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SHORT REPORT

# Second trimester amniotic fluid myo-inositol concentrations in women later developing gestational diabetes mellitus or pregnancy-induced hypertension

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**Abstract**

*Objective*: To evaluate myo-inositol concentrations in amniotic fluid in women later developing gestational diabetes and hypertension.
*Methods*: A retrospective study was carried out with three groups of amniotic fluid samples (15–18 gestational weeks): 30 gestational hypertension pregnancies, 30 gestational diabetes pregnancies, and 30 normal pregnancy.
*Results*: A significant difference was observed in myo-inositol concentrations between the median gestational diabetes values (124.0 μmol/L, IQR 90.0–162.5) and the control group values (79.0 μmol/L, IQR 62.0–107.5), but also with gestational hypertension median values (79.0 μmol/L, IQR 67.75–92.0) ($p < 0.001$).
*Conclusions*: This study has shown that myo-inositol concentrations in amniotic fluid increased significantly in women later developing gestational diabetes compared to the control group.

**Keywords**

Amniotic fluid, fetal metabolism, gestational diabetes, gestational hypertension, myo-inositol

**History**

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## Introduction

Myo-inositol (MI) is one of the nine inositol isomers; it is a polyalcohol deriving from glucose and it is synthesized by only a few organs such as the testes, kidney and brain [1]. Inositol is found in the membrane cells as inositol phosphoglycans (IPGs). The essential structure of IPG is a phospho-inositol group (chiro-inositol in the P-type, myo-inositol in the A-type) and a variable glycan moiety containing glucosamine/galactosamine [2]. MI has been proposed to have a role in many conditions, including insulin resistance, diabetes mellitus and hypertensive disorders [3]. The aim of our study was to evaluate MI concentrations in amniotic fluid at the time of amniocentesis in women who subsequently developed Pregnancy Induced Hypertension (PIH) or Gestational Diabetes Mellitus (GDM), to verify whether MI modifications may occur so early in pregnancy.

## Methods

A retrospective study was carried out with two groups of amniotic fluid samples stored at –80 °C, over a 3-year period, after amniocentesis, performed from 15 to 18 weeks gestation at our Prenatal Diagnosis Center: 30 PIH pregnancies and 30 GDM pregnancies. PIH is a condition characterized by increased blood pressure (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg) which occurs in the second half of gestation [4]. GDM is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. GDM diagnosis was performed with a 2-step screening: a glucose challenge test with 50 g of oral glucose and eventually a complete oral glucose tolerant test with 100 g of glucose. A control group of 30 samples of normal pregnancy amniotic fluid was selected and matched with the cases for maternal age and Body Mass Index (BMI). The protocol was consistent with the principles of the Declaration of Helsinki and all the participants gave their written informed consent, which allowed 1 ml of their amniotic fluid to be used for experimental purposes. The study was approved by the Institutional Review Board. MI quantification was performed by Chelab Pharma Division using gas chromatography–mass spectrometry (GC–MS) analysis after extraction with organic solvents and derivation. Injection (1.0 μl) was performed in a split-less mode at 270_C and a capillary column Agilent 122–5532 DB-5 ms (Agilent Technologies, Inc., Santa Clara, CA) (0.25 mm _ 30 m _ 0.25 μm) was used. Total run-time was 15 min: oven at 70_C from 0 to 1 min; 20_C/min to 150_C; 10_C/min to 240_C;

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Table 1. General characteristics of the three groups: mean (±SD) and (%).

| | Controls ($n=30$) | PIH ($n=30$) | GDM ($n=30$) |
|---|---|---|---|
| Maternal age (y) | 36.0 (±3.4) | 36.4 (±3.0) | 36.2 (±5.3) |
| BMI (kg/m$^2$) | 28.1 (±4.1) | 28.8 (±4.6) | 28.1 (±4.7) |
| Birth weight (g) | 3271 (±246) | 2749 (±601)* | 3184 (±451) |
| GA at delivery (d) | 273 (±7) | 258 (±15)* | 266 (±13)** |
| Nulliparous (%) | 43.3 (%) | 53.3 (%) | 46.7(%) |
| Preterm birth (%) | 0 (%) | 30 (%)* | 13.3 (%)** |
| Obese (%) | 30 (%) | 36.7 (%) | 26.7 (%) |

*$p<0.05$ versus the control group. **$p<0.05$ versus the control group.

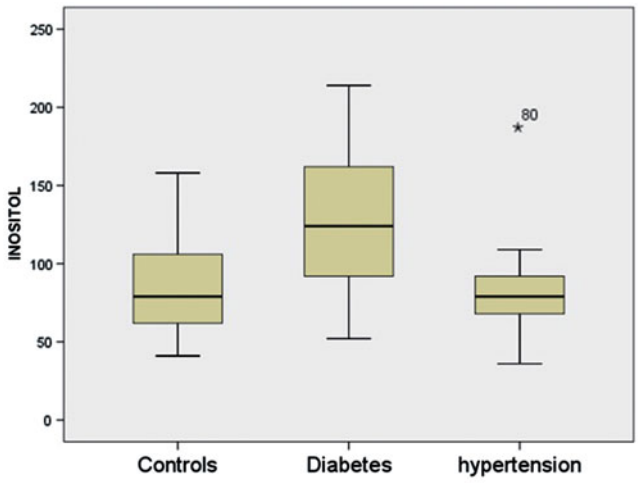


Figure 1. Myo-inosital concentration between the median values of the control group, GDM, and PIH.

4 min at 320_C post run. The flow rate was fixed at 1.2 ml/min and the results were analyzed by an MS 5973 Network Series detector (Agilent Technologies, Inc., Santa Clara, CA) in a sim mode. Statistical analysis was carried out with an SPSS statistical package version 17 (SPSS Inc., Chicago, IL). Data are expressed in median and interquartile range (IQR). The Mann–Whitney test was used to compare amniotic fluid values between groups and Spearman's test was used to correlate normal pregnancy amniotic fluid values with maternal age, BMI, parity, gestational age at amniocentesis and neonatal weight.

## Results

The general characteristics of the three groups are reported in Table 1. There were no statistical differences between groups in BMI, obese women rate, parity, and maternal age. A significant difference was highlighted between the control group and the PIH group in birth weight, gestational age at the delivery and in preterm birth rate ($p<0.05$) (Table 1). A significant difference was observed in MI concentrations between the median GDM values (124.0 μmol/L, IQR 90.0–162.5) and the control group values (79.0 μmol/L, IQR 62.0–107.5), but also with PIH median values (79.0 μmol/L, IQR 67.75–92.0) ($p<0.001$) (Figure 1). No significant difference was shown in MI concentrations between PIH and the control group ($p=0.65$). In the control group, no correlation was highlighted between amniotic fluid values and maternal age: $r=0.15$ ($p=0.43$); BMI: $r=0.84$ ($p=0.65$); parity: $r=0.08$ ($p=0.64$); gestational age: $r=0.23$ ($p=0.21$), and neonatal weight: $r=-0.028$ ($p=0.88$)

## Discussion

A pilot study on free inositol essays in amniotic fluid was carried out, for the first time, about 60 years ago [5]. The researchers failed to demonstrate a placental transfer or a placental synthesis of inositol; the study hypothesized a sole fetal production of MI in the amniotic fluid; furthermore, its concentration in amniotic fluid was higher than in fetal blood [5]. Today it is known that in normal pregnancy inositol concentration in the amniotic fluid is the result of a balance between a fetal production (fetal side) and trans placental intake (maternal side) [6]. In our study, normal pregnancies MI amniotic fluid concentrations (control group) did not correlate with maternal parameters (age, BMI, and parity), suggesting a possible fetal origin. lnositol and its isomers are some of the many molecules and hormones (such as insulin and insulin-like growth factors) that probably act as nutritional and maturational signals, adapting fetal development to prevailing intrauterine conditions, and becoming progressively more responsive to stimuli throughout the gestation [7]. To date, the role of inositol in fetal development has not yet been clarified. Scioscia et al. [8] have speculated that inositol is involved in increasing insulin sensitivity at the level of fetal tissues, promoting growth and development as insulin and other growth factors normally do. Its importance in fetal hormonal milieu is argued by the increased production and metabolism of inositol during preeclampsia as seen in all fetal compartments (blood, amniotic fluid, and placenta) [6]. PIH and preeclampsia often lead to fetal growth restriction particularly when the onset of the hypertensive syndrome is before 34 weeks of gestation. It is important to consider the cellular and molecular processes that underlie fetal adaptations to hypoxic intrauterine stress as it may occur in hypertensive disorders of pregnancy. Paine et al. [6] have shown that in women with hypertensive disorders, the inositol equilibrium between the fetal and the maternal side of the placenta has been disrupted, with an accellerate fetal metabolism of this molecule, probably to counteract the incipient growth restriction. This situation could determine a consequent reduction of the amniotic concentration of MI for its increased conversion into d-chiro inositol (its metabolically active isomer) by an epimerase enzyme [9]. This study has not shown a significant difference in amniotic fluid MI concentrations between PIH cases and controls; probably because the hypertensive cases were not particularly serious (we had only four cases of IUGR) with limited involvement of placental function. Furthermore, the results may be influenced by the fact that these assays were performed months before the manifestation of the syndrome. Instead, a different MI metabolic behavior in the GDM group has been observed. MI is synthesized from glucose, thus the increased level in amniotic fluid could be the consequence of an early higher fetal glucose supply. In diabetic patients, the extracellular glucose in excess might inhibit MI uptake, as a result from a competition between MI and glucose from MI transporters.

Therefore, under hyperglycemic conditions, high glucose ambience could impair extracellular MI uptake and so contribute to the MI intra-tissular depletion observed in diabetes. The consequence is the ''inosituria'', which may explain the increased concentration of MI in amniotic fluid due to urine production. The significant difference highlighted not only with the normal pregnancies but also with the PIH group, reinforces our hypothesis of two different metabolic pathways in these two different maternal pathologies. This study is the first that correlates MI amniotic fluid concentration with conditions such as PIH and GDM so early, and they may affect not only maternal health but also fetal outcome. If our hypotheses about the metabolic behavior of MI will be confirmed by larger studies, we could define this molecule better in the broad context of the network of metabolically active substances for both the mother and the fetus, and also be able to better understand its pathological changes in pregnancy.

## Declaration of interest

A grant has been given from Lo.Li. Pharma for the cost of the assays. Dr Unfer and Dr Carlomagno are employed in Lo.Li Pharma.

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