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EXPERT OPINION ON DRUG METABOLISM & TOXICOLOGY
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REVIEW

Check for updates

An update on the use of inositols in preventing gestational diabetes mellitus (GDM) and neural tube defects (NTDs)

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ABSTRACT

Introduction: Obstetric history and maternal body composition and lifestyle may be associated with serious complications both for the mother, such as gestational diabetes mellitus (GDM), and for the fetus, including congenital malformations such as neural tube defects (NTDs).

Areas covered: In view of the recent knowledge, changes in nutritional and physical activity habits ameliorate glycemic control during pregnancy and in turn improve maternal and neonatal health outcomes. Recently, a series of small clinical and experimental studies indicated that supplemenation with inositols, a family of insulin sensitizers, was associated with beneficial impact for both GDM and NTDs.

Expert opinion: Herein, we discuss the most significant scientific evidence supporting myo-inositol administration as a prophylaxis for the above-mentioned conditions.

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Article highlights

- Gestational diabetes mellitus (GDM) and neural tube defects (NTDs) may be highly dependent on maternal nutritional habits, especially on the lack of glycemic control.
- Myo-inositol (MI), because of its role as second messenger of insulin and insulin sensitizer, can prevent the onset of GDM, as well as reduce the risk of recurrence of NTDs, leading to the achievement of a healthy pregnancy.
- After insulin stimulation, MI is transformed into DCI, through an NAD-NADH-dependent epimerase, to maintain a suitable MI:DCI ratio, required for tissue metabolism.
- The concomitant administration of MI and DCI seems to perform less well than MI alone, and when the two stereoisomers were directly compared, women treated with MI alone compared to those who received MI plus DCI or DCI alone showed largest benefit.
- For congenital malformations like NTDs, MI peri-conceptional supplementation could represent a novel means of prevention, particularly in those women resistant to folic acid (FA) supplementation.

1. Introduction

Several factors can be decisive in the onset of unfavorable maternal and fetal outcomes, lifestyle among them [1]. In particular, poor maternal nutritional habits, and especially a lack of glycemic control, may represent leading causes of complications including gestational diabetes mellitus (GDM), large for gestation age babies (LGA) [2,3], and congenital abnormalities affecting the fetal central nervous system, namely neural tube defects (NTDs) [4].

Recent scientific evidence [5,6] has shown that the supplementation of inositols is correlated with achievement of a healthy pregnancy. In particular, myo-inositol (MI) may prevent the onset of GDM as well as reduce the risk of recurrence of NTDs. Here, the authors have gathered data from their own and others' studies to provide an overview of up-to-date knowledge on the mechanisms by which MI may prevent GDM and NTDs, and the implications of these findings for future clinical practice.

2. The inositols family

2.1. Biochemical properties

Inositols are sugar alcohols (polyols) that occur in nine different stereoisomeric forms. MI, the most common naturally occurring one, can be transformed into numerous derivatives mainly through either epimerization or phosphorylation of one or several of its hydroxyl groups. The conversion to D-*chiro*-, scyllo-, muco-, neo- occurs by means of specific epimerases. In particular, the conversion rate of MI to D-*chiro*-inositol (DCI) ranges from 7% to about 9%, as measured by the analysis of radiolabeled [3 H]-MI, whereas the production of other isomers is minimal, not exceeding 0.06% of total radiolabeled MI [7]. The human body can actively synthetize MI: in particular, the kidneys produce up to 4 g/day [8]. MI derives from the isomerization of glucose-6-phosphate (G6P) to inositol-3-phosphate (Ins3P) by the enzyme D-3-myo-inositol phosphate synthase (inositol synthase, Ino1 or MIPS1) [9]. Then, inositol-3-phosphate is dephosphorylated to free MI by means of inositol-monophosphatase-1 (IMPA-1 or IMPase) [10]. Free MI can also be generated by the recycling of inositol-1,4,5-trisphosphate (InsP3) and inositol-1,4-bisphosphate (InsP2) generated in inositol phosphate signaling [11]. Endogenous production of MI and DCI varies depending on tissue-specific needs [12], and it is known that certain organs, such as brain, need high MI concentrations (10- to 15-fold higher values than are detected in blood) [9].

Inositol transporters are responsible for uptake and intracellular distribution of inositol. They were classified into two groups according to their transport mechanism. Sodium/myo-inositol cotransporter 1 and 2 (SMIT1 and SMIT2) are coupled with sodium ions while proton/myo-inositol cotransporter (HMIT), coupled with protons [13]. They have a different tissue distribution. So far, only SMIT2 was detected in the duodenum and jejunum, the two districts of intestine where they were found [14].

2.2. Physiological activities

Inside the cells, inositols are not only present as free molecules, but also as components of membrane phosphoinositides (inositol-containing lipids), including phosphatidyl-inositol phosphate and phosphatidyl-inositol bisphosphate (PIP2), both compounds with important physiological roles [15]. Hydrolysis of PIP2 by phospholipase C (PLC) produces inositoltrisphosphate (Ins-1,4,5P3, InsP3), which regulates activities of hormones such as FSH, TSH, and insulin as a second messenger [15]. The interaction between InsP3 and the membrane receptors of mitochondria and endoplasmic reticulum stimulates calcium influx into the cytosol. This leads to the activation of protein kinase C (PKC) and mediates specific cellular responses.

Inositols are involved in insulin signaling, with both MI and DCI functioning as insulin second messengers, although they mediate different actions of insulin in humans [16–19]. There is an interplay between MI uptake and cellular glucose uptake, and MI content is elevated in tissues such as brain, heart, and ovary that have high glucose utilization and consumption [18,20]. MI also inhibits adenylate cyclase, thus reducing the release of free fatty acids from adipose tissues [21]. Conversely, DCI levels are elevated in tissues specialized in glycogen storage, including liver, muscle, and fat, whereas DCI has low abundance in tissues with high glucose utilization [7].

DCI and MI glycans (IPG-P and IPG-A, respectively) shift glucose metabolism toward glycogen synthesis or glucose catabolism, respectively. In the latter case, MI enhances phosphate-dehydrogenase activity (PDH), thus stimulating pyruvate catabolism. IPG-P seems preferentially produced in metabolic stress, following an increase of insulin release. Indeed, after insulin stimulation, MI is transformed into DCI, through an NAD-NADH-dependent epimerase, to maintain a suitable MI:DCI ratio, as required for tissue metabolism [19,22,23].

MI significantly inhibits glucose duodenal absorption and therefore counteracts a rise in blood glucose. This finding can

be explained by a mutual interference of intestinal uptake by MI and glucose [19,24]. In addition to the above-cited effects, MI also acts as one of the FSH second messengers in the ovary, as well as of the TSH in the thyroid, both mechanisms mediated by adenylate cyclase [19,25].

2.3. Pharmacokinetics

Some patients, identified as 'inositol-resistant', exhibit a weaker than normal response to MI treatment, owing to poor absorption by the oral route.

Aiming to improve MI clinical efficacy, Monastra et al. planned a pharmacokinetics study [26] in human healthy volunteers to test the effects of α-LA on MI bioavailability, considering the wide range of α-LA activities, especially the effects on improved mineral bioavailability [27].

When administered alone, MI average peak plasma concentration at 180 min increased about threefold *versus* the baseline, whereas when associated with α-LA, it augmented fourfold.

After administration of MI and α-LA, MI plasma concentrations were significantly higher than after administration of MI alone: the increase of Cmax (μmol/l) was 32.4%, while AUC (0–300) increased by 27.5% [26].

Moreover, with the aim to explain the mechanism underlying this effect, the authors carried out an *in vitro* experiment. In detail, they observed that in the presence of digested α-LA, MI passage across a monolayer of human Caco-2 cells, used to simulate the intestinal barrier, significantly increased, thereby achieving a higher plasma concentration compared with MI administration alone [26].

This combination of MI with α-LA has proven to be useful for improving the treatment of Polycystic Ovary Syndrome (PCOS) MI-resistant patients [28]. A goal for the future will be to investigate further this combination of MI and α-LA, for its ability to enhance the potential beneficial effects of MI on complex pathologies, such as GDM and NTDs (described below).

3. The safety profile of inositols

Before discussing the efficacy of inositols in some pathological conditions, it is important to consider the available data on inositol's safety as a supplement. As stated by the United States Food and Drug Administration (FDA), MI is included in the list of compounds that are 'generally recognized as safe' (GRAS). This means inositol has proven to be free of side-effects and, importantly, is safe for use in pregnancy [19,29].

A recent meta-analysis on 965 pregnant women affected by GDM, who were randomized to receive MI, placebo, or no treatment, revealed no adverse maternal events, and no congenital malformations in the fetuses or newborns [30]. Moreover, a Cochrane Review of the relationship between inositol and GDM reported no adverse events associated with inositol antenatal supplementation [31].

4. MI in management of GDM

4.1. GDM diagnosis

GDM, defined as glucose intolerance solely diagnosed during pregnancy, is characterized by increased insulin resistance and hyperglycemia. It represents a worldwide public health problem with a variable prevalence ranging from 2% to 30%, depending on the diagnostic criteria and the population studied [32]. Several clinical risks have been associated with GDM, both for the mother, such as hypertension, cesarean section, and possibility of developing type 2 diabetes, as well as for the newborn, such as preterm birth, macrosomia, shoulder dystocia, neonatal hypoglycemia, respiratory distress syndrome, and congenital abnormalities [33]. Owing to the strong correlation between GDM and maternal/perinatal/neonatal complications, an early diagnosis is highly recommended.

In a recent review and meta-analysis, it was shown that risk for perinatal mortality, and neonatal hypoglycemia was greater among early-onset GDM women compared to late-onset GDM women, despite treatment [34].

As a result, screening for GDM should be done as soon as pregnancy is confirmed, especially in high-risk patients, by using fasting plasma glucose cutoff values [35].

The oral glucose tolerance test (OGTT) remains the gold standard for a GDM diagnosis, even though not devoid of limitations, principally associated with patient compliance [36]. One approach, adopted in some countries is a two-step vs one-step testing procedure, although it appears to have similar diagnostic efficiency [37]. Depending on resources, a universal screening of pregnant women has been implemented in some locations while others choose selective screening based on risk factors (age, obesity, obstetric history, and ethnicity) [38]. Some serum proteins like insulin, adiponectin, C-reactive protein, sex hormone globulin, and glycosylated fibronectin are widely investigated as markers of this pathological condition [39–41]. Although these results are promising, such predictive biomarkers are yet to achieve clinical applicability.

Advances in molecular biology have recently identified novel biomarkers, which offer the potential to improve GDM risk prediction and, consequently, enable application of intervention protocols. These biomarkers can be readily measured in biological fluids, such as blood, plasma, and serum, allowing accurate low-cost diagnosis with improved patient compliance [42]. For example, it was observed that genetic variants, such as single nucleotide polymorphisms (SNPs) in genes involved in specific metabolic pathways may predispose pregnant women to develop GDM [43]. This potentially would allow analysis of a set of SNPs that provide indicators for GDM screening [44].

DNA methylation could also represent an interesting potential parameter correlating with a range of pathophysiological processes, including GDM [45]. Indeed, it was demonstrated that DNA methylation is highly altered in the placenta and cord blood of women with GDM [46]. Micro-RNAs have also proven to be important metabolic regulators during pregnancy, playing a role in GDM onset [47], which would make them ideal candidates for GDM detection. However,

establishment of the specificity and sensitivity of potential new biomarkers for GDM diagnosis is required – including external validation and population studies – before they can be used in clinical practice.

4.2. Why GDM should be treated

The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study evaluated the risk of adverse outcome associated with degrees of glucose intolerance during pregnancy and confirmed the link between maternal glucose and neonatal adiposity and suggested that this relationship is mediated by fetal insulin production influencing fetal growth [48].

Moreover, if not diagnosed and/or untreated, GDM can lead to short-term risks including labor induction, shoulder dystocia, C-section, macrosomia, large for gestational age (LGA) babies, neonatal hypoglycemia, NICU admission [49]. From a lifelong perspective, approximately 40% of all women who are diagnosed with GDM progress to type 2 diabetes within 5 years post-delivery, in addition to their increased risk of GDM in future pregnancies [50,51].

Noteworthy, infants born to mothers with gestational diabetes are at increased risk of impaired glucose regulation, obesity, and diabetes, leading to a vicious cycle of accumulated risks in the next generation [52,53]. A recent follow-up study reported the longitudinal effects of GDM in the offspring. It included 4.160 children aged 10–14 years, whose mothers had a 75-g OGTT at ~28 weeks of gestation with blinded glucose values and underwent an OGTT. Offspring exposed to untreated GDM in utero are insulin-resistant with limited β-cell compensation compared with offspring born to mothers without GDM. GDM is significantly and independently associated with childhood intolerance to glucose [54].

4.3. Lifestyle changes

With regards to management, before any therapeutic intervention, the mainstay for GDM approach is based on lifestyle changes with adequate diet and exercise, aiming to achieve glycemic targets. A recent individual patient data meta-analysis reported that such an approach was efficacious in containing gestational weight gain while the impact on GDM diagnoses was reported only for selected populations. No other perinatal outcomes were reported as significantly affected by lifestyle changes [55]. This was mainly due to the heterogeneity of approach in the primary trials which included different diets and physical activity programs. Moreover, adherence to lifestyle changes is generally poor and it is inversely correlated with efficacy, namely on GDM onset [56]. Anyway, if glycemic targets are not met, then insulin treatment should be considered without delay, which may also improve the woman's health-related quality of life [57]. Indeed, insulin treatment is associated with less risk of neonatal hypoglycemia and provides a good plasma glucose control [58].

4.4. Insulin sensitizers

Earlier observational studies [59–61] suggested that metformin treatment was associated with a lower risk of GDM, although the designs of these studies appear prone to different sorts of bias. Indeed, the above findings were not confirmed in a trial of 274 pregnancies among 257 women with PCOS who were randomly assigned to receive metformin (2000 mg/day) or placebo from the first trimester until delivery. The patients treated with metformin (17.6%) and placebo (16.9%) presented a very similar prevalence of GDM. No significant differences were observed in the prevalence of preeclampsia or preterm delivery [62].

Two further trials investigated metformin for the prevention of large for gestational age babies. They included a total of >800 pregnant obese mothers (BMI >30) who received 1500 mg (till 2500 mg) oral metformin daily (or placebo), starting early in the second trimester. No efficacy of intervention was reported on birthweight and the rate of GDM was similar in metformin- and placebo-treated groups [63,64]. Interestingly, a reduction of preeclampsia was reported with metformin (3.0%) in respect to placebo (11.3%) [63].

On the other hand, several clinical trials demonstrated the significant efficacy of inositols, especially MI, in GDM management (Table 1). Indeed, the concomitant administration of MI and DCI seems to perform less well than MI alone. When the two stereoisomers were directly compared, the non-inferiority analysis demonstrated the largest benefit for women treated with MI alone compared to those who received MI plus DCI or DCI alone [65]. This effect can also be explained referring to the competition between MI and DCI at the transporter site in the intestine. Indeed, SMIT2 in human cells exerts a similar affinity for both compounds with a Km value between 100 and 160 μM, depending on the cell model used to determine it [13]. An *in vivo* pharmacokinetic study demonstrated the DCI inhibitory effect on MI bioavailability [66]. Obviously, a significant increase of DCI concentration in the gut, due to exogenous administration, hinders MI absorption with consequences on its bioavailability and, implicitly, its efficacy.

A recent systematic review and meta-analysis supported the prophylactic treatment with MI in the prevention of GDM. Among the included studies, all but one administered MI alone (2 g MI plus 200 μg folic acid, twice daily), while another one used a combination of lower MI dose (1100 mg) plus 27.6 mg DCI/day. The findings demonstrate that the administration of 2 g MI twice daily was associated with improved glycemic homeostasis, reduced GDM, and preterm delivery rate, with respect to control groups, all supplemented with folic acid, except one trial [30].

Moreover, a secondary analysis from three trials reported that the same dose of MI supplementation in women at risk for GDM affected birthweight, reducing the rate of both macrosomic newborns and LGA babies [76]. Such neonatal impact agrees with the evidence that dietary glycemic index directly correlates with LGA in newborns [77] and indirectly demonstrates that MI supplementation improves glycemic control in those pregnancies.

Table 1. Clinical trials investigating the effects of inositols supplementation in pregnant women.

Study	Population	Active Intervention	Main outcome	Delivery outcomes	Perinatal outcomes
Matarrelli et al 2013 [67]	Non-obese women with an elevated fasting glucose in the first or early second trimester N = 36 (treated) N = 39 (control)	MI	Lower abnormal OGTT and insulin therapy	Lower rates of Polyhydramnios	Lower neonatal hypoglycemia, birth weight, fetal abdominal circumference, higher GA at delivery
D'Anna et al 2013 [68]	Outpatients with a parent with type 2 diabetes N = 110 each group	MI+FA	Lower GDM rate	No differences in CS and PTB rates	Significant differences between groups on macrosomia rate and birth weight
Malvasi et al 2014 [69]	Non-obese healthy women at 13th –24th week of pregnancy N = 24 each group	MI+DCI+FA $+Mn^{2+}$	Improvement of glycemia and blood parameters (except diastolic blood pressure	–	–
D'Anna et al 2015 [70]	Obese women at 12–13 weeks of gestation N = 110 each group	MI+FA	Lower GDM rate	No differences in CS and PTB rates	No differences in birth weight and macrosomia
Santamaria et al 2016 [71]	Overweight, non-obese women N = 110 each group	MI+FA	Lower GDM rate	No differences in CS and PTB rates	No differences in birth weight and macrosomia
Lubin et al 2016 [72]	Women with GDM uncontrolled by diet N = 32 (treated) N = 28 (control)	MI+FA	Lower need of insulin treatment	Lower labor induction	No differences in birth weight and macrosomia
Farren et al 2017 [73]	Women with a family history of diabetes N = 120 each group	MI+DCI+FA	No changes in GDM rate	No differences in CS and PTB rates	Higher incidence of neonatal hypoglycemia and lower neonatal jaundice
Fraticelli et al 2018 [74]	Women with GDM N = 20 each group	MI+FA DCI+FA MI+DCI+FA	Lower HOMA index and weight gain with MI Lower need for insulin therapy in MI and MI+DCI	No differences in CS, induction and PTB rates	Lower birth weight in inositol groups
Pintaudi et al 2018 [75]	Women with GDM N = 6 each group	MI+FA	Reduction of glucose variability	No differences in CS and PTB rates	No differences in birth weight and macrosomia
Celentano et al 2020 [65]	Non-obese women with elevated fasting glucose in the first or early second trimester N = 105 (treated) N = 52 (control)	MI DCI MI+DCI	Lower abnormal OGTT with MI	No differences in CS and PTB rates	Lower birth weight, fetal abdominal circumference, and higher GA at delivery with MI

Finally, in a condition where insulin-resistance is pathogenetic like PCOS, the beneficial effects of MI supplementation on hormonal and reproductive disturbances [20] were specifically reported also in pregnancy, where GDM rate was reduced [78].

From the experimental point of view, recent animal studies showed that combined inositols treatment (MI/DCI 40:1) in pregnant mouse complicated by metabolic syndrome and obesity improved blood pressure, glucose levels at the glucose tolerance, as well as leptin levels [79]. In addition, the same combination of inositols treatment improved not only maternal outcomes, but also offspring weight at birth as well as glucose tolerance test, and vascular reactivity in their adult life, thus reducing the vicious cycle of dysmetabolism [80].

Finally, it should be highlighted that inositols administration in pregnancy was well tolerated and found to be safe, unlike metformin which has been associated with several gastrointestinal symptoms [63].

5. Prevention of NTDs: the role of MI

5.1. Background to NTDs

Neural tube defects (NTDs) are a group of congenital malformations affecting the brain and spinal cord that originate at various times during gestation. Some authors use the term 'NTDs' to denote defects that specifically result from faulty neural tube closure, particularly myelomeningocele (i.e. open spina bifida) and anencephaly. However, NTDs are sometimes considered to also include defects that arise by other embryonic and fetal mechanisms, including encephalocele (a brain herniation defect) and skin-covered low spinal 'dysraphic' conditions (secondary neurulation defects) [81]. Hydrocephalus and Chiari II malformation are closely associated with myelomeningocele and so are frequently present in individuals with NTDs.

Many different factors affect susceptibility to NTDs, including genetic variants and environmental influences, including the antiepileptic drugs valproic acid and carbamazepine [81]. Nutritional status is important, with folate and vitamin B12 levels in maternal blood being independent risk factors for NTDs [82]. When taken together with the findings of the randomized controlled trial of vitamin usage in NTD prevention [83], this evidence has led to the recommendation that all women planning a pregnancy should take folic acid (FA)-containing supplements to minimize the risk of NTDs [84]. Moreover, many countries now mandate addition of FA to staple foods, to counteract folate deficiency on a population-wide basis, and so enhance NTD prevention [85].

5.2. Obesity and diabetes as risk factors for NTDs

Maternal obesity and poorly controlled diabetes mellitus during pregnancy are established risk factors for NTDs [86,87],

although the complexity of the diabetic milieu has made it difficult to pinpoint the precise mechanism(s) by which the diabetic state enhances NTD risk.

The polyol pathway became of attention in the last century. Aldose reductase, key enzyme in this pathway, converts glucose to sorbitol, which is further processed to fructose. Under normal conditions, this enzyme has a low affinity for glucose, and it processes small amount of substrate. However, in diabetes mellitus, the hyperglycemia in some cells causes a marked production of sorbitol with a concomitant reduction of MI concentration.

The depletion of MI has long been thought to be the underlying defect responsible for decreased nerve conduction velocity in experimental diabetes, leading to increased embryo malformations, such as neural tube defects.

Therefore, the potential of aldose reductase inhibitors has been investigated, with the aim to restore MI tissue content. However, treatment with aldose reductase inhibitors failed to correct MI reduction, did not prevent malformations in the embryos and was found associated with significant side effects [88–90].

The failure of aldose reductase inhibitors to prevent diabetic malformations suggested that the polyol pathway was not involved; in contrast, supplementation with MI restored MI tissue content and reduced the incidence of neural tube defects, suggesting the involvement of MI in the mechanism of diabetic embryopathy [91,92].

While hyperglycemia alone is sufficient to cause NTDs in cultured rodent embryos [93], other features of the diabetic environment, including the ketone β-hydroxybutyrate, can also produce NTDs [94].

The effects of the diabetic milieu on the developing rodent neural tube include intracellular oxidative stress and neuroepithelial cell apoptosis, with many studies showing that antioxidant treatment can protect against these effects [95,96]. Nevertheless, precisely how such effects lead to NTDs is unclear. One possibility is a reduction in cellular expression of genes that are vital for neural tube closure, either as a result of cell death or down-regulation of gene expression. For example, *Pax3* loss of function causes both cranial and spinal NTDs [97], and some studies have identified disruption of *Pax3* expression in mouse embryos of diabetic mothers [98].

In humans, hyperinsulinemia has been suggested as a possible mechanism leading to elevated NTD risk in obese mothers who often have type II diabetes [99]. Even in the absence of diabetes, NTDs have been significantly associated with maternal periconceptional increased intake of simple sugars and a high glycemic index, as well as with features of the 'metabolic syndrome' [100–102]. Hence, there is plentiful evidence linking dysregulation of glucose metabolism, in both types 1 and 2 diabetes, with NTDs.

5.3. Inositols and NTDs: evidence from animal models

Diabetic tissues tend to be inositol-deficient, in conjunction with elevated glucose levels [103], and rat embryos cultured under hyperglycemic conditions exhibit diminished MI levels [90]. At neurulation stages, MI deficiency but not other 'vitamin' deficiencies leads to failure of cranial NTDs in cultured rat embryos [104]. Hence, inositol is essential for the neural tube closure process. In terms of prevention, MI supplementation can diminish the frequency of NTDs resulting from hyperglycemic conditions in rats, both in vivo and in embryo culture [105,106].

Perhaps of most relevance to human NTDs, inositol can reduce the frequency of NTDs in a mouse genetic NTD model [107]. A multifactorial etiology underlies most human NTDs, with genetic predisposing variants interacting with environmental factors. In the *curly tail* mouse, a hypomorphic allele of the *Grhl3* gene is the major genetic change, with 'modifier' genetic variants and various environmental influences affecting frequency and severity of NTDs [108,109]. Importantly, NTDs in the *curly tail* strain are FA-resistant, therefore mimicking FA non-responsive human NTDs. Both MI and DCI are preventive in the *curly tail* model, an effect that is dependent on specific isoforms of protein kinase C [110,111]. This preventive action of MI in the *curly tail* strain has been replicated in an independent laboratory [112]. Furthermore, in a related strain in which cranial NTDs were inducible by folate deficiency, these defects were prevented by maternal MI supplementation [113]. These findings suggest potential overlap in response to FA and MI for some NTDs.

5.4. Inositols and NTDs: evidence from human studies

Diminished MI concentrations have been detected in the plasma of pregnant Dutch women with NTD-affected fetuses. The odds ratio for NTD risk associated with extremely low maternal MI concentration (10–13.2 microM/L) was 2.6 (95% CI, 1.1–6.0) [114]. Moreover, a genetic connection between inositol metabolism and NTDs was suggested by findings that cranial NTDs arise in mice with loss of function of *Itpk1*, encoding inositol 1,3,4-trisphosphate 5/6-kinase which produces the highly phosphorylated metabolite, inositol hexakisphosphate (IP6) [115]. A subsequent study in a high-risk area of China found an association of a maternal polymorphism of *ITPK1* increased risk of NTD pregnancy [116].

5.5. Inositol in the prevention of human NTDs

Population-wide supplementation with FA through food fortification has significantly reduced the prevalence of NTDs in many countries [85]. However, FA is only partially effective, and NTD cases continue to occur worldwide, with or without voluntary or mandatory FA consumption. Hence, additional strategies are needed to achieve further prevention.

Building on the data from animal models, an inositol supplementation study was conducted in Italy, among high-risk women who had experienced 1 or 2 previous NTD-affected pregnancies. In their next pregnancy, the women took 500–1000 mg/day MI plus 5 mg/day FA, from 2 months preconception, until 60 days of pregnancy. No NTD recurrences were observed among 29 pregnancies of 27 women, whereas 2–8 recurrent NTDs would be expected, based on typical population recurrence frequencies [117]. Most of the women were likely folate-resistant, having undergone appropriate peri-conceptional folate intake in their previous NTD-affected

pregnancies. Hence, these findings support a preventive effect of MI on NTD recurrence in high-risk pregnancies.

A phase I/II double-blind, case-control clinical trial (the PONTI study; EudraCT2006-000157-22) was performed to gain further experience of MI supplementation in human pregnancy [118]. The subjects were UK women with a previous NTD pregnancy who planned to become pregnant again. Of 117 women contacted, 99 proved eligible, and 61 undertook a detailed screening questionnaire of whom 47 (77% of those screened) agreed to be randomized to periconceptional supplementation with MI (1 g/day) plus FA (5 mg/day), or placebo plus FA. Of 33 randomized pregnancies, there was one NTD recurrence in the placebo plus FA group (n = 19) and no recurrences in MI plus FA group (n = 14). Of 52 women who declined randomization, the periconceptional supplementation regime and outcomes of 22 further pregnancies were documented. Two NTDs recurred in women who took only FA in their next pregnancy (n = 3), whereas there were no recurrences in women who took MI plus FA in their next pregnancy (n = 19). Overall, NTDs recurred among 0/33 MI-supplemented pregnancies and 3/22 FA-only pregnancies (p = 0.06, Fisher's Exact test).

Combining data from the Italian and PONTI studies, with post-PONTI experience of MI use, a total of 76 women at high risk of NTDs (52 with one and 24 with two or more previous affected pregnancies) have taken MI plus FA in a subsequent pregnancy, with no recurrences. Assuming a 3% recurrence risk after one NTD pregnancy and 10% after two [119], as used in genetic counseling, this indicates that 4 recurrences would be expected among 76 pregnancies. The observed 0/76 recurrences among MI-supplemented pregnancies are significantly different from this expectation (Single Proportion Fisher's Exact, p = 0.016). These observations, although on small patient numbers are encouraging that MI may have value in increasing NTD prevention beyond that achievable by FA alone.

5.6. Inositol and NTD prevention: next steps

A definitive answer to the question of the efficacy of MI in enhancing NTD prevention is likely to require a fully powered, randomized clinical trial (RCT). However, this will necessitate large numbers of pregnancies, owing to the relatively low recurrence rate for NTDs. Given the current state of knowledge about inositol and NTD prevention, it is important to consider whether an RCT is ethically acceptable, given that the study design would require withholding inositol supplements from a significant number of pregnancies.

Glasziou et al. [120] argue that expensive and time-consuming RCTs may be unnecessary in cases where a novel treatment has an obvious effect (i.e. a large 'signal-to-noise' ratio). However, for NTDs, the effect of inositol at the population level is likely to be small, as NTD frequency is relatively low (0.1% for first occurrence; 2–3% for recurrence), and any effect of inositol will be additional to FA-mediated prevention. On the other hand, for individual women who exhibit FA-resistance, inositol is currently their only option to increase the likelihood of a normal pregnancy.

Freedman [121] suggested that 'clinical equipoise' should be considered to exist when there is genuine uncertainty within the expert medical community about the preferred treatment. Under such circumstances, an RCT would be ethically justified. In the case of NTDs, MI + FA supplements certainly have not superseded FA-only supplements; worldwide, the great majority of low-risk and high-risk women receive FA only (or no supplement at all). To date very few pregnancies have been supplemented with inositol, and this has been used largely where high-risk women have sought advice from the authors of this article.

We conclude that the Glasziou criterion for avoiding an RCT is not met by MI supplementation for NTDs, and that currently the expert field is in a state of clinical equipoise with regard to use of MI in addition to FA. Hence, an RCT appears indicated to provide a definitive answer to the efficacy of MI as an adjunct preventive supplement for NTDs.

6. Conclusions

Gestational diabetes mellitus (GDM) and congenital abnormalities of fetal central nervous system, such as neural tube defects (NTDs), are strongly correlated to glycemic status of pregnant women.

Before starting a therapeutic intervention to achieve a healthy pregnancy, the principle approach to reach the glycemic targets is based on lifestyle changes by means of an adequate diet and exercise.

When glycemic targets are not addressed, an intervention is reasonable to avoid complications both for the mothers and for the fetus.

A supplementation of MI starting at least one month prior to conception and continued until 36th week of pregnancy is expected to prevent NTDs as well as GDM onset and its related negative perinatal outcomes. As a side effects free molecule at the usually administered doses, MI therefore becomes a candidate for being one of the first tools to be used in pre-conceptional medicine.

7. Expert opinion

The data reported in this brief narrative review are highly encouraging over the use of inositols, namely MI, in pregnant women. To date, the controlled studies available in literature are reassuring about its safety and tolerability. Adverse effects in the short-term period have not been reported yet, either for mothers or babies, despite the widespread use of these supplements since several years, in many countries.

Although confirmatory studies will be necessary to elucidate the mechanisms involved [122], there is a general agreement that MI administered early in pregnancy effectively prevents GDM onset (Figure 1). Proofs are available for different categories of women at risk, namely overweight, obese, and patients affected by PCOS. In addition, significant reduction of gestational hypertensive disorders, preterm birth, and large for gestational age babies suggest a perinatal benefit of MI supplementation. Whether this would apply to low-risk population requires the design of appropriate controlled trials, hopefully comparing different inositols combinations.

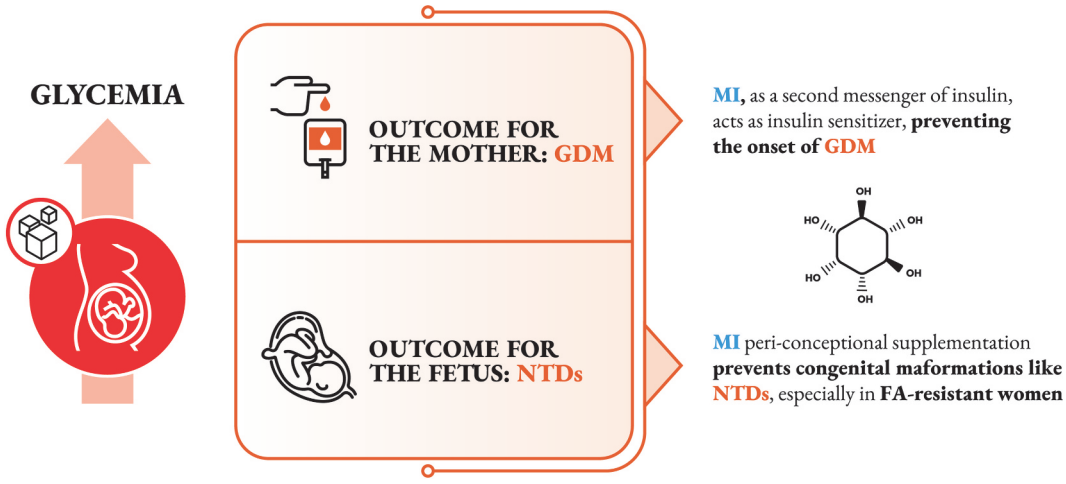

Figure 1. Schematic representation of the effectiveness of MI supplementation in preventing GDM and NTDs.

To date, although several clinical trials are ongoing, MI supplementation has not yet been inserted in clinical guidelines for GDM prevention/treatment.

For congenital malformations like NTDs, MI can offer a novel means of prevention via peri-conceptional supplementation, particularly in those women whose previous pregnancies have proven resistant to FA supplementation (Figure 1).

Thus, given the extensive data documenting the safety of peri-conceptional MI supplementation derived from trials done in Assisted Reproduction, a combination of MI and FA could be recommended for every woman at high risk for NTDs.

Furthermore, it would be interesting to carry out studies to reduce the dose and perhaps improve absorption and bioavailability. Recent research using co-administration of MI and α-LA [26] is an example of how MI usage may be clinically improved. We believe that this association in the next years could conquer a relevant position as insulin-sensitizing treatment for GDM and NTDs prevention, due to both efficacy and absence of side effects.

Declaration of interest

V Unfer is an employee at Lo. Li. Pharma s.r.l., Rome, Italy. F Facchinetti has been a consultant of the same company. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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