

Prevention of neural tube defects and maternal gestational diabetes through the inositol supplementation: preliminary results

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Abstract. – OBJECTIVE: Our study aims to demonstrate that the use in the preconceptional period until the 24th week of pregnancy of inositol and folic acid, first of all, preserves the product of conception from neural tube defects (NTDs) and then, thanks to inositol supplementation, it possibly counteracts and prevents the onset of maternal gestational diabetes (GDM).

PATIENTS AND METHODS: We have collected data derived from pregnant women arrived at our laboratory, from January 2014 to January 2016, with no family history of type 2 diabetes and hypertension. The first group (n = 68 women) was treated from the preconceptional period until the 24th week of pregnancy with 1.75 g/day myo-inositol, 250 mg/day D-chiro-inositol, 12.5 mg/day Zinc pidolate, 100 mg/day methylsulfonylmethane, 120 mg/day Vitamin C and 400 mcg/day (6S)-5-methyltetrahydrofolic acid. The control group (n = 72) was only treated with 400 mcg/day folic acid. The main outcome measure was the prevalence of maternal GDM. Secondary outcome measures were the prevalence of NTDs and fetal macrosomia.

RESULTS: A significant difference was found regarding body mass index (BMI), fasting oral glucose tolerance test (OGTT), after 1-h-glucose OGTT, 2-h-glucose OGTT, glycated hemoglobin (HbA1c) and serum folate, between the two groups. Five infants, in the control group, weighted greater than 4 kg. Moreover, we found a positive correlation between HbA1c and OGTT at the 24th week of pregnancy.

CONCLUSIONS: This study shows the efficacy of preconceptional supplementation of inositol to reduce the risk of the onset of GDM and to confirm the importance of folic acid supplementation to avoid NTDs development. Moreover, the positive correlation between HbA1c and OGTT may be useful to consider the use of HbA1c as a single tool for GDM prevention and diagnosis in selected woman in pregnancy.

Key Words:

D-chiro-inositol, Myo-inositol, Folic acid, Neural tube defects, Maternal gestational diabetes, HbA1c.

Introduction

Pregnancy, due to placental hormones action, is characterized by a physiological increase of insulin resistance (IR), which has the aim to promote the use of nutrients by the fetus, especially in the second and third trimesters of pregnancy¹. However, this physiological mechanism could lead to the onset of gestational diabetes mellitus (GDM)².

Several risks are associated to GDM. The fetus could face macrosomia and birth injuries for shoulder dystocia, the newborn could head for neonatal hypoglycemia, respiratory distress syndrome and childhood obesity. Worthy of note are also the risks met by the mother, who could experiment a caesarean delivery, hypertensive disorders and an increased risk of developing type 2 diabetes later in life³. Therefore, there is a need for a safe, simple and an efficient intervention to prevent the development of GDM.

In order to prevent perinatal complications of mothers and infants mentioned above, the goal of glycemic control during pregnancy should be to bring plasma glucose levels as close to normal as possible without the development of hypoglycemia^{4,5}. Inositol, or cyclohexane-1,2,3,4,5,6-hexol, is a chemical compound present both in animal and plant cells, either in its free form or as a

bound-component of phospholipids or inositol derivatives⁶. It exists under nine stereoisomeric forms depending on the spatial orientation of its six hydroxyl groups.

Whereas intracellular inositol pool is almost (>99%) constituted by myo-inositol in most tissues, significant differences have been recorded in the concentration of myo-inositol and d-chiro-inositol, another important stereoisomer, in fat, muscles, and liver. This different distribution reflects the distinct functions that probably the two isomers play in those tissues⁶. Abnormalities in myo-inositol and in d-chiro-inositol metabolism have been involved in the development of several diseases and in particular in the development of IR and diabetic complications^{7,8}.

Moreover, as described by Crawford et al⁹, myo-inositol has many benefits due to its role as a second messenger. It helps to maintain a normal production of thyroid hormones (i.e. euthyroidism) when used as a co-treatment in patients with subclinical hypothyroidism and autoimmune thyroiditis. Myo-inositol has also been associated with an improvement in premenstrual dysphoric disorder (PMDD); moreover, it has been linked to ameliorations in a range of symptoms of polycystic ovary syndrome (PCOS), including improved insulin sensitivity and ovulatory function in young women affected by PCOS. Therefore, myo-inositol has been associated with improvements in hyperandrogenism in women with PCOS, and increased the number and the quality of oocytes in women undergoing *in vitro* fertilization (IVF) treatment for a previous history of infertility¹⁰.

OGTT measurement is an important part of glycemic control during pregnancy; however, its applicability is difficult due to low patients' compliance; therefore, it may be necessary to perform the glycemic control using alternative indicators (HbA1c, glycated albumin, fructosamine)^{11,12}.

In particular, HbA1c detection has been recommended in the 59th Annual Meeting of Diabetes Association of USA and is currently the most accepted measure of chronic glycemia outside of pregnancy¹³.

Worldwide, birth defect or congenital anomalies are a leading cause of fetal death, infant mortality and morbidity in childhood (approximately 104,000 out of 5.2 million (2%) newborns in Europe per year show congenital anomalies)¹⁴. Of the 5.2 million births in Europe each year, approximately 104,000 (2%) will be born with congenital anomalies¹⁵.

In the last report¹⁶, EUROCAT showed that neural tube defects (NTDs), the most frequent congenital abnormalities of the central nervous system, which are largely preventable by raising preconceptional folate status, have not decreased in prevalence over the last 10 years: in fact the prevalence was 9.47 per 10,000 births in 2003-2004 and 9.10 per 10,000 births in 2011-2012 indicating that preventive measures and preconceptional cares should be reinforced.

The scientific community agrees on the protective effect of folic acid against NTDs; in fact, women in reproductive age worldwide are recommended to take 400 mcg/day folic acid from preconception until the end of the first trimester of pregnancy.

Folate is a water-soluble B-vitamin that functions as an acceptor or donor of one-carbon groups. 5-methyltetrahydrofolate [5-methylTHF, (6S)-5-methyltetrahydrofolate] is the most available folate form in plants, human plasma and whole blood: in fact, constitutes 95-98% of folate in serum or red blood cells¹⁷.

This study shows the efficacy of preconceptional supplementation of inositol to reduce the risk for onset of GDM and confirms the importance of folic acid supplementation to avoid NTDs development. Moreover, the positive correlation between HbA1c and OGTT may be useful to propose the use of HbA1c as a single tool for GDM prevention and diagnosis in selected women in pregnancy. Results of data in the literature encourage a large-scale controlled trial of inositol for NTDs prevention, but indicate the need for a careful study design in view of the unwillingness of many high-risk women to be randomized¹⁸.

Patients and Methods

Over the past 2 years, from January 2014 to January 2016, we have collected data from 140 pregnant women who reached our laboratory to undergo the bi-test (also known as combined test: it is a first trimester screening that, by combining nuchal translucency sonography data and analysis of proteins and placental hormones, allows us to provide the future parents the probability their child is affected by aneuploidy. A statement of informed consent was signed by all women according to principles of Helsinki Declaration.

We have consecutively observed 140 women in pregnancy distinguished in two different groups.

The first group ($n = 68$ women) was treated from the preconceptional period until the 24th week of pregnancy with 1.75 g/day D-myo-inositol, 250 mg/day D-chiro-inositol, 12.5 mg/day Zinc pidolate, 100 mg/day methylsulfonylmethane, 120 mg/day Vitamin C and 400 mcg/day (6S)-5-methyltetrahydrofolic acid (commercially named: Ginfast, Logus Pharma S.r.l., Chiesanuova, Republic of San Marino). The control group ($n = 72$) was only treated with 400 mcg/day folic acid.

The main outcome measure was the prevalence of maternal GDM. Secondary outcome measures were the prevalence of NTDs and fetal macrosomia.

Inclusion Criteria: Preconceptional (1 month before conception) supplementation until the 24th week of pregnancy; Caucasian women with singleton pregnancy without GDM, according to IADPSG recommendations¹⁹; no previous NTD-affected pregnancy; women whose first degree relative was not affected by type-2 diabetes and hypertension; women whose BMI was below 30 kg/m²; fasting plasma glucose below 126 mg/dl and random glycemia below 200 mg/dl.

Exclusion Criteria: BMI greater than 30 kg/m²; previous GDM; pre-gestational diabetes; first trimester glycosuria; first degree relative affected by type 2 diabetes or hypertension; fasting plasma glucose greater than 126 mg/dl or random glycemia greater than 200 mg/dl; hemoglobin (Hb) below 10 g/dl; carrier of thalassemic trait; sickle cell anemia; twin pregnancy; therapy with corticoids; not Caucasian and with PCOS.

Eligible participants underwent a standard 2 h 75 g OGTT in the 24 weeks' gestation. Women were given instructions by doctors to follow the WHO procedures to fast overnight (8-14h) before testing. Before drawing blood, nurses confirmed with the pregnant women that they had had an overnight fasting. 6 ml blood samples were collected at fasting, 1h, and 2h after the women were administered with 300 ml water, in which 75 g of anhydrous glucose were dissolved, respectively, using NaF/EDTA tubes. Blood samples were stored at room temperature before 3 time-points of glucose were all drawn. Once the samples were sent to the laboratory, they were centrifuged, and plasmatic glucose was at once measured by a hexokinase method using eligible participants undergoing a standard 2h 75 g OGTT between 22 and 28 weeks of gestation. Women were provided instructions by doctors to follow the WHO procedures to fast overnight (8-14h) before testing.

Before collecting blood, nurses asked for confirmation to the pregnant women they had and overnight fasting nurses were asked for confirmation that the pregnant women had had an overnight fasting. 6 ml blood samples were collected at fasting, 1h, and 2h after the women were administered with 300 ml water, in which 75 g of anhydrous glucose were dissolved, respectively, using NaF/EDTA tubes. Blood samples were stored at room temperature before 3 time-points of glucose were all drew. Once the samples were sent to the laboratory, they were centrifuged and plasma glucose was immediately measured by a hexokinase method using UniCel DxC 800 Synchron Clinical Systems (Beckman Coulter®, Fullerton, CA, USA). At each batch, quality control plasma was set to calculate the coefficients of variation. The coefficients of variation for low and high value were 1.63% and 1.43%, respectively. If values were outside 3SD, recalibration and retest were performed to confirm the result. HbA1c assay was performed on venous blood collected in test tubes containing EDTA-K3 (anticoagulant) using High Performance Liquid Chromatography (HPLC) through the Tosoh HPLC G8 System (Tosoh Bioscience S.r.l., Turin, Italy).

Statistical Analysis

Statistical analysis was carried out with SPSS statistical package version 17 (SPSS Inc., Chicago, IL, USA). Data are expressed as means $\pm$ Standard Deviation (SD) for categorical variables. The means of independent groups were compared using Student's *t*-test after checking for normal distribution. A value of $p < 0.05$ was considered statistically significant.

Results

We collected data from 150 pregnant women, even if ten of them were excluded from the study: four women who had an Hb value below 10 g/dl, three women because of miscarriage in the first trimester of pregnancy, and other three because they changed the reference clinicians. Therefore, we collected data from 68 women treated with Ginfast (treated group) and from 72 women with supplementation only with folic acid (control group). No statistical difference was found between the two groups at baseline (Table I).

After the 24th week of pregnancy, we did not find any clear difference between groups in

Table I. Baseline parameters of both groups.

Parameters	Treated group	Control group	<i>p</i> *
Age (y)	30.60 ± 4.76	31.34 ± 3.78	0.556
BMI (kg/m ²)	24.52 ± 4.03	25.28 ± 3.12	0.887
SBP (mmHg)	110.77 ± 12.04	112.56 ± 10.45	0.124
DBP (mmHg)	67.61 ± 8.43	66.79 ± 8.86	0.215
FBG (mg/dL)	80.66 ± 8.46	79.87 ± 8.69	0.706
HbA1c (mmol/mol)	29.10 ± 8.30	29.55 ± 8.12	0.812
HB	12.64 ± 0.64	12.16 ± 1.05	0.930
Serum Folate (nmol/L)	12.56 ± 4.42	12.47 ± 4.74	0.209
HCy (μmol/l)	8.09 ± 3.80	7.59 ± 3.76	0.456

Data are mean ± standard deviation; **p* < 0.05 was considered statistically significant. BMI = Body Mass Index; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; FBG = Fasting Blood Glucose; HbA1c = Glycated haemoglobin; HCy = Homocysteine.

terms of systolic and diastolic blood pressure, homocysteine, NTDs, birth weight, neonatal hypoglycemia, preterm delivery and APGAR score (Table II).

A significant difference was found in terms of BMI (*p* = 0.045), Fasting glucose OGTT (*p* = 0.050), after 1-h-glucose OGTT (*p* = 0.020), 2-h-glucose OGTT (*p* = 0.037), HbA1c (*p* = 0.010) and plasma folate (*p* = 0.035).

Five infants weighted greater than 4 kg and were in the control group; none were in the treated group (Table II).

Moreover, Spearman correlation showed a positive association between HbA1c and Fasting Blood Glucose (FBG) (*p* < 0.05) at baseline, and a positive significant correlation between Fasting glucose OGTT (*p* < 0.0001),

1h-OGTT (*p* < 0.0001), 2h-OGTT (*p* < 0.0001) and HbA1c performed at the 24th week of pregnancy (Table III).

Discussion

Due to its role as a second messenger in intracellular signal transduction, several clinical trials have suggested a beneficial effect of inositol (myo- or chiro-) supplementation in improving ovarian function, hormone status and glucose homeostasis, with no reported side effects: for this reason it is potentially suitable to be used on a population-wide level.

We found a statistically significant difference in term of glycemic control biomarkers (FBG, OGTT and HbA1c) between women in pregnancy

Table II. Parameters of both groups at 24th week of pregnancy.

Parameters	Treated group	Control group	<i>p</i> *
BMI (kg/m ²)	26.74 ± 4.18	27.58 ± 5.68	0.045
SBP	108.50 ± 7.54	111.50 ± 10.91	ns
DBP	67.65 ± 7.51	68.03 ± 7.45	ns
Fasting glucose OGTT (mg/dL)	75.93 ± 9.29	82.12 ± 7.65	0.050
1-h-glucose OGTT (mg/dL)	125.26 ± 23.60	133.95 ± 21.42	0.020
2-h-glucose OGTT (mg/dL)	98.56 ± 26.65	104.82 ± 28.62	0.037
HbA1c (mmol/mol)	23.46 ± 8.69	27.20 ± 9.42	0.010
Serum folate (nmol/L)	17.47 ± 3.09	14.25 ± 3.27	0.035
HCy (μmol/l)	7.33 ± 2.13	7.68 ± 1.76	ns
NTD %	0	0	ns
Birth weight (kg)	3.23 ± 0.44	3.36 ± 0.79	ns
Macrosomia (> 4.kg) %	0	5	0.045
Neonatal hypoglycemia %	0	0	ns
Preterm delivery %	4	7	ns

Data are mean ± standard deviation; **p* < 0.05 was considered statistically significant; ns = no significative. BMI = Body Mass Index; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; FBG = Fasting Blood Glucose; HbA1c = Glycated haemoglobin; HCy = Homocysteine; NTD = Neural tube defects.

Table III. Spearman correlation between OGTT and HbA1c at baseline and after supplementation.

Parameters		FBG (mg/dL) baseline	HbA1c mmol/mol baseline	Fasting glucose OGTT (mg/dL)	OGTT 1h (mg/dL)	OGTT 2h (mg/dL)	HbA1c (mmol/mol)
FBG (mg/dL) baseline	Correlation Coefficient	1.00					
	<i>p</i> *	•					
HbA1c (mmol/mol) baseline	Correlation Coefficient	0.239	1.00				
	<i>p</i> *	0.04	•				
Fasting glucose OGTT (mg/dL)	Correlation Coefficient	0.260	0.05	1.00			
	<i>p</i> *	0.05	0.71	•			
1-h-OGTT(mg/dL)	Correlation Coefficient	-0.19	0.07	0.314	1.00		
	<i>p</i> *	0.15	0.60	0.02	•		
2-h-OGTT (mg/dL)	Correlation Coefficient	-0.04	-0.13	0.20	0.585	1.00	
	<i>p</i> *	0.79	0.37	0.16	0.00	•	
HbA1c (mmol/mol)	Correlation Coefficient	0.11	0.434	0.373	0.789	0.594	1.00
	<i>p</i> *	0.40	0.00	0.00	0.00	0.00	

FBG = Fasting blood glucose. HbA1c = Glycated haemoglobin. **p* < 0.05 was considered statistically significant.

treated with D-myo-inositol and D-chiro-inositol and control group, with a marked improvement in the treated group.

To prevent the possible risk of GDM onset, our results suggest that treatment with 1.75 g/day myo-inositol, 250 mg/day D-chiro-inositol, 12.5 mg/day Zinc pidolate, 100 mg/day methylsulfonylmethane, 120 mg/day Vitamin C and 400 mcg/day (6S)-5-methyltetrahydrofolic acid could be useful for selected pregnant women (Caucasian, singleton pregnancy without GDM, no previous NTD-affected pregnancy, whose first degree relative was not affected by type-2 diabetes and hypertension; BMI below 30 kg/m²; fasting plasma glucose below 126 mg/dl and random glycemia below 200 mg/dl, Hb greater than 10 g/dl, no carrier of thalassemic- trait, no sickle cell anemia).

One of the aims of our study was to demonstrate that the use of (6S)-5-methyltetrahydrofolic acid in the preconceptional period until the 24th week of pregnancy contrasts the product of conception from NTDs.

Unlike (6S)-5-methyltetrahydrofolic acid, folic acid needs to be reduced and substituted with one-carbon residues, a process involving 5,10 methylenetetrahydrofolate reductase (MTHFR), before entering the systemic circulation as 5-methyltetrahydrofolic²⁰.

Therefore, folic acid might have a smaller effect compared with (6S)-5-methyltetrahydrofolic acid on plasma folate in individuals with the thermolabile variant of MTHFR (single nucleotide polymorphism: MTHFR C677T)²⁰.

For this reason it was chosen the supplementation with (6S)-5-methyltetrahydrofolic acid, because is not influenced by the MTHFR genotype respect to folic acid²¹.

After the 24th week of pregnancy, we have observed a higher serum folate values in the group treated with (6S)-5-methyltetrahydrofolic acid respect to the control group treated with folic acid. These results confirm the efficacy of the (6S)-5-methyltetrahydrofolic acid respect to the folic acid.

However, the use of folic acid in the periconceptional period can prevent about 70% of NTDs; in the remaining cases, no medical prevention is available, and those conditions should be defined as folate-resistant NTDs²².

Rodent models at first²² and then some study in humans^{21,23,24}, have suggested that some folate-resistant NTDs can be prevented by inositol (myo-inositol and chiro-inositol) supplementation prior to pregnancy.

Cavalli et al²² have suggested that mothers with a previous NTD-affected pregnancy, despite correct periconceptional folic acid intake, should

be considered at risk of folate-resistant NTDs and should be treated with myo-inositol and folic acid in the next pregnancy.

The OGTT, performed at the 24-28th gestational week, is the most common test used to screen and diagnose GDM, but it is often poorly tolerated by pregnant women.

The gold standard indicator of glycemic control in patients with diabetes mellitus is HbA1c, but in the scientific literature there are several conflicting data on its use in pregnancy²⁵.

Our preliminary data would seem to encourage the use of HbA1c as a screening tool for GDM, however, population reference ranges need to be established prior to universally implementing HbA1c as a screening test in gestational diabetes.

The importance to identify specific reference populations explains the reason for which, in our study, we have selected pregnant women who had specific characteristics.

Conclusions

To prevent perinatal complications for mothers and infants and to avoid the risk of adverse outcomes in pregnancy and in the long term, there is an urgent need to reinforce the preconceptional care. Our investigation shows the potential benefit of antenatal supplementation with the association of D-myo-inositol and D-chiro-inositol on glycemic parameters of pregnant women and in preventing the occurrence of GDM.

Furthermore, the improvement of serum folate confirms the efficacy of (6S)-5-methyltetrahydrofolic acid supplementation, in order to prevent NTDs.

Moreover, the positive correlation between HbA1c and OGTT may be useful to integrate HbA1c as a single tool for GDM prevention and diagnosis in pregnant women with specific characteristics.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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