol. Inositol exists under 9 stereoisomeric forms through epimerization of its hydroxyl groups. *myo*-Inositol (framed) is the most common isomer of inositol in foodstuffs and animal tissues.

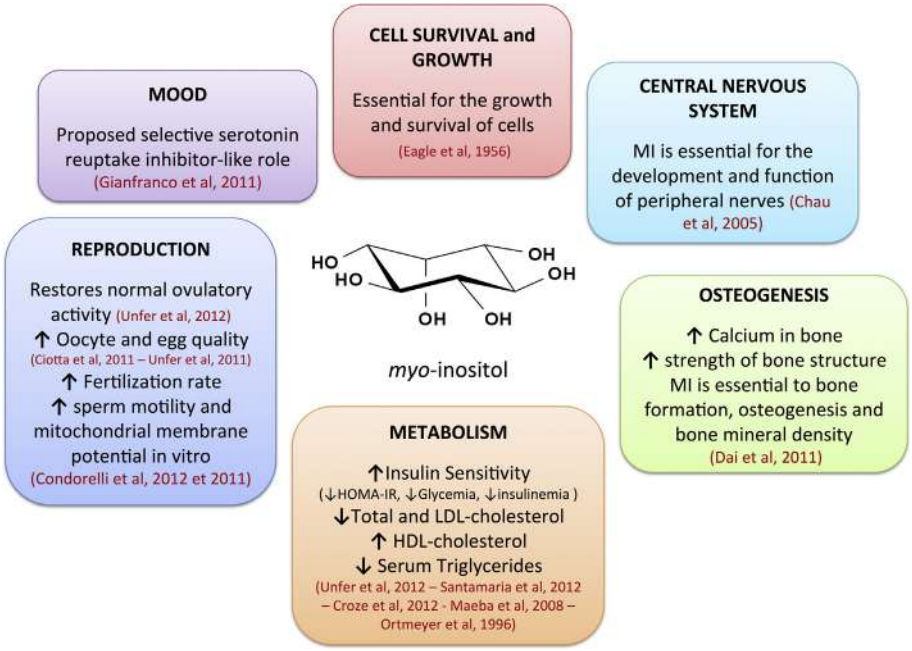

Fig. 1. Functions and benefits of a myo-inositol diet supplement for human health.

myo-inositol present in the 2500 kcal American diet is approximately 900 mg, of which 56% is lipid-bound. However, the *myo*-inositol intake provided by common foods can range from 225 to 1500 mg/day per 1800 kcal depending on the composition of the diet [16].

3. Dietary MI uptake and metabolism

3.1. Digestion and absorption

myo-Inositol from phytic acid can be released in the gut of monogastric animals by the enzymes phytases, which occurs in the intestinal mucosa. Phytases (*myo*-inositol hexaphosphate phosphohydrolase, EC 3.1.3.8 and EC 3.1.3.26) are found in plants, microorganisms and in animal tissues [15]. These enzymes are capable of releasing free inositol, orthophosphate, and intermediary products including the mono-, di-, tri-, tetra- and penta-phosphate forms of inositol. Much of the ingested inositol hexaphosphate is hydrolyzed to inositol. A considerable fraction of the ingested *myo*-inositol is consumed in the form of phosphatidylinositol (PI) that may be hydrolyzed by a pancreatic phospholipase A in the intestinal lumen. The resulting lyso-phosphatidylinositol (lysoPI) may be then reacylated via acyltransferase activity upon entering the intestinal cell or further hydrolyzed with the release of glycerylphosphorylinositol [14].

Virtually all of the free *myo*-inositol ingested (99.8%) is absorbed from the human gastrointestinal tract, through an active transport system involving a Na^+/K^+-ATPase [14]. In normal and healthy subjects, the circulating fasting plasma *myo*-inositol concentration has been found to be approximately 30 μM and it turns over with a half-life of 22 min Refs. [14,16]. *myo*-Inositol is also present in small but significant amounts in phospholipids in association with the circulating serum lipoproteins, and as phytic acid at a level of about 0.1–0.4 μM.

3.2. Organ and tissue incorporation

Lewin et al. [17] followed the distribution of radiolabeled *myo*-inositol after intraperitoneal injection in male rats. Radiolabeled *myo*-inositol accumulated rapidly (within 1 h) and in large amounts in the thyroid, coagulating gland and seminal vesicles. Other tissues, such as the pituitary, prostate gland, liver and spleen, also concentrated *myo*-inositol quite actively. Of note, all the organs of the male reproductive tract (the vas deferens, epididymis, coagulating gland, seminal vesicle and prostate) except testis had radioactivity levels that were approximately 10–30 fold those of blood serum. The muscle tissues studied (diaphragm and heart) concentrated little inositol and adipose tissue (epididymal fat pad) was apparently unable of concentrating it from the blood, as well as the brain and testis which are, however, organs with high levels of endogenous inositol. Most of the radioactivity was found in the aqueous trichloroacetic acid extract, largely as free *myo*-inositol in most organs, with the exception of the liver where the lipid fraction contained most (approximatively 60%) of the radio-labeled *myo*-inositol accumulated.

Lewin et al. also reported that in bilaterally nephrectomized rats, *myo*-inositol catabolism did not occur since the sole pathway of inositol catabolism in the rat takes place in the kidney. As expected, nephrectomized rats were essentially unable to convert inositol into CO_2, whereas the sham-operated rats catabolized about 16% of the injected inositol to CO_2. The nephrectomized rats accumulated more radioactivity in most of the organs tested, presumably because significant amounts of the administered inositol are normally metabolized or excreted by the kidney. An interesting exception to the rule was the brain, which accumulated more radioactivity in sham-operated than in nephrectomized animals. This may be due to the presence of metabolites of inositol, produced by the kidney, which are more prone to cross the blood/brain barrier than is inositol [14].

3.3. Cellular uptake

Cells normally derive inositol from three sources: (1) *de novo* biosynthesis from glucose-6-phosphate by 1-D-*myo*-inositol-phosphate synthase (MIPS) and inositol monophosphatase (IMPase), (2) dephosphorylation of inositol phosphates derived from breakdown of inositol-containing membrane phospholipids; or (3) uptake from the extracellular fluid via specialized *myo*-inositol transporters [18].

Inositol can be transported from extracellular fluid via three specialized *myo*-inositol transporters: sodium-dependent *myo*-inositol transporters 1 and 2 (SMIT1/2), and H^+-*myo*-inositol transporter HMIT, that co-transports *myo*-inositol with H^+ [19]. SMIT1 and SMIT2, co-transport two sodium ions along the concentration gradient, to generate enough energy to actively transport *myo*-inositol. SMIT1 and SMIT2 are both expressed in the brain and may be responsible for regulating brain *myo*-inositol level that is about 100-fold greater than those found in the periphery. Active *myo*-inositol transport through SMIT2 also mediates *myo*-inositol uptake in apical membrane of rat small intestine (although SMIT1 is present) and is responsible for *myo*-inositol reabsorption in rabbit kidney [20,21]. This active transport is inhibited by D-glucose and phlorizin and accounts for the inosituria occurring in diabetes mellitus. Of note, SMIT2 (but not SMIT1) also transports D-*chiro*-inositol.

3.4. Metabolism

3.4.1. MI de novo biosynthesis

myo-Inositol can be synthesized endogenously from D-glucose in rat testis, brain, kidney and liver [22,23] in three steps: first glucose is phosphorylated by hexokinase, second, glucose-6-phosphate is converted to *myo*-inositol-1-phosphate by MIPS, and finally, *myo*-inositol-1-phosphate is dephosphorylated by IMPase to produce free MI (See Fig. 3). The second step is the rate limiting step of MI biosynthesis in most organisms [24]. In human, this endogenous biosynthesis of inositol is rather important in the kidney since it produces about 2 g/day so the endogenous daily production is about 4 g in the binephric human, which is significantly above the daily dietary intake (about 1 g/day). Extrarenal tissues can also contribute to the endogenous production of inositol in human and animals. Indeed, one half of the free inositol content of the rabbit brain comes from endogenous production *in situ*, the other half being transported from the blood.

3.4.2. MI conversion to isomers and derivatives or incorporation into phospholipids

myo-Inositol can lead to numerous derivatives through either epimerization, phosphorylation or methylation of one or several of its hydroxyl groups. Nonetheless, several of these derivatives cannot be obtained from *myo*-inositol in animal cells. For example, the inositol isomers *allo*-, *cis*-, and *epi*- are synthetically prepared compounds. Methylated inositol derivatives such as D-pinitol, sequoyitol or quebrachitol can be found in some plant species but these compounds are unlikely produced from *myo*-inositol in human body. In cells, *myo*-inositol exists under many phosphorylated forms from monophosphorylated forms (Ins-1-P, Ins-3-P or Ins-4-P) to the hexaphosphorylated form (IP_6 or phytic acid) and even to pyro-phosphate forms (PP-$InsP_4$, PP-$InsP_5$, $[PP]_2$-$InsP_3$ or $[PP]_2$-$InsP_4$). Even so, the mono-, di- and tri-phosphorylated forms

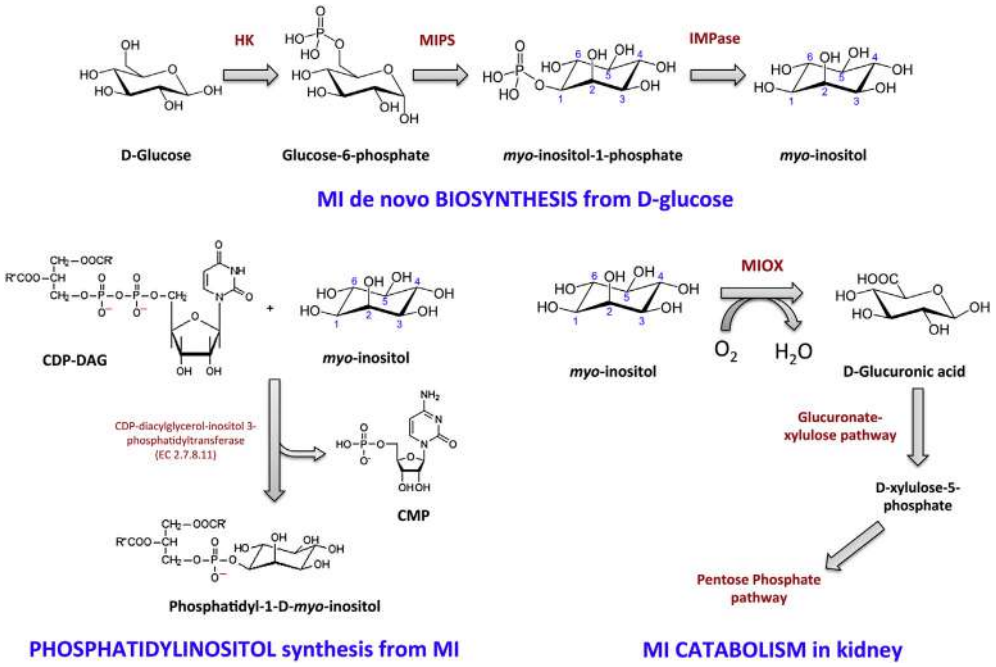

Fig. 3. *myo*-Inositol de novo biosynthesis, incorporation into phospholipids and catabolism. Abbreviations: HK, hexokinase; MIPS, 1-D-*myo*-inositol-phosphate synthase; IMPase, inositol monophosphatase; CDP-DAG, cytidin diphosphate-diacylglycerol; CMP, cytidin MonoPhosphate; MIOX, *myo*-inositol oxygenase.

cannot come directly from the phosphorylation of *myo*-inositol by kinases, since such enzymes do not exist in human cells, but they can come from the dephosphorylation of more phosphorylated forms by specific phosphatases, and/or from phosphoinositide hydrolysis (i.e. inositol-1,4,5-triphosphate comes from the hydrolysis of phosphatidyl-inositol-(4,5)-biphosphate by Phospholipase C) (see Fig. 4).

The naturally occurring inositol isomers are *myo*-, *chiro*- (L-*chiro* and D-*chiro*), *scyllo*-, *muco*- and *neo*-. *In vivo* conversion of *myo*-inositol to D-*chiro*-inositol can occur in tissues expressing the specific epimerase. Pak et al. measured a conversion rate of radiolabeled [^{3}H]-*myo*-inositol to [^{3}H]-D-*chiro*-inositol of about 7.6% in rat blood and 8.8% in rat muscle and liver [25]. An epimerase interconverts *myo*- and *scyllo*-inositol with simultaneous production of *neo*-inositol in bovine brain [26]. However, in the study of Pak et al., labeling of inositol isomers other than D-*chiro*, namely *scyllo*-, *neo*- and *muco*-inositol, was minimal, approximately 0.06% of radiolabeled *myo*-inositol [25].

Finally, only a small amount of *myo*-inositol or none is converted to other isomers or methylated derivatives in mammalian tissues and cells and it is primarily found as free *myo*-inositol or bound covalently to phospholipids, as the structural basis for a number of secondary messengers, including inositol triphosphates (IP_3), phosphatidylinositol (PI) and polyphosphoinositides (i.e. PI(4)P, PI(4,5)P_2 and PI(3,4,5)P_3, in much lower concentrations than phosphatidylinositol). Phosphatidylinositols (PI) are synthesized *in vivo* from *myo*-inositol and cytidine diphosphate-diacylglycerol (CDP-DAG) (Fig. 3). This synthesis is catalyzed by phosphatidylinositol synthase with a relatively high K_m (1.5–2.5 mM) [27,28] for *myo*-inositol making intracellular MI homeostasis potentially important to numerous cell functions. Phosphatidylinositol phosphate lipids (PIPs) are a product of class I, II and III phosphoinositide 3-kinases (PI 3-kinases) acting on phosphatidylinositol. As explained above, many inositol phosphates are produced through the hydrolysis of phosphatidylinositol phosphates by phospholipase C and may also be synthesized or remodeled by many kinases and phosphatases (See Fig. 4).

myo-Inositol and D-*chiro*-inositol can also be bound components of glycosyl-phosphatidylinositol (GPI) anchors and of inositol phosphoglycans (IPGs) that would constitute second messengers of insulin action in the GPI/IPG pathway. See Section 5.3 of this review for further details on this putative secondary signaling pathway of insulin.

3.4.3. MI catabolism

The kidney is the sole organ of importance in the catabolism of *myo*-inositol since [2-^{14}C]-inositol was not degraded to $^{14}CO_2$ in nephrectomized rats in contrast with sham operated rats [17,29]. *myo*-Inositol is catabolized to D-glucuronic acid by *myo*-inositol oxygenase (MIOX) exclusively in the kidney. Through subsequent metabolic steps, D-glucuronic acid can lead to D-xylulose-5-phosphate which can enter the pentose phosphate cycle (See Fig. 3). In human subjects, urinary excretion accounts for a small fraction of the disposal of inositol by the kidney. Therefore, the kidney appears to be an important regulator of plasma inositol concentration in human subjects.

3.5. Tolerance

myo-Inositol supplementation is well tolerated and relatively safe since *myo*-inositol LD50 in mouse is 10 000 mg/kg body weight when orally administered [30]. In human, *myo*-inositol in a daily dose up to 18 g per os for 3 months or 2 g/day for 1 year is safe and well tolerated. Side effects, when present, are mild and mainly gastrointestinal in nature (nausea, flatus and diarrhea) [31,32].

4. Inositol metabolism abnormalities associated with insulin resistance

MI and DCI are involved in an array of cellular functions and abnormalities in their metabolism have been involved in the development of several diseases states (e.g. Bipolar, Panic and Obsessive Compulsive Disorders, Depression, Alzheimer's Disease) and in particular in the development of insulin resistance and

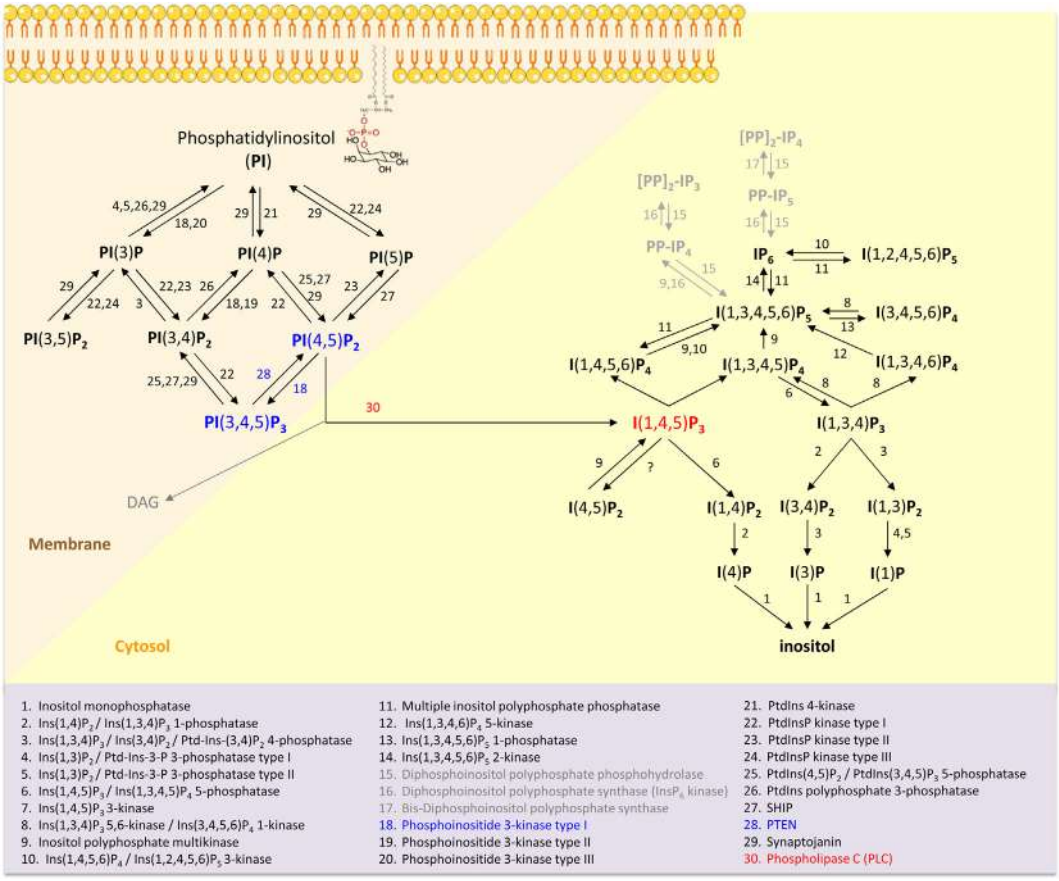

Fig. 4. Phosphoinositides metabolism in eukaryotic cells. Figure adapted from K. Abel, et al., J. Cell Sci. 114 (2001) 2207–2208.

diabetic complications. Indeed, in primary sites for the development of diabetic microvascular complications (kidney, sciatic nerve, retina and lens) a concomitant depletion of intracellular *myo*-inositol and accumulation of intracellular sorbitol is commonly observed in diabetic animal models and human subjects [33,34]. In addition to this tissue-specific *myo*-inositol depletion, type 2 diabetic human subjects [35] and experimental models (rhesus monkeys [35], Goto Kakizaki (GK) rat [36]) excrete excessive amounts of MI and decreased amounts of DCI in urine (a phenomenon called inosituria). This urinary excretion pattern leads to a decrease in DCI to MI urinary ratio. The same inositol abnormal pattern is observed in insulin sensitive tissues (liver, muscle, fat and kidney) of human [37] and animal [36] diabetic subjects.

4.1. Intracellular MI depletion

4.1.1. Putative mechanisms of MI intracellular depletion

MI intracellular concentration is regulated through processes such as extracellular MI uptake, *de novo* biosynthesis, regeneration (phosphoinositide cycle), efflux and degradation (See Fig. 5). Alteration of one or several of these processes can lead to inositol intracellular abnormalities. In diabetes mellitus, inhibition of cellular MI uptake, altered MI biosynthesis, enhanced MI efflux due to sorbitol intracellular accumulation and increased MI degradation are putative mechanisms of MI intracellular depletion [34].

Indeed, a reduction of MI uptake was observed in cells (aorta, nerve cells and brush border vesicles) cultured in medium containing ambient glucose [38–40]. This glucose-induced MI uptake inhibition results from a competition between MI and glucose for MI transporters since MI and glucose exhibit structural similarities [41]. Therefore, under hyperglycemic conditions, high glucose ambience could impair extracellular MI uptake and so contribute to the MI intra-tissular depletion observed in diabetes. However, hyperglycemia *per se* is not sufficient to explain this intra-tissular MI depletion since the use of aldose reductase inhibitors (which selectively inhibit the conversion of glucose to sorbitol) corrected sorbitol intracellular accumulation and concomitantly inositol intracellular depletion, without affecting hyperglycemia [34].

In tissues possessing osmolyte efflux systems such as neuronal tissues, a rapid intracellular sorbitol accumulation can result in an osmotic stress that may favor the net efflux of osmolytes such as MI through the volume-sensitive organic osmolyte anion channels and thus reduce intracellular MI levels [34]. This sorbitol-induced osmotic stress was important in diabetic lens but may not mediate MI depletion in other tissues [42,43].

In the testes of diabetic animals, a significant reduction (50%) in the activity of MIPS, the enzyme regulating the first and critical step of MI biosynthesis, was observed [44]. However, no changes in MIPS activity were observed in the other organs (kidney, brain, and nerves) and even if the rate of MI biosynthesis is intrinsically greater in testes, the contribution of this MIPS activity reduction in MI intracellular depletion remains unclear [34].

Finally, an up-regulation of MIOX, the enzyme that breaks down MI, was observed at both mRNA and protein levels in the kidney of animal models of diabetes (STZ-diabetic rat [34], db/db mice [45]), insulin resistance (high fat diet-induced insulin resistant C57BL6 mice [34]) or hypertension (SHR rat). In all cases (normoglycemic hypertensive rat, insulin resistant mice or hyperglycemic STZ-rat), MIOX up-regulation was associated with an intra-renal MI deficiency [34]. These findings suggest that MI depletion in the kidney

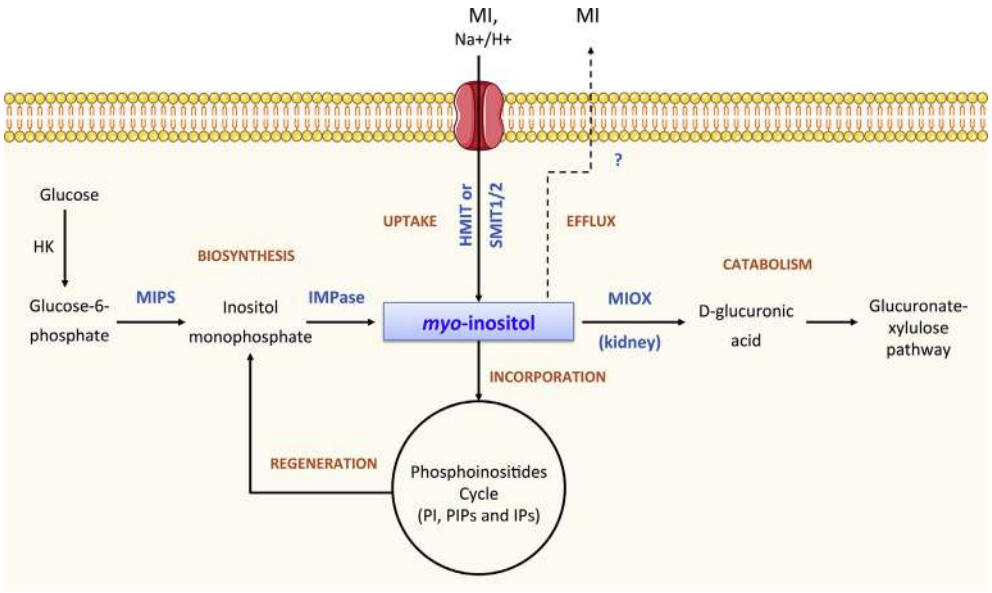

Fig. 5. *myo*-Inositol intracellular level regulation. *myo*-Inositol intracellular level depends on its extracellular uptake through specific transporters (HMIT/SMIT1/SMIT2), its *de novo* biosynthesis from glucose (HK, MIPS and IMPase), its regeneration or entry into the phosphoinositide cycle, its efflux and/or its catabolism by MIOX (in kidney). Figure adapted from H.H.G. Chang PhD Thesis, 2011. Abbreviations: HK, hexokinase, HMIT, H^+/*myo*-inositol transporter, IMPase, inositol monophosphatase, MIOX, myo-inositol oxygenase MIPS, 1-D-*myo*-inositol-phosphate synthase, SMIT1/2, sodium-dependant *myo*-inositol transporter 1/2.

is not directly attributable to hyperglycaemia *per se* and may instead reflect in one aspect the up-regulation of the glucuronate-xylulose pathway as indicated by the elevated MIOX expression and activity. In addition, the activation of MIOX and its subsequent glucuronate-xylulose pathway have been implicated in the development of diabetic nephropathy through the activation of fibronectin [46].

4.1.2. Consequences of MI depletion: possible role in diabetic microvascular complications

As explained above, inositol is involved in many cell functions, especially as a precursor of phophatidylinositol and phosphoinositides. Since the K_m for the biosynthesis of PI from MI is relatively high (i.e. in the millimolar range), a depletion of intracellular *myo*-inositol could have a negative impact on the synthesis and availability of PI and PIPs in cells. Indeed, altered PI metabolism associated to MI deficiency has been observed in the sciatic nerve of streptozotocin-diabetic rat model [47]. Since altered PI turnover is associated with impaired Na^+/K^+-ATPase activity, abnormal Na^+/K^+-ATPase activity may be a direct consequence of intracellular MI deficiency and a possible mechanism of diabetic microvascular complication development. Indeed, in neuronal cells, Na^+ and K^+ ions are essential for the maintenance of membrane potential for neurotransmitter-induced excitement and altered Na^+/K^+-ATPase activity has been associated to impaired nerve conductivity [48,49] and could be linked to the pathological changes observed in diabetic neuropathy (axonal degeneration and demyelination) [50] through a possible inhibition of cell growth, transformation and differentiation [51].

The hyperglycemia-induced MI depletion is also associated with the hemodynamic disturbances in the diabetic kidney, which are believed to be directly responsible for the development of glomerulosclerosis and its attendant proteinuria [34]. On the other hand, the depletion of MI may also affect the normal physiological function of renal tubular epithelial cells, resulting in increased accumulation of extracellular matrix (ECM), which may lead to renal tubulointerstitial fibrosis. Therefore, the depletion of MI plays an important role in the development and progression of diabetic nephropathy [46,52].

Despite a lack of well-defined aetiological mechanisms, the MI depletion observed under hyperglycemic conditions in insulin insensitive tissues seems to contribute to the development of diabetic microvascular complications, together with the four major and more recognized pathways, namely: increased Advanced Glycation End products (AGEs) formation, activation of protein kinase C (PKC), increased hexosamine and sorbitol pathways [34].

4.2. Inosituria and decreased DCI to MI ratios in insulin target tissues

4.2.1. Putative mechanisms of inosituria and altered inositol profiles

Larner and colleagues described a decreased urinary excretion of DCI and an increased urinary excretion of MI in human subjects and rhesus monkey with Type 2 Diabetes (10 times higher than in healthy subjects) [35]. A similar urinary excretion profile was observed in studies of the Goto Kakizaki rat [53]. Although this fact was known since 1859 (Neukomm et al., 1859 quoted by Ref. [54]), the cumbersome analytic procedures for inositol prevented a thorough study of the mechanism of this abnormality at this time. In 1954, the increased inositol clearance observed in diabetes mellitus was related to glycosuria rather than polyuria [55]. In monkeys the inositol excretion pattern became more marked with the progression of the disease from normal to obese non-diabetic to diabetic [56] and additional studies on humans and monkeys demonstrated that this altered inositol profile in urine was more directly related to the underlying insulin resistance rather than to the type 2 diabetes *per se* (with a correlation between the decrease in urinary DCI and the severity of insulin resistance measured by five distinct parameters [10]). Altered ratios of increased *myo*-inositol to decreased *chiro*-inositol in urine have even been proposed as an index of insulin resistance in human subjects [57].

An altered DCI to MI ratio was also found in autopsy and biopsy muscles of type II diabetic subjects. In autopsy muscle, urine and hemodialysate samples, *chiro*-inositol was decreased about 50% compared to control subjects [37]. In the muscle biopsy specimens, no DCI was detected in the type II diabetic samples either before or after insulin administration. MI, in contrast, was present in the type

II diabetic samples in increased amounts over controls and was further increased with insulin administration [35]. To explain this inositol imbalance associated with insulin resistance, a defect in MI to DCI epimerization activity was postulated. To test this hypothesis, the existence of such a MI to DCI conversion was demonstrated *in vivo* in rats [25] and *in vitro* in fibroblasts [58] in a process stimulated by insulin. It was then shown that this epimerase activity is dependent on time, pH, tissue (liver and kidney being more active enzymes sources) and co-factors availability, full activity being obtained with the co-factors NADH and NADPH [36]. In keeping with this hypothesis, a strikingly decreased conversion of [^{3}H]-MI to [^{3}H]-DCI was observed in muscle, liver and fat cytosolic extracts of Goto Kakizaki type 2 diabetic rats compared to Wistar control rats (conversions of 20–25% in controls were reduced to basal levels of 5% or less in GK rat) [36]. This 2–3 fold decreased epimerase activity in insulin target tissues of GK rat is consistent with the decrease in DCI content (and so in DCI to MI ratio) observed in the same tissues and also seen in human muscle autopsy of type 2 diabetics [37]. Finally, the decreased MI to DCI epimerase activity observed in GK rat insulin target tissue extracts may play a role in explaining the decreased urine and tissue DCI content (and decreased DCI to MI ratios) related to insulin resistance.

4.2.2. Consequence of inosituria and altered tissues DCI to MI ratios in diabetes

Excessive urinary MI excretion could reduce MI plasma level and consequently emphasize MI intracellular depletion, particularly in tissues heavily dependent on extracellular MI import. Decreased production of DCI from MI reduces the availability of intracellular DCI for its incorporation into IPGs, putative downstream second messengers of insulin. Indeed, type 2 diabetes mellitus patients display decreased IPG levels in muscle biopsies as compared to healthy controls [35]. Therefore, the decreased DCI content in insulin target tissues could reduce insulin signal transduction involving IPGs and so further enhance or contribute to the insulin resistance in those tissues. Depleted plasma levels of DCI observed in PCOS (a syndrome characterized by insulin resistance and hyperinsulinemia, see Section 5.1) patients further emphasize the correlation between impaired plasma DCI and insulin resistance.

To sum up this section, insulin resistance and diabetes are associated with 1) abnormally low levels of DCI in urine, plasma and insulin target tissues (liver, muscle, fat); 2) excessive MI urinary excretion and 3) intracellular MI deficiency in insulin insensitive tissues (kidney, sciatic nerve, lens and retina). DCI deficiency could emphasize insulin resistance in liver, muscle and fat while MI depletion in specific tissues could play a role in the development or aggravation of diabetic microvascular complications (neuropathy, nephropathy and retinopathy). Therefore, it seems reasonable to speculate on a possible beneficial effect of MI and/or DCI supplementation in diabetes to restore depleted MI and/or DCI intratissue levels.

5. MI supplementation benefits for some metabolic disorders associated with insulin resistance

5.1. Polycystic ovary syndrome (PCOS)

Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder affecting 5–10% of women in reproductive age and characterized by hyperandrogensim, polycystic ovaries and ovulatory dysfunction. According to the 2003 Rotterdam Consensus Criteria, PCOS can be diagnosed after exclusion of other conditions that causes hyperandrogenism and if at least two of the following three criteria are present: chronic oligo- or anovulation (manifesting as oligoamenorrhea or amenorrhea), elevated serum androgen levels or clinical manifestations of hyperandrogenism (hirsutism, acne or androgenic alopecia) and polycystic ovaries on ultrasonography [59]. The cause of PCOS is unknown but studies suggest a strong genetic component that is affected by gestational environment, lifestyle factors or both.

Insulin resistance with compensatory hyperinsulinemia and central obesity are frequent metabolic features associated with PCOS and are key factors in the pathogenesis of anovulation and hyperandrogenism. Indeed, hyperinsulinemia could produce hyperandrogenism in PCOS women via two distinct and independent mechanisms: 1) by stimulating androgen production by the ovary and 2) by directly reducing the liver secretion of testosterone transporter (SHBG for Sex Hormon Binding Globulin) thereby reducing its serum level [60]. The net result of these actions is to increase circulating levels of free (active form) testosterone. In addition to promoting hyperandrogenism, recent evidence indicates that hyperinsulinemia contributes to the anovulation of PCOS. Indeed, hyperinsulinemia could adversely affect folliculogenesis and impede ovulation by increasing intra ovarian androgen production, altering gonadotropin secretion, or directly affecting follicular development. Confirming the important role of hyperinsulinemic insulin resistance in the pathogenesis of PCOS, insulin reduction, whether achieved by inhibition of pancreatic insulin release (diazoxide or octreotide) or improvement in peripheral insulin sensitivity (metformin, troglitazone, DCI), is associated with a reduction in circulating androgens, an improvement in ovulatory function, and enhanced fertility in women with PCOS.

myo-Inositol, through its insulin-sensitizing effect, was also found to be effective in improving metabolic and hormonal parameters in PCOS women. Previous studies have demonstrated that MI supplementation can restore spontaneous ovarian activity (spontaneous ovulation, menstrual cyclicity restored), and consequently fertility in most women patients with PCOS [13,61–65]. A significant improvement of typical hormonal parameters was observed in PCOS women after MI treatment: decreased LH, FSH, and testosterone circulating levels, and increased SHBG, estrogens and progesterone circulating levels [62–64,66]. Insulin peripheral sensitivity and insulinemia were improved (reduced HOMA-IR index and/or reduction of the AUC of glucose and insulin during an oral glucose tolerance test). Markers of cardiovascular risk were also improved with a decrement in systolic and diastolic blood pressure, a decrease in plasma triglycerides, an increase in HDL cholesterol, and a decrease in LDL and total cholesterol concentrations [64,67]. In some studies [64,68], anthropometric measurements showed a significant decrease in the Body Mass Index (BMI) and a decrease in circulating leptin concentration in the MI group after at least 16 weeks of treatment. Unfer et al. [13] reviewed and analyzed the six Randomized Controlled Trials (RCTs) focused on MI supplementation to improve PCOS hormonal and metabolic disturbances and they provided level Ia evidence of *myo*-inositol effectiveness (with a dosage of 2–4 g/day for 12–16 weeks in those studies and no side effects reported in these conditions). *myo*-Inositol mechanism of action appears to be mainly based on improving insulin sensitivity of target tissues, resulting in the reduction of insulinemia which has a positive effect on the reproductive axis (ovulation restoration and oocyte quality improvement) and hormonal functions (reduction of clinical and biochemical hyperandrogenism and of dislipidemia). Of note, *myo*-inositol positive effect on the reproductive axis could also be related to the pivotal role of I(1,4,5)P_3 in the regulation of Ca^{2+} release during oocytes development which conditions the acquisition of meiotic competence and drives oocytes to the final stages of maturation [4].

In conclusion, MI supplementation seems to be a simple, safe and effective first-line treatment for women with PCOS (See for review Unfer et al., 2012 [13]).

5.2. Diabetes, gestational diabetes and metabolic syndrome

5.2.1. Lessons from clinical studies

Since inositol, under the isoform MI or DCI, has been reported to improve insulin sensitivity and ovulatory function in young women affected by polycystic ovary syndrome, the ability of a MI supplementation to prevent or reduce insulin resistance was investigated in post-menopausal women with metabolic syndrome and in pregnant women with gestational diabetes or at risk of developing one [69–73] (See Table 1). A supplement of MI (2 g/day) to a controlled diet for 8 weeks in gestational diabetes [73] and for 6–12 months in post-menopausal women with metabolic syndrome [71,72] further improved fasting serum insulin and blood glucose levels, and consequently the HOMA-IR index compared to the diet treatment alone (−75% at 6 and 12 months compared to baseline in post-menopausal women with MI vs. −42% for the placebo group with the diet only; about −50% at 8 weeks with MI in women with gestational diabetes vs. −29% in the placebo group with diet only). In pregnant women with a family history of Type 2 Diabetes [70], a 4 g/day MI supplement throughout the pregnancy also significantly reduced the fasting and 1 h-glycemia at OGTT, and reduced the incidence of gestational diabetes by 40% (6% cases vs. 15.3% in the placebo group). A reduction of 65% of the risk for gestational diabetes (odd ratios 0.35) with MI was registered in this study. The improvement in glucose control obtained in the women supplemented with MI resulted in a significant reduction of some hyperglycemia-related pregnancy outcomes, in particular fetal macrosomia and high mean fetal weight. In postmenopausal women, the cardiovascular risk parameters were also further improved with the MI supplement with a reduction in blood pressure, in total and LDL-cholesterol, in serum triglycerides (−34%) and an enhancement of the HDL-cholesterol (+21%). Finally, after 6 months or one year of supplementation, the MI added to the diet of the post-menopausal women improved significantly almost all the metabolic parameters studied compared to the placebo group and even treated the metabolic syndrome of 20% of the women of the study group (8 on 40) while only one patient on 40 (2.5%) had no longer a metabolic syndrome in the placebo group with the diet alone.

Comparing MI with other insulin-sensitizing substances, MI is more effective than rosiglitazone in reducing serum triglycerides but less effective than pioglitazone (Pioglitazone (−50%) [74] > *myo*-inositol (−34%) > Rosiglitazone (−20%) [75]). In a study performed with metformin for 12 months [76], no change in triglycerides was noted, but only a slight improvement of HDL cholesterol (+2.4%) while in the study of Santamaria et al., one year of MI supplement enhanced HDL cholesterol concentrations by 21% which was also better compared to others obtained with pioglitazone [74] and rosiglitazone [75]. Hence MI supplementation seems to be a valuable and effective method to reduce cardiovascular risk in a context of metabolic syndrome or PCOS. The most important result of Santamaria and Giordanno et al. studies is the critical reduction in serum insulin and, consequently, insulin resistance (HOMA-IR index), which was about double compared to other insulin-sensitizing substances such as pioglitazone [74], rosiglitazone [75] and metformin [76] that are or were, at the moment, the gold standard of therapy for patients with impaired glucose tolerance.

Finally, according to those four randomized controlled trials (and to the 6 RCTs on PCOS), MI dietary supplementation (2–4 g/day) seems to be a safe and effective mean of fighting insulin resistance and associated cardiovascular risk in women in a context of gestational diabetes, post-menopausal metabolic syndrome or PCOS. However, those studies only include women and more precisely women with a special hormonal status (gestation, menopause or PCOS) which enables us to conclude on the effect of MI supplementation in other contexts since the true mechanism of action is still unclear. Moreover, those four studies have been done only on Caucasian women and mostly (3 on 4) in open-label trials. Additional studies on larger populations, from different origins, sex and in double-blind trials should be done to really and powerfully prove the efficacy of *myo*-inositol supplementation as an insulin-sensitizing treatment.

5.2.2. Lessons from animal studies

The effectiveness of certain inositol isomers or derivatives, especially DCI and D-pinitol, in lowering post-prandial blood glucose level had been reported in several cases of diabetes mellitus (STZ-diabetic rat [77], rhesus monkey [11], ob/ob mice [77] and human [78,79]). Studies on DCI showed that this effect was related to its insulin sensitizing activity. The herbal constituent sequoyitol, the 5-*O*-methyl form of *myo*-inositol, also exerts anti-diabetic effects in mice when administered chronically. Indeed, both subcutaneous and oral administrations of sequoyitol (80 mg/kg per day) for 8–10 weeks improved hyperglycemia, glucose intolerance and enhanced insulin signaling in liver of ob/ob insulin resistant mice [80]. The blood glucose lowering effect of high doses of MI in post-prandial condition was first established in the insulin resistant Rhesus monkey [12] and gave rise to a patent in 1998 [81] for the treatment of hyperglycemia in diabetes. Earlier studies on streptozotocin diabetic rats had however failed to show any improvement in hyperglycemia with dietary MI [82]. This could be explained by the fact that MI hypoglycemic effect passes through an improvement in insulin sensitivity [83] and so could not counteract hyperglycaemia in animal models of type 1 diabetes wherein insulin is missing. In addition, MI cellular uptake is competitively inhibited by glucose so its action is probably mitigated under hyperglycemic conditions. Later studies on healthy mice confirmed the ability of high doses of MI (given acutely [84] or chronically [83]) to reduce blood glucose level after a glucose load. This effect was associated with an improvement in peripheral insulin sensitivity established *in vivo* during an insulin tolerance test and further confirmed by the observation of an enhanced GLUT-4 translocation to the plasma membrane in response to hyperglycemia in the skeletal muscle [84].

5.3. Insight into the MI mode of action on insulin sensitivity – possible role of inositol glycans as second messengers of insulin

The exact mechanisms of action of MI and other inositol isomers (DCI) or derivatives (e.g. D-pinitol, sequoyitol) with insulin-mimetic activities are still unclear. A putative mechanism of action implies inositol phosphoglycans (IPGs) containing MI or DCI as insulin mediators.

The discovery of IPGs has emerged from the observation that the canonical model of insulin signaling invoking phosphorylation of insulin receptor substrates (IRS), phosphoinositide 3 kinase (PI3K) and protein kinase B/Akt (PKB/Akt) accounts for most, but not all, intracellular actions of insulin. Non-oxidative and oxidative glucose disposal by activation of glycogen synthase (GS) and mitochondrial pyruvate dehydrogenase (PDH) remain indeed incompletely explained by such model. Moreover, insulin stimulates both cellular glucose uptake and glycogen synthesis but these actions sometimes occur in a disconnected manner suggesting the possible existence of not only one but also two parallel signaling pathways connecting the insulin receptor to the activation of glucose uptake and its metabolic intracellular disposal. Consequent research on second messengers of insulin action led to the discovery of two IPGs as putative insulin mediators, extracted from rat liver and released in response to insulin. Their chemical nature was revealed later: the

Table 1
Clinical trials in which MI supplementation has been evaluated for the treatment of metabolic diseases (Note that PCOS were excluded[a]).

Reference	Study design	Duration	Treatment	No of subjects	Inclusion criteria	Exclusion criteria	Assessment of the response	Results
D'Anna et al., 2013 [70]	Randomized, controlled vs. placebo (FA 200 μg twice/day), open-label	From the 1st trimester through the whole pregnancy	2 g MI + 200 μg FA twice/day	$N = 220$ Placebo: 110 MI: 110	1) First-degree relatives (mother, father or both) affected by T2D; 2) prepregnancy BMI < 30 kg/m^2; 3) fasting plasma glucose <126 mg/dL and random glycemia <200 mg/dL; 4) single pregnancy; 5) Caucasian race	1) Prepregnancy BMI ≥ 30 kg/m^2; 2) previous GDM; 3) pre-gestational diabetes; 4) first trimester glycosuria; 5) first-degree relatives not affected by T2D; 6) fasting and random glycemia ≥126 and 200 mg/dL respectively 7) twin pregnancies; 8) associated therapy with corticosteroids; 9) not Caucasian race; 10) PCOS women	*Main outcome*: Incidence of gestational diabetes (diagnosed with IADPSG recommendations); *Secondary outcomes*: prevalence of fetal macrosomia (fetal weight > 4000 g at delivery), caesarean section, gestational hypertension, preterm delivery, neonatal hypoglycemia (<45 mg/dL), shoulder distocia and distress respiratory syndrome	Incidence of gestational diabetes significantly reduced in the MI group compared with the placebo group: 6 vs. 15.3%, respectively ($P = 0.04$) and reduction of gestational diabetes risk occurrence (odds ratio 0.35). Significantly reduced fasting ($p < 0.001$) and 1 h-glycemia ($p < 0.02$) at OGTT in the MI group. A statistically significant reduction of mean fetal weight at delivery in MI group and absence of fetal macrosmia (vs. 7 cases in placebo group). No difference between the groups for the other secondary outcomes studied
Matarrelli et al., 2013 [69]	Randomized, controlled vs. placebo, double-blind	For the entire pregnancy period	2 g MI + 200 μg FA twice/day taken with at least 6 h interval	$N = 75$ Placebo: 39 MI: 36	Consecutive singleton pregnant women with an elevated fasting glucose (glycemia ≥5.1 mmol/L or 92 mg/dL and ≤7.0 mmol/L or 1.26 mg/dL) in the 1st or early 2nd trimester	Pregestational obesity (BMI above 35) and refusal to participate were the only exclusion criteria	OGTT at 24–28 weeks' gestation. BMI, need for maternal insulin therapy, macrosomia, polyhydramnios, neonatal birth weight and hypoglycaemia	The incidence of gestational diabetes in mid pregnancy was significantly reduced ($p = 0.001$) in women who received MI compared to placebo (relative risk 0.127). Women supplemented with MI required less insulin therapy, delivered at a later gestational age, had significantly smaller babies with fewer episodes of neonatal hypoglycaemia.

(*continued on next page*)

Table 1 (*continued*)

Reference	Study design	Duration	Treatment	No of subjects	Inclusion criteria	Exclusion criteria	Assessment of the response	Results
Santamaria et al., 2012 [71]	Randomized, controlled vs. placebo	12 months	MI 2 g/day	$N = 80$ Placebo: 40 MI: 40	Postmenopausal women with MetS (at least 3 criteria of the ATP III of the National Cholesterol Education Programme); age between 50 and 60 years old and at least a 12-month period from the last menstruation	Use of glucose-lowering drugs and/or lipid-lowering drugs	Serum glucose, insulin, HOMA-IR, TG, total and HDL-CST, and BP at baseline and after 6 and 12 months of treatment	Serum glucose, insulin, HOMA-IR, TG, total and HDL-CST and BP significantly improved with MI compared to placebo. A significant difference from basal values was highlighted only in the MI group ($p < 0.0001$) for both BMI and WC at 12 months. In the MI group, the number of women without MetS was eight (20%) vs. only one in the control group after 12 months of diet
Giordano et al., 2011 [72]	Randomized, controlled vs. placebo	6 months	MI 2 g/day	$N = 80$ Placebo: 40 MI: 40	Postmenopausal women with MetS (at least 3 criteria of the ATP III of the National Cholesterol Education Programme); Age between 50 and 60 years old and at least a 12-month period from the last menstruation	Use of glucose-lowering drugs and/or lipid-lowering drugs	Serum glucose, insulin, HOMA-IR, TG, total and HDL-CST and BP at baseline and after 6 and 12 months of treatment	In the group treated with MI, significant improvements in diastolic BP (−11%), HOMA index (−75%), serum TG (−20%) and in HDL cholesterol (+22%) were observed
Corrado et al., 2011 [73]	Randomized, controlled vs. folic acid (FA) 400 μg/day, open label	8 weeks	2 g MI + 200 μg FA twice/day—Inofolic® (MI + FA)	$N = 69$ Placebo: 45 MI: 24	Gestational diabetes (diagnosed with an OGTT performed between 24 and 28 weeks of gestation)	Insulin therapy; premature delivery (before 35 weeks of gestation)	Fasting HOMA-IR and adiponectin blood level	Fasting glucose and insulin, and consequently HOMA-IR, decreased in both groups (50% in the MI group vs. 29% in the control group), but the decline in the MI group was significantly greater than that in the control group ($P = 0.0001$). Adiponectin increased in the MI group while it decreased in the control group ($P = 0.009$).

Maeba et al., 2008	Not a placebo controlled study	2 weeks	5 g MI/day the 1st week, 10 g MI/day the 2nd week	$N = 17$	Male ($n = 15$) or female ($n = 2$) hyperlipidemic subjects with ($N = 8$) or without ($N = 9$) MetS defined according to Japanese guidelines	Medications	Fasting Serum Plasmalogens, TG, Total-, LDL-, HDL- and sdLDL-cholesterol levels and fasting blood glucose levels.	After MI treatment, significant increase in plasmalogen-related parameters, and significant decrease in atherogenic cholesterols including sdLDL were observed. Among the hyperlipidemic subjects treated with MI, subjects with MetS had a significant increase in plasmalogens and a tendency toward reduced sdLDL, hsCRP and blood glucose levels compared to subjects without MetS.

Search procedure: we searched in the MedLine® database (using PubMed as a search engine) with the thesaurus terms "*myo*-inositol", "*myo*-inositol supplementation" or "dietary *myo*-inositol" in combination with "insulin", "diabetes", "metabolic syndrome" and/or "clinical trials". Papers were restricted to those published in English. Studies on women with polycystic ovary syndrome were excluded. Preference was given to randomized controlled trials. Abbreviations: ATP, Adult Treatment Panel; BMI, Body Mass Index; BP, Blood Pressure; CST, cholesterol; FA, Folic Acid; HDL, High Density Lipoprotein; HOMA-IR, HOmeostasis Model Assessment of Insulin Resistance; IADPSG, International Association of Diabetes in Pregnancy Study Group; LDL, Low Density Lipoprotein; MetS, Metabolic Syndrome; MI, myo-inositol; OGTT, Oral Glucose Tolerance Test; sdLDL, small dense LDL; TG, triglycerides; WC, Waist Circumference.

[a] See the review of Unfer et al., 2012 for the clinical trials evaluating MI supplementation for the treatment of PCOS.

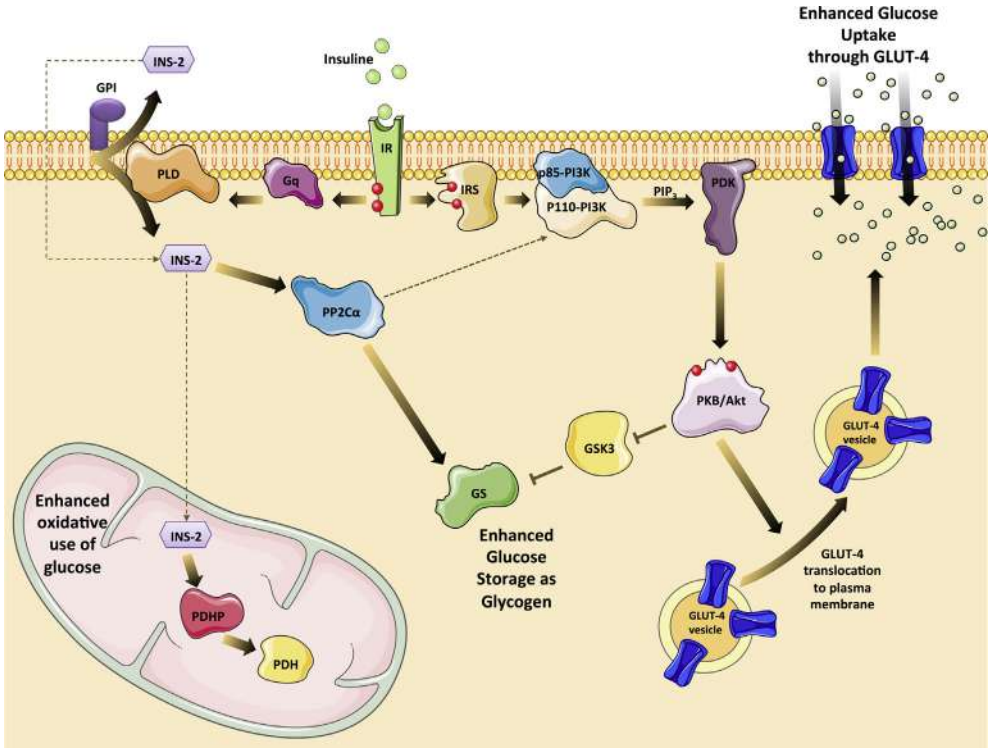

Fig. 6. New model of insulin-signaling proposed by Larner and co-workers – Inositol glycans as putative second messengers (INS-2) of insulin (inspired from the review of Larner et al, 2010). In this model, insulin binding to its receptor (IR) leads to autoactivation of the receptor and the activated IR can transduce the signal through two parallel signaling pathways. The first and well-accepted pathway of insulin implies the recruitment and activation of substrates of insulin receptor (IRS) by the activated IR. Subsequent protein activations (PI3K, PDK-1) finally lead to PKB/Akt recruitment and activation at the plasma membrane. Activated PKB/Akt induces GLUT-4 translocation to the plasma membrane and so enhances glucose entry into the cell. In the second putative pathway, the IR is coupled to a G protein itself coupled to a phospholipase (possibly PLD or PLC) that catalyzes the hydrolysis of a GPI. The insulin-induced hydrolysis of the GPI releases an inositol phosphoglycan containing D-*chiro*-inositol which acts as a putative second messenger of insulin (INS-2) mediating insulin effects on glucose oxidative and non-oxidative disposal. INS-2 binds and allosterically activates two Mg^{2+}-dependent protein phosphatases: PP2Cα in the cytosol and PDHP in the mitochondria. Activated PP2Cα stimulates glycogen synthase directly and also indirectly through possible activation of PI3K, Akt and subsequent inhibition of GSK3. In the mitochondria, activated PDHP stimulates PDH and so glucose oxidative use. Those two pathways act together to mediate insulin action in a complementary and synergistic manner. Abbreviations: IR, insulin receptor, GLUT-4 glucose transporter 4, GPI, glycosyl phosphatidylinositol, GSK3, glycogen synthase kinase 3, INS-2, insulin second messenger, PDH, pyruvate dehydrogenase, PDHP, pyruvate dehydrogenase phosphatase, PDK-1, phosphoinositide-dependent kinase 1, PI3K, phosphoinositide 3 kinase, PKB/Akt, protein kinase B/Akt, PLC, phospholipase C, PLD, phospholipase D, PP2Cα, phosphoprotein phosphatase 2C alpha.

first glycan termed IPG-P (Inositol Phosphoglycan-Phosphatase stimulator) contained methylated DCI (i.e. D-pinitol) and galactosamine and activated PDH phosphatase; the other one termed IPG-A (Inositol Phosphoglycan-AMP kinase inhibitor) contained MI and glucosamine and inhibited cAMP-dependent protein kinase (PKA) and adenylate cyclase (AC) [85]. Both types of IPG have been shown to additionally contain neutral sugars and phosphate residues. IPG-A and IPG-P were both insulin mimetic when administered *in vivo* in normal or diabetic rats: they reduced hyperglycemia dose-dependently by intravenous injection in low-dose STZ type 2 diabetic rat and stimulated glucose incorporation into glycogen in rat diaphragm muscles by intraperitoneal injection [86]. The origin of their insulin-stimulated production was first brought to light in 1986 by the discovery that inositol glycans release from hepatic plasma membranes in response to insulin was reproduced by addition of a phosphatidylinositol-specific phospholipase C [87]. Numerous reports then confirmed that insulin, other growth factors and classical hormones, stimulated the hydrolysis of glycosyl-phosphatidylinositol (GPI) generating water-soluble inositol phosphoglycan (IPG) second messenger. The origin of IPG-A is thought to be *myo*-inositol-containing GPI, as both PLC and PLD mediated hydrolysis of GPI yield biologically active IPG molecules (reviewed in Ref. [88]). Point-mutated and kinase-deficient insulin receptors fail to couple the generation of IPG-A through GPI hydrolysis, implying in some way that IPG-A release from GPI is controlled by insulin-stimulated tyrosine phosphorylation events. For DCI glycans (IPG type P like), Larner and colleagues propose that they are hydrolyzed from membrane phospholipids and/or GPI linked proteins such as alkaline phosphatase [89]. Indeed, bovine liver has four GPI lipid species with 1:1 M ratios of chiro-inositol and galactosamine like IPGs of P type.

Further evidence for a possible role of IPGs as insulin second messengers comes from studies on isolated rat adipocytes, in which IPGs released from GPI extracted from rat liver or purified from hemodialysate (Actovegin®) mimicked the anti-lipolytic and lipogenic effects of insulin [90,91]. The anti-lipolytic effect of such IPGs was associated with a reduction in the cAMP production stimulated by isoproterenol [90]. This effect is probably related to an inhibitory effect of these IPGs on adenylate cyclase and/or cAMP kinase, likely to IPG-A type. The stimulation of lipogenesis from glucose might be explained by PDH activation, similarly to IPG-P type; PDH and ACC are indeed two important control points for *de novo* lipogenesis from glucose. In addition, a purified *chiro*-inositol-containing IPG mediator (IPG-P type) from beef liver directly activated G3PAT in cell-free preparations of BC3H1 myocytes and Wistar rat adipocytes and is probably a mediator of this insulin action [92].

Further efforts allowed the identification of a novel putative mediator of insulin purified from beef livers and termed INS-2 [93]. Its exact chemical structure was determined and further confirmed by its chemical synthesis: it was a 4-*O*-(2-amino-2-deoxy-beta-D-galactopyranosyl)-3-*O*-methyl-D-*chiro*-inositol. This unique pinitol β-1,4-galactosamine structure is contrasted with the more common

myo-inositol α-1,6-galactosamine structure determined in other *myo*-inositol glycans. The bioactivity of this galactosamine *chiro*-inositol pseudo-disaccharide Mn^{2+} chelate was studied. This allowed the proposition of a new model of insulin signaling depicting the production of INS-2 in response to insulin and its role as second messenger of insulin action.

This new model proposed by Larner and colleagues (Fig. 6) (Reviewed in Ref. [89]) incorporates how insulin activates GS and PDH via a *chiro*-inositol glycan second messenger like INS-2 and how insulin activates GLUT-4 translocation to the plasma membrane. As depicted in Fig. 6, binding of insulin activates insulin receptor (IR) tyrosine kinase that autophosphorylates, recruits IRS proteins and phosphorylates them on Tyr residues to serve as scaffolds. A principal IR/IRS target is PI3K that generates PIP_3 to activate the phosphorylation of PKB/Akt by the PDK. After several steps, Akt activation leads to the translocation of GLUT-4 vesicles to the plasma membrane to increase glucose transport into the cells. In parallel of this IRS/PI3K/Akt pathway, IR activation would be also coupled to the heterotrimeric G protein Gq itself coupled to a GPI-phospholipase (possibly GPI phospholipase D, PLD) (See the review of Larner et al., 1999 [94] for further details). Activation of the phospholipase would release an inositol glycan second messenger INS-2 from a GPI lipid precursor in the inner and/or outer leaflets of the plasma membrane. INS-2 could be released either directly into the cytosol or outside the cell and then transported back into a neighboring cell or into the original cell via an ATP-dependant inositol glycan transporter (anti-inositol glycan antibodies inhibition studies [95], cultured cell dilution experiments [96] and impermeant fluorescent tag experiments [97] all supporting the extracellular generation/release of IPGs hypothesis). Inside the cell, INS-2 would bind and allosterically activates two members of the Mg^{2+}-dependent protein phosphatase family: cytosolic PP2Cα and mitochondrial PDHP. In the cytosol, activated PP2Cα stimulates glycogen synthase directly and indirectly via PI3K/PDK/Akt/GSK3 pathway. Indeed, it has been reported that PP2Cα dephosphorylates the Ser-608 residue of PI3Kα p85 regulatory subunit, resulting in activation of PI3Kα p100 catalytic subunit. The consecutive activation of PKB/Akt leads to the inactivation of glycogen synthase kinase 3 (by phosphorylation on its Ser-9 residue) resulting in activation of glycogen synthase. Other signaling events occur downstream of activated Akt and lead in particular to the activation of mTOR kinase and GLUT-4 translocation to the plasma membrane. Not depicted in Fig. 6 but possibly important, it has been observed that PP2Cα also inactivates AMPK by dephosphorylation of its Thr-172 in hepatocytes, heart and hypothalamus. In the mitochondria, allosteric activation of PDHP by INS-2 as a manganese chelate dephosphorylates Pyruvate dehydrogenase (PDH) thereby enhancing the oxidative glucose disposal.

The model depicted in Fig. 6 and the studies on IPGs provide a possible explanation for the observed effect of insulin on GS and mitochondrial PDH activation that were not fully explained by the conventional model. It also proposes a conceptual framework for the origin, production and actions of *chiro*-inositol-containing glycans as second messengers working in a complementary and synergistic manner with the better-accepted pathways of insulin signaling. However, this model does not integrate *myo*-inositol-containing glycans second messengers and their mode of action still remains elusive. In addition, the exact structure–activity relationship of IPGs is not precisely defined and a recent study yielded conflicting results since over a wide range of synthetic IPGs, none were insulin mimetic in both *in vivo* and *in vitro* studies [98] contrary to previous studies [99–102].

The insulin sensitizing effect of a MI, DCI or pinitol supplementation could possibly results from their intracellular enhanced availability for the production of membrane IPGs precursors, but real evidence for this phenomenon is lacking. Moreover, a recent study showed an enhanced GLUT-4 translocation to the plasma membrane in baseline conditions (e.g. in the absence of a direct or glucose-induced insulin stimulation) in mice skeletal muscle *in vivo* as well as in rat muscle and L6 myotubes *in vitro* with several inositol isomers [84,103]. Unless a baseline production of IPGs can be obtained with inositol supplement and independently of insulin stimulation, this result cannot be explained by the production of second messengers induced by insulin. In addition, 12 h pretreatment of HepG2 hepatocytes or 3T3-L1 adipocytes with 100 μM *myo*-inositol or sequoyitol (5-*O*-methyl-*myo*-inositol) directly enhanced IR, IRS-1 and Akt activation in response to insulin stimulation (10 nM, 5 min) [80]. Considering that IPGs act on insulin signaling downstream of IR and IRS-1 phosphorylation events, these results cannot be explained by an enhanced production of IPGs.

It is worth noting that part of the MI supplementation effect on insulin sensitivity may come from its partial *in vivo* epimerization to DCI. However, the MI to DCI epimerase activity was reported to be reduced in insulin-resistant tissues. Hence, if MI efficiency mainly relies on its *in vivo* conversion to DCI, the MI supplementation effects will be mitigated in contexts of insulin resistance (unless a supplement of MI epimerase substrate could enhance its activity).

To conclude, MI and other inositol isomers insulin mimetic properties are still not fully understood. Numerous evidences support the hypothesis of a role of inositol glycans insulin-second messengers in insulin mimetic properties of some inositol isomers. However, many questions remain unanswered and deserve further investigations and/or explanations.

5.3.1. Some outstanding issues

- Why a GLUT-4 translocation and a glucose uptake enhancement were observed in response to several inositol isomers without insulin stimulation in some animal and *in vitro* studies [84,103]? Is there a baseline production of IPGs independently of insulin stimulation?
- How sequoyitol (a potential *myo*-inositol precursor) and *myo*-inositol pretreatments (100 μM, 12 h) of hepatocytes and adipocytes cell lines directly enhance IR, IRS-1 and Akt in response to insulin (10 nM, 5 min)?
- What is the phospholipase releasing inositol glycans and from which precursor lipids and proteins?
- How many inositol glycan second messenger of insulin exist? Are they different depending on the species, tissue or cell type? Is the structure of plasma IPGs different from that of tissular IPGs?
- Are inositol glycans released extracellularly and/or intracellularly?
- If existing, what is/are the inositol glycan transporter(s) in plasma and mitochondrial membranes.
- Does a MI or DCI supplementation increase IPGs production and how?
- Is MI conversion to DCI essential for its efficiency on glucose homeostasis?

6. MI supplementation effects on diabetic complications

In diabetes, tissues likely to develop long-term microvascular complications (kidney, sciatic nerve, lens and retina) are depleted in MI and this MI intracellular deficiency could play a pivotal role in the development and progression of these complications. Restoring MI intracellular levels with dietary MI supplement could then be a suitable strategy to prevent or delay the development of diabetic neuropathy, nephropathy or retinopathy.

6.1. MI supplementation and neuropathy

Several animal and human studies showed a beneficial effect of a MI supplementation in the diabetic nerve. First, Green et al. demonstrated that a 1% (w/w) MI supplemented diet (vs. 0.011% or 0.069% free MI in normal diets) restored MI intracellular level in the nerve of STZ-diabetic rat model. On this supplemented diet, the development of impaired Motor Nerve Conduction Velocity (MNCV) by the 14th day after STZ administration was moderated or totally prevented, despite persistent hyperglycemia and elevated nerve intracellular levels of sorbitol and fructose [82]. In this type 1 diabetes rat model, insulin treatment from day 3 after STZ administration failed to prevent impaired MNCV in the sub-group of diabetic rats in which hyperglycemia and weight loss were improved. However, insulin treatment prevented the development of MNCV in the other sub-group of diabetic rats in which the tail vein glycemia never exceeded 160 mg/dl and average glycemia was 75 mg/dl during the days 6–14 after STZ injection. In this latter group of STZ-diabetic rats, insulin treatment also prevented MI nerve depletion. This study suggests that insulin deficiency and possibly hyperglycemia are primary factors in the development of impaired MNCV and that this MNCV impairment appears to be related to a disruption of MI intracellular level regulation in the nerve.

Although some discrepancies were published on MI supplement benefit for MNCV in diabetes [104,105], Greene et al. finally proposed a partial explanation to those differences showing that dietary MI supplementation ameliorated the diabetes-induced MNCV impairment in both sciatic and tibial motor nerves but with different time courses (suggesting metabolic or physiologic heterogeneity among populations of large myelinated motor fibers).

Finally, beneficial effects of MI supplementation on nerve function were confirmed in latter studies. Mayer and colleagues demonstrated that a dietary supplement of MI (0.667 g/kg per os, daily) or an aldose reductase inhibitor treatment (ICI 105552; 50 mg/kg per os, daily) prevented defects of both axonal transport and MNCV in STZ-diabetic rat [106]. Aldose reductase inhibitor treatment also normalized sorbitol levels in motor nerve of diabetic rats. Altered Na^+/K^+-ATPase activity was also corrected by 1% (w/w) MI supplementation, such as nerve conductivity [48]. The effect of MI content in diet (low, normal or high) was studied on patients with symptomatic distal symmetrical diabetic polyneuropathy. It was found that ingestion of high-MI diet increased significantly the median sensory and the sural sensory nerve conduction velocities (+1.92 m/s and +6.67 m/s respectively, $p < 0.001$). In contrast, ingestion of low-MI diet led to a decrease in the median and peroneal MNCV ($p < 0.001$). Thus ingestion of diet enriched in MI may have a positive effect upon peripheral nerve function in the patients with symptomatic diabetic neuropathy.

6.2. MI supplementation and nephropathy

In an *in vitro* model of diabetic renal disease, it has been previously demonstrated that elevated glucose levels stimulate pro-collagen transcription and secretion in proximal tubule cells in culture while inducing cellular hypertrophy and reducing cellular proliferation. MI supplementation (800 μM) in a high glucose culture medium (4.5 g/L) reverses the glucose-induced reduction in cell proliferation and the increase in pro-collagen transcription and secretion [107]. On the other hand, it did not prevent the glucose-induced cellular hypertrophy. In animal models of diabetes (STZ rat), Na^+/K^+-ATPase activity was significantly increased in the cortex of untreated diabetic rats compared with nondiabetic control rats at both 1 and 2 weeks after STZ injection. This increased Na^+/K^+-ATPase activity was prevented by MI (0.65 g/kg, p.o.) or Sorbinil (an aldose reductase inhibitor) treatment [108]. The effect of MI supplements on Na^+/K^+-ATPase appeared independent of glomerular filtration rate (GFR), since the increase in inulin clearance measured in diabetic rats was unaffected by MI. A long term supplementation (4 months) with 1% MI to the Cohen diabetic (type 2 diabetes) rat also reduced the increased renal Na^+/K^+-ATPase activity but had no effect on blood glucose levels, body weight, increased kidney weight, or creatinine clearance and did not prevent or reduce the development of renal glomerular pathology [109]. There was no correlation between the level of Na^+/K^+-ATPase activity and the degree of nephropathy. It is then possible that some renal pathological changes are due to metabolic and humoral factors resulting from hyperglycaemia, other than MI depletion.

6.3. MI supplementation and cataracts

MI supplementation restored intracellular MI content in the lens of STZ-induced diabetic rats (otherwise undetectable after 14 weeks post STZ injection) [34,110]. The MI supplemented STZ group displayed minor structural changes and early stages of cataract formation while the untreated group displayed apparent structural changes and well-established cataracts [34].

To conclude, MI supplementation seems to be efficient to counteract the diabetes-induced MI depletion in kidney, nerves and lens. Correction of MI deficiency by dietary MI supplement prevented or delayed the development of some microvascular complications of diabetes in the motor nerves and lens in type 1 diabetes animal models. The fact that MI treatment was shown to have a beneficial effect on restoring impaired conduction velocity and on the disruption of structural elements in the nerve but had no effect on the development of renal pathological changes indicates that the effect of the biological changes ensuing from hyperglycemia vary in different tissues depending on local conditions. Additional clinical studies would be useful to evaluate the efficiency of a MI supplementation against long-term complications of diabetes in human.

7. Concluding remarks

myo-Inositol is a polyol naturally present in eukaryotic cells and is a component of numerous biological molecules, including second messengers like IP_3, PIP_2/PIP_3 and IPGs which makes it essential for numerous biological processes. Abnormalities in its metabolism are associated to pathological states and in particular, MI intracellular deficiency in sciatic nerve, kidney, lens and retina of diabetic subjects probably contribute to the development or aggravation of some diabetes complications in those tissues. Correction of MI intracellular depletion by MI supplement prevented or delayed the development of some microvascular complications of diabetes in the motor nerves and lens in animal models but was inefficient for diabetic nephropathy. Insulin resistance is also associated to excessive urinary excretion of MI and decreased urinary level of DCI. A correlation has even been observed between the decrease in urinary DCI and the severity of insulin resistance. Insulin resistant tissues like skeletal muscles also presented altered DCI to MI ratios and decreased IPGs content, activity and production in response to insulin. Since IPGs are putative mediators of insulin action, their deficit in insulin target tissues probably participate to the development or progression of insulin resistance. Dietary supplement of inositol isomers DCI, D-pinitol or MI were found to be efficient in lowering post-prandial plasma glucose in several animal models of diabetes or insulin resistance. The insulin-mimetic properties of dietary inositol supplements is mainly believed to be related to the production of inositol glycan secondary messengers containing

either MI or DCI. However further investigations are required to unravel the exact molecular mechanisms of action of MI and to confirm or infirm this IPGs hypothesis. Randomized control trials on MI dietary supplement gave positive results in fighting insulin resistance and reducing cardiovascular risk in women with PCOS, gestational diabetes mellitus or metabolic syndrome in post-menopause. However, larger studies, in double-blind trials, including populations with other than a Caucasian origin and also including men would be necessary, 1) to confirm the previous results for women with GDM, PCOS or post-menopausal MetS; 2) to test a possible application for a more generalized population of subjects already presenting an insulin resistance or at risk of developing one because of genetic predisposition.

Disclosure

The authors declare no conflict of interest.

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Review

Contribution of myo-inositol to reproduction

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ABSTRACT

Myo-inositol is involved in several aspects of human reproduction.

Elevated concentrations of myo-inositol in human follicular fluids appear to play a positive function in follicular maturity and provide a marker of good quality oocytes.

Nevertheless its positive role in PCOS women is a consequence of a defect in the insulin signaling pathway (inositol-containing phosphoglycan mediators) that seems to be primarily implicated in the pathogenesis of insulin resistance.

This article will review the involvement of inositol in female reproduction. After describing the biologic function of inositol and its derivatives, studies are quoted in which the role of inositol in fertility, oogenesis, and polycystic ovary syndrome are examined.

Contents

1. Introduction

Since Beemster review in 2002 [1], increasing evidence supports the physiological and therapeutic role of myo-inositol in human reproduction and in particularly in oogenesis.

Myo-inositol (MYO) is one of nine stereoisometric of a C6 sugar alcohol that belongs to the vitamin B complex group [2,3].

MYO plays an important role in cell morphogenesis and cytogenesis, lipid synthesis, structure of cell membranes and cell growth [4,5].

Other studies have shown that MYO is incorporated into phosphoinositides and inositol phosphates in rabbit embryos [6] and can enhance bovine blastocyst development from in vitro culture with medium supplemented with MYO [7]. Taken together, the results from these studies support the notion that MYO serves as a precursor for the synthesis of phosphoinositides. This constitutes the phosphatidylinositol (PtdIns) signal transduction system known to be involved in the regulation of diverse cellular functions including cell proliferation [8].

Human adults consume approximately 1 g of inositol per day in different biochemical forms [9]. Free inositol is actively transported across the intestinal wall by a mechanism dependent on sodium and energy, a process that can be inhibited by glucose. Circulating free inositol is taken up by most tissues by a membrane-associated sodium-dependent inositol co-transporter [10].

Since the deciphering of the molecular details and importance of the inositol phospholipids-calcium second messenger system [11], the amount of cellular processes known to be directly or indirectly controlled by this class of lipids has tremendously expanded in recent times [12,13]. The long standing evidence indicates that this signal transduction system involves a receptor-dependent hydrolysis of phosphatidylinositol 4,5-bisphophate to

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form the two second messengers, inositol 1,4,5-trisphosphate ($InsP_3$) and diacylglycerol (DAG) [8]. $InsP_3$ diffuses through the cytosol and binds to $InsP_3R$ on the surface of endoplasmic reticulum where it triggers the release of intracellular Ca^{2+} whereas DAG activates protein kinase C (PKC) which alters the cell function by phosphorylating a variety of cell proteins [11,12]. These signaling pathways will operate throughout the life of a cell to regulate a variety of cellular processes including gametogenesis, fertilization, cell proliferation and development, secretion and contraction and neural activity [14–16].

1.1. The role of inositol in oogenesis

Increasing evidence has indicated that $InsP_3Rs$ play a primary role in generating calcium signals in mammalian oocytes [11]. Two types of receptor-operated channels, namely inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$) and ryanodine receptors (R_yR) are found in mammalian cells known to mediate intracellular Ca^{2+} release [17–19]. Of the three isoforms of $InsP_3Rs$ that have been identified, type I $InsP_3Rs$ are the major receptors present in mouse oocytes [20]. Subsequent studies have demonstrated that similar receptors are also present in human oocytes [21]. Besides, calcium release mechanisms are shown to undergo modification during oogenesis and maximal sensitivity of calcium release is acquired during the final stages of oocyte maturation in preparation for successful activation at the time of fertilization [18,22]. This evidence points to the putative role of the inositol phospholipids-calcium second messenger system in oocyte development.

Calcium signalling in oocytes has been extensively studied in various species because of its putative role in oocyte maturation and the early stages of fertilization [23,24,18]. It had been demonstrated that fully grown mammalian GV oocytes exhibiting spontaneous intracellular calcium oscillations are associated with a higher incidence of germinal vesicle breakdown (GVBD) and supplementation of MYO can promote meiotic progression of these GV oocytes [25]. Depletion of inositol will desensitize PtdIns signaling by slowing down the re-synthesis of the PtdIns [4,5] P_2 precursor used to release $InsP_3$ as proposed by Berridge and Irvine [8]. This, in turn, can disrupt the dynamics of the intracellular calcium transients necessary for proper initiation of oocyte maturation. Thus supplementation of MYO can avoid inositol depletion and also enhances the intracellular inositide-calcium second messenger system involves in meiotic progression of mammalian oocytes. By the same token, we have demonstrated that follicles containing higher levels of MYO have better quality of oocytes which may be related to the intricate relationship between MYO and inositol phosphates involve in the PtdIns cycle activation for oocyte maturation. The presence of higher levels of MYO can indicate the well being of the follicle and the quality of the oocytes [26].

Indeed, a recent review has provided evidence to support the important regulatory roles of inositol phospholipids in nearly all aspects of cell physiology including cellular signal transduction, regulation of membrane traffic, nuclear events, cytoskeleton organization and transport functions of membranes [27].

1.2. The role of inositol in polycystic ovary syndrome

Some actions of insulin may involve low-molecular weight inositolphosphoglycan (IPG) mediators (also known as putative insulin mediators or second messengers) [28–30].

Further supportive evidence of the association between insulin sensitivity and D-Chiro-Inositol-IPG release can be gathered from clinical trials comparing oral DCI or MYO supplementation (which can be converted to DCI intracellularly) vs placebo in women with PCOS.

DCI administration led to a reduction in serum testosterone levels and an improvement in ovulation and metabolic parameters such as blood pressure and triglycerides in women with PCOS [31]. These findings have since been supported independently by Gerli et al. [32] who conducted a randomized, double-blind, placebo-controlled trial of 283 women with PCOS. Frequency of ovulation was increased by almost 2-fold in women who received DCI; and serum HDL cholesterol increased, effects consistent with improved insulin sensitivity. Similar findings were found after oral administration of MYO, a precursor of DCI in vivo [33] and [34]. Although the above data suggest that decreased DCI concentrations, and/or bioactive DCI-IPG release, may contribute to insulin resistance, the association between an increase in DCI-IPG release and improvement in insulin sensitivity has not been directly assessed.

Those hypothesis had been demonstrated in a study in which myo-inositol administration improved reproductive axis functioning in PCOS patients reducing the hyperinsulinemic state that affects LH secretion [35].

In this study all out of 20 overweight PCOS patients, after 12 weeks of MYO administration, presented plasma LH, PRL, T, insulin levels and LH/FSH reduced. Nevertheless insulin sensitivity, expressed as glucose-to-insulin ratio and HOMA index resulted significantly improved. Furthermore menstrual cyclicity was restored in all amenorrhoic and oligomenorrhoic subjects.

In fact both American and Greek women affected by polycystic ovary syndrome present an increased urinary clearance of inositols that reduce tissue availability of DCI and decrease the release of DCI-IPG mediator, which could contribute to insulin resistance and compensatory hyperinsulinemia [36,37].

In patients with PCOS, undergoing standard protocol of ovulation induction for intracytoplasmic sperm injection, the treatment with myo-inositol and folic acid, but not folic acid alone, reduces germinal vesicles and degenerated oocytes at ovum pick-up without compromising total number of retrieved oocytes. This approach, reducing oestradiol levels at hGC administration, could be adopted to decrease the risk of hyperstimulation in such patients.

Taking into account dermatological disorders as an additional end-point of treatment in PCOS women, MYO was tested to evaluate the effects on the lipid pattern and insulin sensitivity of hirsute women [39].

Its administration to 46 hirsute women significantly reduced hirsutism and hyperandrogenism and ameliorated the abnormal metabolic profile of those patients. Total androgens, FSH and LH concentrations decreased while oestradiol concentrations increased. Insulin resistance, analysed by homeostasis model assessment, was reduced significantly after therapy.

2. Discussion

MYO may represent one of the maturational factors in follicular fluid responsible for the in vitro growth of human oocytes. Perhaps, the content of MYO in follicular fluids may represent a more appropriate physiological indicator than follicular volume for monitoring the status of the developing follicles. Follicles containing good quality oocytes have higher concentrations of MYO in follicular fluids, probably due to the intricate relationship between MYO and inositol phosphates in the PtdIns cycle activation for oocyte maturation.

Regarding embryo development the animal models shows some contradictory results. For example while different glucose

concentration can inhibit embryo development in bovine, on the contrary short-term treatment of in vitro produced bovine preimplantation embryos with Insulin-like growth factor (IGF-I) can block induction of apoptosis caused by heat shock through signaling events requiring phosphatidylinositol 3-kinase (PI3K) [40].

In cryopreserved mouse oocytes, maturation, and fertilization, are positively related to the structure and function of the endoplasmic reticulum. These oocytes had the capacity to release Ca(2+) following injection of inositol 1,4,5-trisphosphate, demonstrating that Ca(2+) release mechanisms developed during meiotic maturation [41].

In human this data are not yet well established, and research in first stage embryo development and early pregnancy is still ongoing.

Recently MYO role powerfully emerged in the pathogenesis of polycystic ovary syndrome and in particularly linked with insulin resistance.

Whilst a significant progress has recently been made in the diagnosis for PCOS [42], the optimal infertility treatment for PCOS women remains to be determined [43,44].

In infertile patients with PCOS who present to reproductive endocrinologists desire pregnancy immediately, and for them time is of the essence, a rapidly acting induction agent such as clomiphene would be most appropriate [42].

On the contrary, young patients with PCOS whose timeline for achieving pregnancy differs from ‘‘immediately’’, often present with concerns unrelated to immediate fertility and might seek to postpone pregnancy; these women may be quite accepting of a pregnancy when it comes. Such women with longer timelines for achieving pregnancy constitute at least one ‘‘well-defined subset’’ for whom MYO, with its gradual onset of action minus the potential risk of multiparity, may be the drug of choice to re-establish ovulatory menses and fertility.

Moreover MYO may ameliorate the availability of DCI in these patients and acts as an ovarian insulin-sensitizing agent whereby a significant reduction in estradiol levels was detected in PCOS women undergoing ovarian stimulation compared to the controls [38]. The serum oestradiol level at hCG injection in MYO treated PCOS patients is well below the recommended normative threshold level of E2 (2500 pg/mL) suggested by Practice Committee of the ASRM [45].

In conclusion long-term co-treatment with MYO for patients with PCOS undergoing ICSI cycles does not improve the response to stimulation but significantly ameliorate oocytes quality and reduces the risk of OHSS.

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Review

Contribution of myo-inositol and melatonin to human reproduction

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ABSTRACT

Diet is a critical factor for the development of both embryo and fetus, as well as maternal health. In particular, two natural molecules have been shown to exert beneficial effects on fertility, pregnancy wellness and embryo development: myo-inositol and melatonin, whose requirements increase during pregnancy.

In the present review, we summarize the most important functions of melatonin and myo-inositol on male and female reproductive systems (oocyte quality and development, sperm quality), on the maintenance of a physiological pregnancy and on embryo development.

Contents

1. Introduction

Diet is one of the major factors influencing the development of both embryo and fetus, as well as maternal wellness and health. In particular, a deficiency in micronutrients has been linked to a significantly high rate of reproductive disorders, ranging from infertility to fetal structural defects and long-term diseases [1]. Maternal micronutrient supplementation is essential to prevent most pregnancy disorders [1]. Prevention of pregnancy disorders should start during the periconceptional period: indeed, the highest rate of malformations and pregnancy-related disorders (i.e., low oocyte quality, pre-eclampsia, congenital abnormalities, insufficient fetal growth, miscarriage, premature delivery) may occur during this period [2].

A proper micronutrient supplementation during both the periconceptional and pregnancy periods will positively affect fetal and maternal wellness, and it may also directly enhance the quality of breast milk [3].

During the last decades, a new awareness has arisen regarding the beneficial effects of two natural substances on male and female reproductive functions: myo-inositol (MI) and melatonin. Both MI and melatonin are synthesized by the cells in physiological conditions, but during the periconceptional and pregnancy periods their physiological requirements have been demonstrated to increase [4,5].

In the present review, we summarize the most important functions of these two natural compounds in supporting female and male gametogenesis and embryo development.

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2. Melatonin

Melatonin, N-acetyl-5-methoxytryptamine, is a small lipophilic indoleamine. It is mainly synthesized by the pineal gland. Pinealectomy leads to a decrease in circulating melatonin concentrations to nearly undetectable levels.

Melatonin is secreted in a circadian manner, with high levels being secreted in all species at night. In mammals, including humans, melatonin rhythm is generated by an endogenous circadian clock in the suprachiasmatic nuclei (SCN) of the hypothalamus. In particular, the activity of the enzyme N-acetyltransferase (NAT) increases from 30- to 70-fold at night and, in most circumstances, is rate-limiting in melatonin synthesis. The period of melatonin secretion is depending on the duration of darkness.

The onset of a circadian melatonin rhythm in human infants appears only after 9 weeks of age. A peak in melatonin secretion is established between 3 and 5 years, with a subsequent decline to adult levels by 15–18 years. Amplitude remains relatively stable until old age, when a marked decline has been demonstrated, due to a general weakening of the circadian system.

3. Myo-inositol

Inositol is a sugar similar molecule, which for long time has been erroneously included among vitamins.

Many studies support the notion that myo-inositol (MI) is one of the precursors for the synthesis of phosphatidylinositol polyphosphates (PIPs). PIPs are key biomolecules belonging to the signal transduction system known to be involved in the regulation of several cellular functions [6]. Indeed, MI plays a crucial role in cell morphogenesis and cytogenesis, it is involved in cell membrane formation, lipid synthesis and cell growth [6].

4. MI biological actions occur via PIPs or via inositol polyphosphates (InsPs)

Each of the seven different PIPs acts as a docking site for several protein effectors such as AKT. PIPs are crucial factors in regulating several cellular processes: indeed, it has been shown that a defect in binding and/or lipid composition results in the onset of several diseases [7].

On the other hand, InsPs are probably the most studied second messengers: indeed, the role of $InsP_3$ in calcium signaling through its receptors ($InsP_3Rs$) is well known [7]. $InsP_3$ is produced via hydrolysis of a specific PydInsPs by phospholipase C; once the reaction has taken place; two different signal transduction molecules are produces: $InsP_3$ and diacylglycerol.

Recently, the complexity of the inositol transduction system induced scientists to look at it as a new biological code that needs to be translated [8]. These signaling pathways will operate throughout the life of a cell to regulate a variety of cellular processes including those related to gamete development, as oocyte maturation, fertilization and early embryonic development [9].

5. Human reproductive functions

5.1. Melatonin

At the end of the 19th century, Hübner demonstrated that a tumor in the pineal gland altered pubertal development. It was the first evidence that something secreted by the pineal gland was able to influence the reproductive function. Only 60 years after the pioneering study by Hübner, Lerner et al. discovered melatonin, thus paving the way for a new field of research in reproductive physiology.

Together with the discovery of melatonin, cytological studies of the pineal gland demonstrated that its metabolic activity was increased during darkness. Additional clinical studies further investigate the involvement of the pineal gland in reproductive physiology. Scientific evidence has been provided to support a role of melatonin in human reproduction. It has been demonstrated that in boys having a precocious puberty, melatonin concentration was higher compared to age-matched children of normal puberty age, while boys with delayed puberty had lower melatonin concentration [10]. Moreover, hypothalamic amenorrhoea was associated with high melatonin concentrations [11].

One of the most important functions of melatonin was discovered in 1993, when it was demonstrated that melatonin has radical scavenger properties; indeed, melatonin was found to be able to reduce concentration of highly reactive hydroxyl radical in vitro and in vivo [12], caused both by oxygen- and by nitrogen-based reactants [12]. Furthermore it was shown that melatonin is able to stimulate antioxidative enzymes [12].

The scavenger properties of melatonin have been shown to be crucial for optimal cell and organ functions, including functionality of the reproductive system [13]. In particular, it has been demonstrated that melatonin protects liver against damage during ischemia-reperfusion [14] and prevents kinate-induced oxidative damage in brain tissue [15]. Furthermore, it was reported that melatonin was able to detoxify the peroxyl radical generated during lipid peroxidation [16] and the peroxynitrite anion [17] which could damage membrane lipids. Melatonin is also known to reduce lipid peroxidation induced by a variety of toxins [18] and is able to preserve plasma membrane fluidity which is normally altered when the membrane contains oxidatively damaged polyunsaturated fatty acids [19].

Melatonin exerts its antioxidant actions as a direct free radical scavenger and by stimulating antioxidant enzymes interacting with its membrane receptors. Melatonin receptors have been identified in hypothalamic neurons that govern the release of pituitary gonadotropins, in the anterior pituitary, and in both female and male gonads, as well as in other reproductive organs [20].

Several studies have shown that sperm is negatively affected by oxidative stress caused by inflammatory processes, and it has been shown that melatonin or its metabolites are able to protect sperm from oxidative damage [21].

Additional studies have shown that human seminal fluid contains melatonin [22], and spermatozoa express melatonin receptors [23]; furthermore, Fujinoki et al. in 2008 demonstrated that melatonin is able to stimulate flagellar motility.

Data obtained by measuring melatonin concentrations in human ovarian follicular fluid (FF), obtained from antra of Graafian follicles showed significantly higher melatonin concentrations compared to plasma levels [20]. Higher melatonin concentration in the follicular fluid is maintained by both active melatonin transport from the blood stream in the FF, and by melatonin ovarian synthesis (possibly by granulosa cells) [24].

Recently, it has been shown that melatonin is important for optimal oocytes development and ovulation has a positive effect on early embryo development. Indeed, ovulation involves processes similar to a local inflammatory response [25], in which both reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced, inducing oocyte oxidative damage. Therefore, since both melatonin and its metabolites are able to quench ROS and RNS, they might be involved in the protection of granulosa cells and oocyte during ovulation [13]. Furthermore, it has been demonstrated that there is a direct correlation between melatonin concentrations in follicular fluid and oocyte quality [13].

The presence of melatonin and its precursors, serotonin and N-acetylserotonin, has been also documented in extracts of human

ovary; furthermore, enzymatic activity of the two melatonin synthesizing enzymes, NAT and hydroxyindole-O-methyltransferase (HIOMT), was detected in human ovarian homogenates [24].

Several trials have reported that endogenous melatonin levels decrease with age [20], inducing a decrease in oocyte quality in women near the end of their reproductive life. Melatonin administration was shown to be able to preserve oocytes quality by increasing melatonin intrafollicular concentrations. Indeed, high melatonin levels in the follicular fluid lead to a reduction of intrafollicular oxidative damage. Reduction of the oxidative stress increases fertilization and pregnancy rates in women who failed to become pregnant due to poor oocyte quality [26].

5.2. Myo-inositol

In recent years, an increasing number of studies have supported the physiological and therapeutic role of MI in human reproductive functions, as highlighted by the review article written by Beemster et al. [27].

The pivotal role of inositol in mammalian cell metabolism is known since several years; in mammalian oocytes Ca^{2+} oscillations play an important role in the acquisition of meiotic competence and drive oocytes to the final stages of maturation. It has been demonstrated that calcium release mechanisms are regulated during oogenesis; in particular, the final stages of oocyte maturation were shown to have an increased sensitivity in calcium release [28]. The interaction between Ins(1,4,5)P_3 and its receptors is responsible to generate calcium release in mammalian oocytes. Indeed, it has been demonstrated that mouse oocytes had the capacity to release Ca^{2+} following injection of Ins(1,4,5)P_3, leading to meiotic maturation [29]. Concerning human oocytes, it has been shown that they express Ins(1,4,5)P_3-receptor (type I), responsible to mediate intracellular Ca^{2+} release [30]. This evidence underlines the role of inositol as second messenger of calcium signaling in oocyte development. The fully grown mammalian germinal vesicles exhibit spontaneous intracellular calcium oscillation, it has been demonstrated that a supplementation with MI can promote meiotic progression into fertilization-competent eggs [31]. On the contrary, depletion of MI will desensitize PtdIns signaling and lower $InsP_3$ release, thus leading to a disruption of intracellular calcium transient dynamics and to interruption of oocyte maturation.

The Ca^{2+} oscillations were also detected in the zygote [9]. The $InsP_3$-receptors are indeed expressed by zygotes, suggesting that Inositol is involved in mediating Ca^{2+} release also in an early stage of development. Ca^{2+} fluctuation occurring in the cleavage stage of the mammalian embryos could influence the preimplantation embryo development [9]. Furthermore, it has been demonstrated that the proportion of fertilized oocytes with 2PN, the number of 2-cell stage embryos developed, and the percentage of normality of the post-implantation embryos were significantly higher when germinal vesicles were cultured in a maturation medium containing MI compared with control medium [31].

Due to the vital role played by Ca^{2+} oscillations during oocyte maturation, fertilization and embryogenesis, it is crucial to have bioavailable MI in the body. In particular, the presence of high concentrations of MI in the follicular fluid has become a marker of good quality oocytes. Indeed, Chiu et al. demonstrated that high concentrations of MI in the follicular fluid play an important role in follicular maturation and in embryonic development [31].

Another important area in which inositol plays an essential role is the treatment of polycystic ovary syndrome (PCOS). This syndrome affects up to 10% of the total female population in reproductive age, and it is responsible of menstrual irregularities, metabolic and hormonal impairments and infertility. The pathogenesis of PCOS is unknown although insulin resistance, which affects about 70% of the PCOS patients, seems to be the main cause. Indeed, high insulin concentration is the leading cause of hyperandrogenism [32]. The granted relationship between insulin and gonadal function led physicians to choose insulin-sensitizing drugs such as troglitazone, inositol, and mainly metformin as treatment for PCOS. In particular, the administration of MI has been shown to normalize menses and restore ovulation in women suffering of PCOS [33]. Furthermore, it was demonstrated that MI treatment improves metabolic and hormonal pattern, being normally altered in PCOS patients [33–36]. On the contrary, an impaired tissue availability or altered metabolism of inositol has been found to be one of the causes of insulin resistance in PCOS [37].

In addition to this, in a recent study it was shown that MI supplementation was able to treat the metabolic syndrome in postmenopausal women [38].

Concerning the role of MI in male reproduction, it was shown that MI concentration in the seminiferous tubules is dramatically higher than in serum [39]; furthermore, MI concentration was increasing during the movement through the epididimus and the deferent duct [40].

Oligoasthenoteratospermia is a reduction in motility and number of spermatozoa and a change in their morphology. It is one of the most relevant causes of infertility in men. Morphologically altered spermatozoa may influence male infertility due to the production of reactive oxygen species (ROS). In particular, ROS can affect motility, morphology and DNA stability of spermatozoa. Spermatozoa of men suffering from oligoasthenoteratospermia appear entirely covered by "amorphous fibrous material", which gives an excessive viscosity to the seminal fluid, and reduces cell mobility. In a recent study, it has been demonstrated that treatment with MI can be useful to reduce the presence of this amorphous material [41].

It has been suggested that MI might play a role in the osmoregulation of seminal fluid. Indeed, both hypo- and hyperosmotic media have been found to significantly decrease sperm progressive motility and velocities [42]. An increased amount of a particular enzyme, Inositol-1 monophosphatase (IMPA-1) involved in the dephosphorilation of phosphatidylinositol has been detected in asthenozoospermic patients [43]. Therefore, it is possible that increased expression of IMPA-1 in asthenozoospermic patients could alter phosphatidyl inositol signaling pathway and therefore induce a reduction in sperm motility [44].

5.3. Pregnancy

Pregnancy is a physiological state in which there is a high metabolic demand for tissue oxygen. This increased oxygen requirement leads to a higher production of reactive oxygen species (ROS), which could damage cell membranes by lipid peroxidation. During pregnancy, the main source of peroxidized lipids is placenta [45], and the concentration of peroxidized lipids increases in the blood of pregnant women [45]. Furthermore, pregnancy has a negative effect on the activity of several antioxidant enzymes such as superoxide dismutase glutathione peroxidase in liver and placenta [46]. This evidence clearly shows that during pregnancy women face an increase in oxidative stress. Several studies have shown that some pregnancy related disorders depend on both high levels of oxidative stress and unbalanced levels of some micronutrients in the maternal blood. Several studies have been performed to investigate the role of MI and melatonin in restoring and maintaining a healthy pregnancy and fetal development.

5.4. Melatonin

An important pathological condition occurring during pregnancy is preeclampsia, which is estimated to affect 5–7% of all

pregnancies worldwide [47]. Preeclampsia is a leading cause of premature delivery and fetal growth retardation [47]. Maternal complications are renal or liver failure, cerebral edema with seizures, HELLP syndrome (hemolysis, elevated liver enzymes, and thrombocytopenia), and, rarely, death. In particular, preeclampsia is associated with increased lipid peroxidation in both maternal circulation and placenta [48,49]. Placenta is the major source of free radicals and lipid peroxidation products, which enter the blood stream and are transported to distant sites, leading to systemic oxidative stress [50]. Many studies have suggested that melatonin could be an effective treatment for preeclampsia, due to its antioxidant properties [51]. Furthermore, other important properties have been described for melatonin, such as antihypertensive [52] and anticonvulsive action [53]. Therefore, melatonin could be able to exert its beneficial effects on several parameters that are altered in preeclampsia.

Ischemia-reperfusion damage is the transitory interruption of the blood flow. Several studies have demonstrated that melatonin is able to increase the recovery due to anoxia or hypoxia that follows an ischemia event in the reproductive system [54].

Additional studies have shown that melatonin has a protective action on both the fetus and the mother. Indeed, melatonin produced by the mother can easily cross the placenta to enter the fetal circulation, thus leading the photoperiodic information to the fetus [55]. Melatonin is transferred from the maternal to the fetal circulation, generating a day–night difference in melatonin concentration in the circulation of the fetus [55]. Melatonin receptors are expressed in the human fetal SCN [56] and in several areas of the fetal human brain [56]. Furthermore, melatonin suppresses the vasospastic effect of H_2O_2 on the human umbilical artery and this suppressive effect is reduced by two antioxidants, mannitol and catalase [57]. Thus, this indole is responsible to preserve the integrity of tissues (placenta, fetus).

Spontaneous abortion is estimated to occurs in 15–20% of identified pregnancies in young women; the frequency rises up to 35% in women older than 38. The causes of spontaneous abortion can be divided into two main categories: chromosomal anomalies or abnormal intrauterine environment. Several studies suggested that the systemic oxidative stress generated by placenta could be the leading cause of abortion and recurrent pregnancy loss [58]. Furthermore, it has been demonstrated that a deficiency in antioxidant defenses is associated with recurrent pregnancy loss [58], and that the concentration of lipid peroxidation products reaches higher levels before abortion [59]. In normal pregnant women, melatonin levels increase during gestation [60], and contribute to reduce oxidative stress and therefore reduce the abortion rate.

Melatonin is a safe molecule that lacks of toxicity even when administrated at high doses ($\leq$200 mg/kg daily) throughout pregnancy [61]. In addition, melatonin might have beneficial effects on early embryonic development as suggested by preliminary studies [62].

Since delivery occurs with a higher frequency at night (when melatonin levels are high), it has been speculated that melatonin might play a role in stimulating the myometrium likely via its own receptor [63].

It was shown that in myometrial cell contractions and gap junction activity are stimulated by a synergic action of melatonin and oxytocin [63]. In particular, gap junction activity is essential in promoting synchronous myometrial contractions [64].

5.5. Myo-inositol

Neural tube defects (NTDs) are the most frequent human malformation occurring during pregnancy. They are characterized by a defective closure of neural tube during the first 4 weeks after conception. Mis-clousure of the neural tube could lead to serious pathologies and malformations in infants, being in certain conditions incompatible with life (spina bifida, anencephaly). Nowadays, it is generally known that folic acid supplementation in the periconceptional period can prevent the majority of neural tube defects (NTDs) [65]. Several studies performed on NTD mouse models provided several evidence about two different subtypes of NTD that are classified as folate sensitive and folate resistant. In particular, NTDs in mice homozygous for mutations of the Pax3, Cart1, and crooked tail genes are prevented by folic acid [66], whereas NTDs in the curly tail mutant mouse are resistant to folic acid [67]. In humans about 30% of NTDs are folic acid resistant [67].

Recently, several studies showed that a novel therapy for folate resistant NTDs is administration of a combined treatment with folic acid and MI. This novel therapy demonstrated to successfully prevent the majority of NTDs cases, even those folate resistant [4].

As outlined above, preeclampsia is a complication of late pregnancy characterized mainly by hypertension and proteinuria. Recent studies found a correlation between polycystic ovary syndrome or gestational diabetes mellitus [GDM], both diseases correlated with insulin resistance, and a greater risk of developing preeclampsia during late pregnancy [68,69]. In addition to hypertension [70], several features of insulin resistance syndrome, such as obesity [71], dyslipidemia [72], cardiovascular disease [73], systemic inflammation [74], and impaired fibrinolysis [75], are also associated with preeclampsia. Collectively, these data suggest that insulin resistance may contribute to the pathogenesis of preeclampsia. The role of Inositol phosphoglycan P-type [P-IPG] in preeclampsia has been extensively investigated, and an increased P-IPG production has been demonstrated [76]. P-IPG is the second messenger of insulin signaling, therefore it is able to enhances the metabolic effects induced by insulin. Indeed, lower P-IPG blood concentration was associated with insulin resistance [37]. MI supplementation has been demonstrated to reduce the risk of enveloping metabolic syndrome and hyperinsulinaemia and to improve pregnancy outcomes in PCOS women [34,36]. Since PCOS strongly correlates with hyperinsulinaemia and metabolic syndrome, it is possible to speculate that MI supplementation could be an effective treatment in women having higher risk of preeclampsia and GDM.

Premature delivery is an important adverse pregnancy outcome that can lead to severe fetal morbidities, such as the neonatal respiratory distress syndrome [RDS]. Infants suffering of RDS have an immature respiratory system that often leads to premature death. In the last 30 years the strategy for the prevention of RDS has been directed towards the acceleration of fetal lung maturity in utero by means of drugs administered to the mother such as glucocorticoids [GC] [77], and to the development of surfactant substitutes for the treatment of surfactant deficiency at birth. Indeed it was shown that RDS incidence can be reduced by stimulating the pneumocites to produce surfactant. Possible severe side effects of GC have led to development and testing of other drugs capable of accelerating fetal lung maturity. Studies aiming at investigating pulmonary surfactant constituents demonstrated that during fetal development the surfactant system contains very high levels of phosphatidylinositol [78]. By gathering all these data further studies demonstrated that myo-inositol, administered to the mother positively affects fetal lung mechanics, reducing GC adverse effects such as the decrease of lung protein content [79].

The available scientific evidence reveal that melatonin and MI are important, and sometimes essential for a physiological pregnancy. They exert their beneficial actions on maternal and fetal systems, protecting both from external and internal threats that could negatively affect pregnancy.

The literature data summarized in this review clearly demonstrate the crucial role that MI and melatonin have throughout

human reproductive life. Indeed, both of them are necessary in order to have high quality gametes, to have a correct pregnancy and development and to have a healthy reproductive life.

Literature data show no side effects for melatonin and reported only occasional gastrointestinal mild side-effects for MI; therefore MI and melatonin supplementation could be considered relatively safe molecules.

Their supplementation could be an effective treatment to achieve pregnancy, especially if we take into account that oocyte quality decreases with age and that the mean age of women who become mothers for the first time has increased over the last 17 years, from 24.3 to 26.0 years [80]. On the other hand, their real therapeutic efficacy could be relevant in women having high risk to undergo though pregnancy complication such as PCOS patients especially prone to develop gestational diabetes, premature delivery with the increased risk for the infant to suffer of RDS and in women having an increased risk of preclampsia.

Scientific evidence strongly supports the therapeutic effectiveness of MI and melatonin, thus suggesting that their supplementation during pregnancy should be highly recommended.

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REVISIÓN:

Involvement of Inositol in Reproduction

Special Article

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Involvement of Inositol in Reproduction

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Inositol is involved in several aspects of reproduction. It affects overall embryogenesis, may prevent neural tube defects, and stimulates the production of lung surfactant. This article will review the involvement of inositol in reproduction. After describing the biologic function of inositol and its derivatives, studies are quoted in which the role of inositol in fertility, embryogenesis, fetal development, and pregnancy outcome are examined.

Key Words: inositol, reproduction, fertility, embryogenesis, fetal development

Introduction

Mounting evidence supports the role of myo-inositol in human reproduction. Myo-inositol, henceforth called inositol, and its various biochemical derivatives are broadly distributed in mammalian tissues and cells where they perform important biologic functions. The human concentration of inositol in various body fluids and compartments remains within a narrow low range. In newborn infants and fetuses, however, serum concentrations of inositol are several times higher than in adults.[1] Diabetic mothers have an increased risk of offspring that suffer from congenital malformations. Recently, some evidence indicated that this could be explained by inhibition of inositol uptake into the cells by the increased glucose concentration.[2] The inositol content of human breast milk is fairly high indicating that inositol may have a specific function in early human development.[3,4] This review will therefore focus on the role of inositol in reproduction.

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Inositol and Derivatives

Structure

Myo-inositol (inositol) is one of nine stereoisometric hexahydroxycyclohexanes.[5] Inositol is the most predominant isomer in mammalian tissue and cells. Its molecular structure is given in Figure 1. Specific inositol kinases can produce phosphorylated forms of inositol. One to six of the carbons can be phosphorylated, producing a variety of inositol mono-, bi-, tri-, tetra-, and hexaphosphates [inositol(..)P_*].

Inositol also exists in the form of various inositol phospholipids as phosphoinositides. Phosphoinositides are membrane-bound glycerophospholipids built around inositol. Phosphatidylinositol (PI), sn-1,2-diacyl-glycero-3-phospho-D-1-myoinositol, is the most abundant phosphoinositide in mammalian cells (accounting for approximately 5–20% of phosphoinositides in cells) (Figure 1).[5]

The specialized functions of phosphoinositides are mainly fulfilled by phosphorylated derivatives of PI (PIPs, Figure 2A).[5] Seven of these lipids have been identified so far.

The derivative glycosyl-phosphatidylinositol (GPI) anchors various proteins to the plasma membrane. Approximately 150 different GPI-anchored proteins have been identified; they belong to a diverse family of molecules, including enzymes, adhesion molecules, antigens, differentiation markers, and protozoan coat components.[6–8] These proteins are located exclusively on the extracellular side of the plasma membrane.[8] Although there is structural and functional heterogeneity of GPI-linked proteins, all GPI anchors have a highly conserved core structure (Figure 3).[6,7]

Absorption and Endogenous Synthesis

Human adults consume approximately 1 g of inositol per day in different biochemical forms.[9] Free inositol is actively transported across the intestinal wall by a mechanism dependent on sodium and energy, a process that can be inhibited by glucose.[10,11] Inositol is transported in blood plasma at a concentration of approximately 30 μM in healthy subjects.[12] Circulating free inositol is taken up by most tissues by a membrane-associated sodium-dependent inositol cotransporter that is temperature-depen-

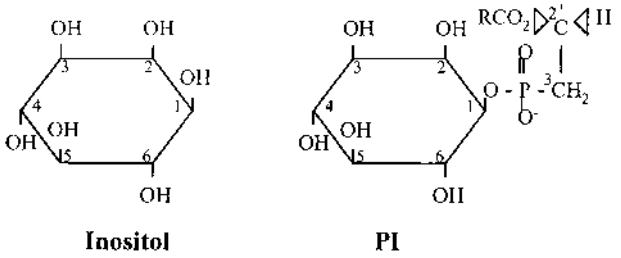

Figure 1. Structures of free inositol (inositol) and phosphatidylinositol (PI).

dent, energy-dependent, and saturable.[9,13] Although this carrier is distinct from the normal glucose transporter in cells, transport of inositol is competitively inhibited by glucose.[13]

Several mammalian tissues such as testes, mammary gland, brain, liver, and kidney are able to synthesize inositol from glucose (Figure 4).[6] It has been estimated that one human kidney can synthesize approximately 2 g of inositol per day.[12] The enzyme inositol-1-phosphatase can be inhibited by lithium.[14]

Cells can also obtain inositol by recycling phosphatidylinositol diphosphate (PIP_2, Figure 2B).[14] The intermediates of this recycling process are several inositol (poly)phosphates. As shown in Figure 2B, some of these enzymes are sensitive to lithium.[14] This means that cells that are exposed to lithium will ultimately be depleted of inositol and will be completely dependent on extracellular inositol.

Clearance

The kidneys play a major role in the regulation of plasma inositol concentration; besides being able to synthesize

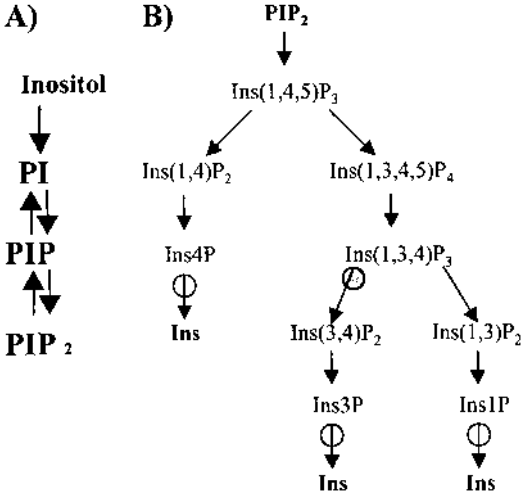

Figure 2. A) A schematic presentation of the conversion of inositol into the phosphoinositides (PI). B) Recycling of phosphatidylinositol diphosphate (PIP_2) to free inositol (inositol) via various intermediate inositol (poly)phosphates (inositol(..)P*). The arrows with circles represent lithium-sensitive enzymes: gray circle = inositol polyphosphate 1-phosphatase, white circles = inositol monophosphate phosphatase.

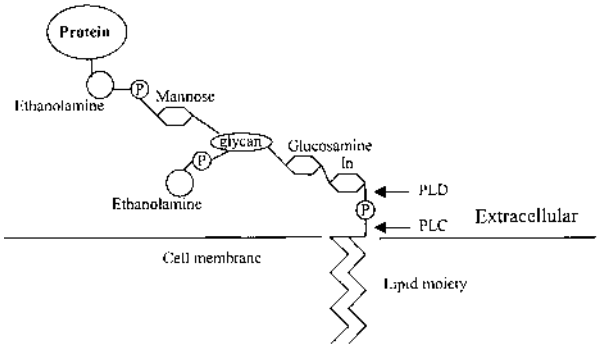

Figure 3. Structure of the GPI-anchor. The anchor can be cleaved by phospholipase C or D (PLC and PLD, respectively).[8] GPI = glycosyl-phosphatidyl inositol, P = phosphate, In = inositol.

inositol, they are the primary sites of clearance. In fact, the kidney is the only organ containing inositol oxygenase (= inositol-1-P synthase), the rate limiting enzyme in the catabolism of inositol.[15] Clearance of inositol by the human kidney was found to be 1 g/day, rising to 21 g/day after exposure to hyperinositolemia.[12] Humans with chronic renal failure show markedly elevated inositol levels that were reduced to normal levels following renal transplantation.[12]

Biologic Function

Free inositol has been implicated in the control of cell volume and cell osmolality.[13] In rats dietary inositol prevents accumulation of triglycerides in the liver, and in gerbils it prevents lipid accumulation in the intestine, suggesting that inositol acts as a lipotropic factor.[16,17]

Several water-soluble inositol phosphates can be distinguished.[18] The most studied inositol phosphate is inositol(1,4,5)P_3. This second messenger is formed by cleavage of PI(4,5)P_2 when cells are stimulated by growth factors or other hormones (Figure 5).[19] The result is the control of the Ca^{2+} concentrations and specific protein phosphorylation processes via protein kinase C (PKC), which modulates a network of various cellular processes.[19] This important signaling pathway is involved in fertilization, cell growth, transformation, secretions, smooth muscle cell contraction, sensory perception, and neural signaling; it has also been implicated in the control of cell proliferation. This information has been reviewed in detail elsewhere.[20,21]

Other inositol phosphates have various biologic functions. Inositol(3,4,5,6)P_4 regulates Ca^{2+}-stimulated Cl^- secretions—a process that is relevant to salt and fluid secretions—and thereby regulates osmolarity, pH, and smooth muscle excitability.[20] Inositol(1,3,4,5,6)P_5 and InositolP_6 are the two most abundant inositol

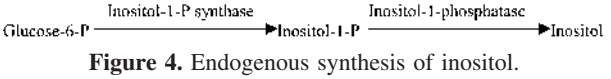

Figure 4. Endogenous synthesis of inositol.

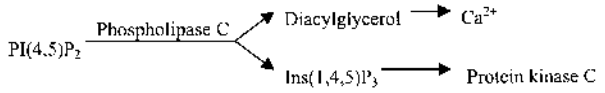

Figure 5. Formation of $Ins(1,4,5)P_3$.

polyphosphates in eukaryotic cells.[21] It has been suggested that $InositolP_5$ and $InositolP_6$ merely serve as stores of inositol and/or phosphate.[22] Their ability to bind to certain proteins, however, might serve several biologic functions. For review of this topic, see Shears[20] and Menneti et al.[22]

PI is enriched in stearic and arachidonic acids, and serves as a source of releasable arachidonic acid for eicosanoid synthesis.[9] In addition PI modulates the activity of membrane-bound enzymes such as Na/K-ATPase, and mediates the transmembrane passage of proteins.[9] PI also affects tyrosine hydroxylase, an enzyme that catalyzes the rate-limiting step in the biosynthesis of catecholamines, dopamine, and norepinephrine.[9] Because phosphoinositides are quickly synthesized and degraded, they seem ideal regulators of the highly dynamic cytoskeleton and endomembrane. There are indications that $PI(4,5)P_2$, $PI(3,5)P_2$, and PI3P may play such a role.[5] Recent studies have revealed that $PI(3,4,5)P_3$ functions as a second messenger of the insulin signaling pathway, leading to increased glucose uptake and glycogen synthesis.[23]

Several other functions of the GPI anchor have been proposed besides anchoring proteins, but it remains obscure why some proteins are anchored by a transmembrane polypeptide and others are anchored by GPI. GPI anchors are clearly susceptible to cleavage by phospholipase C or D (Figure 3).[8] This allows for rapid downregulation of the protein from the cell surface and/or for the generation of its soluble form.[6] This release may be hormonally or developmentally regulated.[6–8] GPI anchors also allow for denser clustering of proteins, and for more lateral mobility in the membrane than transmembrane polypeptide anchors.[6] The GPI anchors can act as targeting signals in polarized epithelial cells, directing the protein to the apical membrane.[8] Furthermore, certain GPI-anchored proteins are involved in the clathrin-independent endocytic process called potocytosis.[6] Transmembrane signaling may occur through GPI-anchored proteins when they interact with protein-tyrosine kinases.[6]

Reproductive Organs

Male

Semen and other secretions of the male reproductive tract are found to be rich in inositol, but inositol's function remains unclear. The testes, which cannot concentrate inositol from blood, seem especially active in inositol synthesis ensuring a high concentration of inositol in the seminiferous tubules.[24]

Hinton et al.[25] identified inositol concentrations in luminal fluids from the ductus deferens from healthy men age 30 to 45 years. The inositol level was within the range of 5.5 to 6.6 mM, which is higher than in blood but lower than in other mammals studied. It has been reported that inositol synthesis in the male reproductive organs is under certain control of the thyroid because inositol-1-P synthase activity is decreased after thyroidectomy in male rats.[24] The testicular inositol synthesis and concentration of rats is not affected by the presence of inositol in the diet or by lowering of the protein content of the diet (from 18% to 7%).[26] The latter situation does, however, decrease the body weight of the rats. Apparently, even under conditions of malnutrition inositol synthesis in the reproductive organs occurs, which contributes to the hypothesis that inositol has a significant role in male reproduction.

Female

Rat studies reveal that the inositol concentration of the uterus and ovaries are under hormonal control.[27] The inositol concentration of female reproductive organs is much higher than in blood serum owing to their ability to concentrate inositol from the blood stream. Interestingly, the inositol concentration in the uterine fluid was much lower than in seminal fluid of the rat meaning that the surrounding inositol concentration for the sperm is lowered when entering the uterus. These high concentrations of inositol in the male and female reproductive tracts suggest that it may influence fertility.

Fertility

In vitro fertilization (IVF) is indicated for some couples with fertility problems. Human (maternal) serum is added to the culture medium for IVF because it contains nutrients, macromolecules, and growth factors that promote the growth of the human embryo. Serum quality varies between patients and seems to be related to the outcome of the IVF procedure.

The correlation between the inositol content of the patients' sera and the outcome of pregnancy using IVF was investigated; results showed that inositol levels increased significantly during IVF treatment compared with the natural cycle.[28] In addition, the embryotrophic properties of the sera were examined by a postimplantational mouse embryo culture showing a strong correlation with IVF outcome. Nevertheless, supplementation of unsupportive sera with inositol indicated that other trophic factors are involved.

Embryogenesis

The function of inositol in embryonic outcome has been investigated in animal studies. These studies revealed that inositol could inhibit the deleterious effects of high NaCl content in culture media; this may be because inositol behaves as an osmolyte, which affects membrane potential and membrane permeability during embryonic development.[29]

The nutritional requirements for exogenous inositol of postimplantational rat embryos in culture decreased as gestational age increased.[30] Moreover, inositol seems to be needed for the development of several parts of the brain. The uptake of inositol for mouse embryos is therefore dependent on the number of cells and the developmental stage.

Outcome

To study the importance of inositol in the outcome of IVF, Chiu and Tam[28] showed that inositol levels before IVF treatment were significantly lower in the group of women who had a spontaneous abortion than in the women who had single or multiple pregnancies; this suggests that inositol levels may be associated with pregnancy outcome.

Studies showed that the blood inositol concentration was not significantly different between nonpregnant women and pregnant women in the first, second, and third trimester.[1] This does not mean however that maternal inositol metabolism is not affected during pregnancy. It is possible that there is increased inositol biosynthesis, decreased renal clearance, or both. To study the fetal inositol concentration, blood was obtained from 18- to 20-week-old fetuses after abortion and from neonates at term by cesarean section.[1] At midgestation the inositol concentration in mixed–umbilical cord serum was fivefold higher than the maternal serum concentration. At term, the serum inositol concentration of the neonates had decreased, but it was still two- to threefold higher than in maternal blood.

To understand whether inositol oxygenase and inositol cyclase are responsible for the high inositol content during perinatal development, the activity of these enzymes in the rabbit fetus was investigated.[15] Results showed that the enzymes in the kidneys are indeed partially responsible for high inositol levels in fetuses. Because the largest increase in oxygenase activity did not parallel the largest decrease in serum inositol level, however, suggests that although inositol oxygenase plays an important role in lowering the inositol level after birth, other unknown factors influence this decrease.

Inositol therefore seems to play a role in the pregnancy outcome: low levels correspond with higher risk of spontaneous abortion. Fetal inositol levels are higher than adult levels, but they decrease soon after birth by action of inositol oxygenase and other unknown factors.

Interaction Between Inositol and Glucose

Infants of diabetic mothers have increased risk of congenital malformations, especially of the central nervous system and the heart. The mechanism whereby hyperglycemia induces embryopathy is not well understood, although it probably has a multifactorial etiology and occurs before the seventh week of gestation.[31]

The inositol uptake from extracellular media by the 10.5-day-old rat conceptus can be inhibited by glucose and by inhibitors of the glucose uptake, suggesting that transporters are responsible for inositol uptake in these conceptuses.[32] Glucose not only inhibits inositol uptake in the water-soluble fraction of the conceptus, but also in the lipid-soluble fraction. It is possible that glucose compromises the complete inositol metabolism in the rat embryo.

In vitro studies have shown that high glucose concentrations in the medium lower the inositol content in the embryo of the cultured rat conceptus, and increases the sorbitol content.[33] This suggests that the high glucose content leads to an increased incidence of dysmorphogenesis caused by the increase in sorbitol, the decrease in inositol, or both. By adding inositol or an aldose reductase inhibitor (reducing the production of sorbitol) to the medium, the effect on the embryos was investigated.[32] By contrast with reduced sorbitol content, inositol supplementation led to an increased inositol content in the embryo and fewer neural tube lesions. The aldose reductase inhibitor also did not restore inositol levels. These findings suggest that the inositol level in the embryo, not the sorbitol level, is involved in the pathogenesis of dysmorphogenesis in diabetic pregnancy.

In one study female Streptozocin-induced diabetic rats were impregnated, after which they were treated with either insulin or oral inositol supplementation from day 6 to day 11.7 of gestation.[33] The inositol content of embryos of the diabetic rats was significantly decreased. Oral inositol supplementation resulted in complete restoration of inositol content in the embryo, but did not improve growth retardation and only partially reduced the incidence of neural lesions compared with the effect of insulin treatment. This may be explained by the fact that glucose competitively inhibits inositol transport into a cell resulting in intracellular inositol depletion. However, another possibility is that insulin directly affects inositol metabolism because insulin treatment was also shown to increase the inositol content of the embryo.

In another study embryos from diabetic rats were examined after their diets were supplemented with 0.08 to 0.5 mg inositol/day between day 6 and day 12 of

gestation.[34] Inositol supplementation did not affect the glucose level or significantly increase the concentration of inositol in the maternal blood. The latter finding is in contrast to the results of Akashi et al.[33] All doses of inositol resulted in a significant decrease in neural tube defects (NTDs) compared with nontreated diabetic rats. The same authors observed that in vitro arachidonic acid supplementation is also able to prevent embryopathy. It is possible that inositol in the arachidonic acid pathway is involved in preventing NTDs in the diabetic rats.

In mouse embryos, arachidonic acid supplementation has been shown to protect against glucose-induced failure of neural tube fusion.[35] Because the arachidonic acid pathway leads to synthesis of prostaglandin (PG), this process may be involved in normal tube fusion. Indomethacin, an inhibitor of arachidonic acid metabolism, was shown to completely inhibit the protective effect of inositol supplementation on glucose-induced neural tube lesions in cultured 8-day-old mouse embryos.[36] To examine whether this effect was caused by the inhibition of PG production, several PGs were added to the medium, namely PGE_2, $cPGI_2$, and $PGF_{2\alpha}$. This showed that PGs are indeed able to prevent neural tube lesions in a medium high in glucose; PGE_2 was the most potent.

Based on these facts it was hypothesized that normal organ growth requires signal transduction, leading to PI turnover, which in turn stimulates the arachidonic acid cascade, and leads to PG synthesis.[31] Any disruption of this biochemical pathway (resulting in a deficiency of PI or arachidonic acid turnover) may lead to a deficiency in PGs that cause congenital anomalies as in diabetic mothers. When the diet of Streptozocin-induced diabetic pregnant rats was supplemented with inositol from day 6 to day 12 of gestation, however, results showed that inositol supplementation lowered, though not completely reduced, the incidence of NTDs to nondiabetic levels.[37] The inositol levels in the yolk sacs of the supplemented group did return to nondiabetic levels, although the inositol levels in the embryos did not. This suggests that hyperglycemia affects the transport between the yolk sac and the embryo. Arachidonic acid levels were lower in the yolk sac and the embryo of both the diabetic inositol-supplemented group and the nonsupplemented group. This suggests that in diabetes, both inositol and arachidonic acid levels are decreased, but that inositol supplementation does not inhibit the decrease in arachidonic levels.

In conclusion, there may be a relationship between the concentrations of inositol, arachidonic acid, and PG, and the occurrence of neural tube lesions in diabetic pregnancies but the exact mechanism involved remain to be elucidated.

Interaction Between Inositol and Lithium

As already mentioned, there are several lithium-sensitive enzymes active in both the synthesis and recycling of inositol. Berridge et al.[14] postulated the "inositol depletion hypothesis" to explain the effects of lithium. They hypothesized that lithium causes depletion of inositol, which ultimately affects the formation of the second messengers diacylglycerol and inositol(1,4,5)P_3.

Because lithium can readily cross the human placenta, several studies have been done on the effect of lithium on embryonal development. In vitro studies demonstrated that 9.5-day-old rat embryos exposed to LiCl for 48 hours have reduced growth, protein content, and differentiation, and delayed development of several organs or even dysmorphogenesis.[38] After addition of inositol to the culture medium the effect of lithium was not attenuated.

Another study also showed that inositol could not counteract the inhibiting effects of LiCl on rabbit blastocyst expansion even at high concentrations. LiCl did affect inositol phosphates: after 1 hour the concentrations of inositolP and inositol(1,4,5)P_3 increased. Although these results do show that extracellular inositol can be incorporated into the rabbit blastocysts, they do seem to argue against the inositol depletion hypothesis of Berridge et al.[14] Because the culture medium does not contain growth factors, however, it is possible intracellular signaling via cleaving of PIP_2 is minimally stimulated, and thus it will take a long time before depletion of phosphoinositide stores is established. Alternatively, it is possible that LiCl acts via another pathway in rabbit blastocysts.

Neural Tube Defects

The NTDs in the *curly tail* (*ct*) mice cannot be prevented by folic acid.[39] Approximately 15 to 20% of the embryos of *ct* mice develop spina bifida aperta. The primary developmental defect in *ct* appears to be causally related to down-regulation of the retinoic acid receptor β (RARβ).[39] It was shown that supplementation of the pregnant *ct* mice with an i.p. injection of 400 mg inositol/kg weight 24 hours before completion of neural tube closure reduced the occurrence of spina bifida by 70%.[39] In vitro, 50 μg/mL inositol also enhanced neuropore closure, which could be eliminated by adding 200 μg/mL LiCl to the culture medium; this suggests that the inositol effect is mediated by the phosphatidylinositol cycle. The LiCl did not worsen the delay of neuropore closure in *ct* embryos in the absence of inositol, however, suggesting that the basic abnormality in *ct* embryos does not involve an inositol deficiency. Investigation of the downstream mechanism of inositol leads to the conclusion that the arachidonic acid pathway does not mediate the effect on the neuropore; arachidonic acid even inhibited the pre-

ventive effect of inositol. The beneficial effect of inositol could be mimicked by a PKC agonist, so inositol could be stimulating this enzyme. Both inositol and the PKC agonist caused up-regulation of RARβ expression. It is possible that inositol supplementation stimulates PKC, which in turn enhances RARβ expression, and prevents NTDs in *ct* mouse embryos.

Fetal Development

Several studies have been performed on the ability of inositol to promote fetal differentiation and growth of the lung because the availability of inositol may control the formation of surfactant phospholipids in immature lung tissue; this may help to prevent neonatal respiratory distress syndrome (RDS). Lung effluent phospholipids can be used to evaluate lung maturity and abnormalities in surfactant after birth.[40]

In one study the food of infants with RDS was supplemented with 40 mg/kg of inositol every 6 hours starting 48 hours after birth.[41] The results showed that 5 days after birth, inositol supplementation resulted in a significant increase in phosphatidylcholine and PI and a significant decrease in sphingomyelin and phosphatidylserine in the tracheal aspirate, demonstrating that surfactant synthesis and secretion is affected by inositol.

In another study, 80 mg inositol kg body weight^{-1} day^{-1} was administered intravenously to premature infants with RDS starting 4 to 12 hours after birth and subsequently every 12 hours for 5 days.[42] Results showed that inositol administration decreased the severity of RDS and mortality owing to respiratory failure, and increased the general rate of survival without bronchopulmonary dysplasia. Moreover, infants supplemented with inositol showed less retinopathy. The role of inositol in the development of the retina is unknown, but it has been shown in animals that the severity of retinopathy in alloxan-induced diabetes is decreased by inositol supplementation.

The free inositol content of milk varies considerably both within and between species. In some species the concentration is much higher than in blood. The inositol content of milk was measured in rats fed diets with different amounts of inositol.[43] After an inositol-free diet, inositol was synthesized and entered the milk. However, the concentration increased approximately linearly when inositol was supplemented 7 to 21 days postpartum. Most of the inositol in milk was a result of active transport from the serum.

The inositol content of human milk has also been investigated. The inositol concentration of 12 preterm infants (27–32 weeks of gestation) was measured from the time of birth to 10 weeks of age, as was the inositol content of their diets.[3] The results showed that inositol concentration in preterm colostrum was highest, followed by full-term colostrum, preterm mature milk, and full-term mature milk, with the lowest inositol concentration found in formulae and parenteral fluids. Serum inositol concentration was highest in infants receiving breast milk. After 2 weeks, there was a significant correlation between inositol intake and serum inositol. Before this period there was no such correlation, possibly owing to prenatal factors, for example, immaturity of renal inositol metabolism.

In another study serum inositol measurements were started directly after birth and the mothers serum inositol concentration was also measured.[4] Results showed that serum inositol concentration in cord blood is higher in premature neonates than in full-term neonates, but that inositol full-term neonates is still higher than the maternal serum concentration. In addition, it was found that the inositol concentration in human milk was inversely correlated with the time of lactation at least during the first 6 weeks of feeding.

In conclusion dietary inositol helps the development of healthy lungs in (premature) neonates. This is possibly the reason why breast milk contains high concentrations of inositol.

Conclusion

The fact that the inositol concentrations in the male and female reproductive organs are several times higher than in serum suggests that inositol has a certain role in human reproduction. This is confirmed by the role of inositol in normal (early) embryonic growth in several animal species. Female diabetic animals especially benefit from dietary inositol supplementation, which lowers the risk of NTDs and other growth defects in their offspring.

NTDs in nondiabetic mutant mice can also be prevented by inositol supplementation, indicating that not only the glucose-inhibited inositol uptake is involved in this protection by inositol. In accordance with the multifactorial etiology, not every NTD can be prevented by inositol; some forms are inositol resistant, whereas others are not. Inositol supplementation can also prevent RDS in preterm infants. This may be why the inositol content of breast milk is so high after preterm deliveries.

The question arises whether inositol should be supplemented to the diet of pregnant women, as is done with folic acid in many countries (i.e., United States, United Kingdom, The Netherlands, Belgium, France, and Spain). It is possible that this treatment could prevent even more NTDs than with folic acid alone. Inositol could also be added to baby formulae and parenteral nutrition formulae to prevent RDS in infants. In our opinion, however, it is too early to recommend giving inositol to women of childbearing age and to neonates because no trials or human studies have been done to

examine the correct dose to be given and the safety aspects of these supplements.

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